

AN EVALUATION OF THE MUTAGENIC POTENTIAL OF SC-19192  
EMPLOYING THE AMES SALMONELLA/MICROSOME ASSAY;  
S.A. 1384

Final Report

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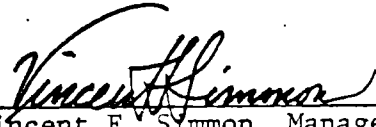
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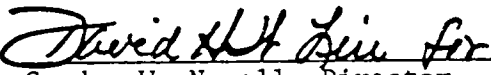
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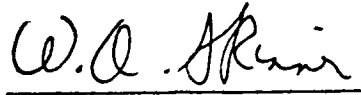
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## SUMMARY

SRI International examined G. D. Searle & Co. compound SC-19192 (Lot TJT 12-32) for mutagenicity using the Ames Salmonella/microsome assay with the tester strains TA1535, TA1537, TA1538, TA98, and TA100. Each assay was performed in the presence and in the absence of a metabolic activation system. No mutagenic response was obtained with SC-19192, either in the presence or in the absence of metabolic activation.

## INTRODUCTION

SRI International examined G. D. Searle & Co. compound SC-19192 (Lot TJT 12-32) for mutagenic activity by in vitro microbiological assays with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100. An Aroclor 1254-stimulated, rat liver homogenate metabolic activation system was included in the assay procedure to provide metabolic steps that the bacteria either are incapable of conducting or do not carry out under the assay conditions.

The Ames Salmonella/microsome assay has proven to be 80 to 90% reliable in detecting carcinogens as mutagens, and it has about the same reliability in identifying chemicals that are not carcinogenic.<sup>1</sup> However, because this system does not always provide 100% correlation with carcinogenicity investigations in animals, neither a positive nor a negative response proves conclusively that a chemical is hazardous or nonhazardous to man.

## METHODS

### Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The Salmonella typhimurium strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium petri plates containing a trace of histidine, only those cells that revert to histidine independence (his<sup>+</sup>) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The his<sup>+</sup> revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar the mutation frequency is increased 2- to 100-fold.

We obtained our S. typhimurium strains from Dr. Bruce Ames of the University of California at Berkeley.<sup>1-3</sup> In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa<sup>-</sup>) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin, biotin (bio<sup>-</sup>), and in the repair of ultraviolet (uv)-induced DNA damage (uvrB<sup>-</sup>). The rfa<sup>-</sup> mutation makes the strains more permeable to many large aromatic molecules, thereby increasing the mutagenic effect of these molecules. The uvrB<sup>-</sup> mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to his<sup>+</sup> by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations

for a given dose of most mutagens.<sup>5</sup> In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cells. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens (e.g., ICR-191, benzo(a)pyrene, aflatoxin B<sub>1</sub>, and 7,12-dimethylbenz(a)anthracene). Strains TA1537 and TA1538 are reverted by many frameshift mutagens. TA1537 is more sensitive than TA1538 to mutation by some acridines and benzanthraces, but the difference is quantitative rather than qualitative. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All indicator strains are kept at 4° C on minimal medium plates, supplemented with a trace of biotin, and an excess of histidine. The plates with the plasmid-carrying strains contain, in addition, ampicillin (25 µg/ml), to ensure stable maintenance of the plasmid pKM101. New stock culture plates are made every two months from single colony reisolates that were checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37° C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures are shaken for 3 to 4 hours to ensure optimal growth.

#### Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals, either of the aromatic amino type or polycyclic hydrocarbon type, are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens.<sup>4,6-8</sup> Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use.<sup>6</sup> In brief, adult male rats (250 to 300 g) are given a single 500-mg/kg intraperitoneal injection of a polychlorinated biphenyl, Aroclor 1254. This treatment enhances the synthesis of enzymes involved in the metabolic

conversion of chemicals. Four days after the injection the animals' food is removed but drinking water is provided ad libitum. On the fifth day, the rats are killed, and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed in an equal volume of cold, sterile 0.15 M KCl (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x g, and the supernatant, referred to as the S-9 fraction, is quickly frozen in dry ice and stored at -80° C.

The metabolic activation mixture for each experiment consists of, for 10 ml:

- 1.00 ml of S-9 Fraction
- 0.20 ml of  $MgCl_2$  (0.4 M) and KCl (1.65M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate (0.2 M, pH 7.4)
- 3.35 ml of  $H_2O$ .

### Assays in Agar

To a sterile 13 x 100 mm test tube placed in a 43° C heating block, we add in the following order:

- (1) 2.00 ml of 0.6% agar\*
- (2) 0.05 ml of indicator organisms
- (3) 0.05 ml of a solution of the test chemical
- (4) 0.50 ml of metabolic activation mixture (optional).

For negative controls, we use steps (1), (2), and (4) (optional) and 0.05 ml of the solvent used for the test chemical. Because the majority of organic compounds are not sufficiently water soluble--particularly at the higher concentrations--we routinely use dimethylsulfoxide (DMSO). Other solvents that are occasionally used are water, ethanol, or benzene. For positive controls, we test each culture by specific mutagens known to revert each strain using steps (1), (2), (3), and (4) (optional).

This mixture is stirred gently and then poured onto minimal agar plates.<sup>†</sup> After the top agar has set, the plates are incubated at 37° C for 2 days. The number of his<sup>+</sup> revertant colonies is counted and recorded.

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\* 0.6 % agar contains 0.05 mM histidine, 0.05 mM biotin, and 0.1 M NaCl.

† Minimal agar plates consist of, per liter, 15 g of agar, 10 g of glucose, 0.2 g of MgSO<sub>4</sub>·7H<sub>2</sub>O, 2 g of citric acid monohydrate, 10 g of K<sub>2</sub>HPO<sub>4</sub>, and 3.5 g of NaH<sub>2</sub>PO<sub>4</sub>·4H<sub>2</sub>O.

## RESULTS AND DISCUSSION

Tables 1 through 3 present the results of the microbiological assays of SC-19192 with S. typhimurium. The data in Table 1 are an average of the two assays conducted on separate days; Tables 2 and 3 present the results of the individual assays and the date when they were conducted.

SC-19192 was tested over a wide range of concentrations, from 50 to 10,000  $\mu\text{g}$  per plate. Dimethyl sulfoxide (DMSO) was used as the solvent. No toxicity was apparent at the highest dose tested (10,000  $\mu\text{g}$  per plate), but some of the sample formed a precipitate on the plate at that dose. SC-19192 was not mutagenic in these assays, either in the presence or in the absence of metabolic activation.



Table 1

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM  
SC-19192 (LOT TJT 12-32)<sup>a</sup>

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Average Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control (DMSO)	-	50,000	20	7	11	21	134
	+	50,000	21	8	23	32	154
Positive controls							
Sodium azide	-	0.5	211				321
9-Aminoacridine	-	50.0		188			
2-Nitrofluorene	-	5.0	20	6	969	580	140
2-Anthramine	+	2.5	172	98	16	21	1292
		2.5			911	991	
SC-19192	-	50	25	6	26	15	121
	-	100	18	8	16	19	136
	-	500	15	3	15	19	131
	-	1000	19	5	10	17	146
	-	5000	19	3	15	22	111
	-	10000	20	9	16	19	123
	+	50	23	8	21	29	140
	+	100	23	8	27	28	150
	+	500	18	8	28	22	164
	+	1000	18	5	18	31	163
	+	5000	20	6	16	26	152
	+	10000	19	9	16	24	136

Table 2

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM  
SC-19192 (LOT TJT 12-32)\*

Experiment 1 - 29 March 1978

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control (DMSO)	-	50,000	17, 15	10, 8	13, 11	17, 31	113, 131
	+	50,000	15, 18	12, 11	27, 19	36, 53	155, 150
Positive controls							
Sodium azide	-	0.5	240				300
9-Aminoacridine	-	50.0		230			
2-Nitrofluorene	-	5.0			846	698	
2-Anthramine	-	2.5	9	3	12	28	140
	+	2.5	188	66	850	871	1044
SC-19192	-	50	14	10	45	20	106
	-	100	18	11	20	24	141
	-	500	14	3	15	21	141
	-	1000	22	5	15	18	149
	-	5000	18	4	24	22	107
	-	10000*	15	14	20	17	130
	+	50	22	11	28	47	120
	+	100	15	10	27	40	146
	+	500	9	10	34	34	172
	+	1000	10	7	15	41	166
	+	5000	15	7	25	32	150
	+	10000*	12	15	21	28	107

\* Precipitate was formed on the plate at 10000 µg per plate.

Table 3

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM  
SC-19192 (LOT TJT 12-32)\*

Experiment 2 - 31 March 1978

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control (DMSO)	-	50,000	23,26	4,5	16, 4	13,21	144,146
	+	50,000	30,22	2,6	18,26	26,14	162,148
Positive controls							
Sodium azide	+	0.5	181				341
9-Aminoacridine	-	50.0		145			
2-Nitrofluorene	-	5.0			1092	462	
2-Anthramine	-	2.5	30	9	19	14	139
	+	2.5	155	130	972	1111	1539
SC-19192	-	50	35	2	7	9	135
	-	100	18	5	12	13	131
	-	500	16	2	14	16	120
	-	1000	15	5	5	15	143
	-	5000*	20	2	6	22	114
	-	10000*	25	4	12	20	116
	+	50	23	5	13	10	160
	+	100	30	5	26	15	153
	+	500	26	5	22	9	155
	+	1000	25	2	20	20	159
	+	5000*	25	5	6	19	153
	+	10000*	26	3	10	20	164

\* Precipitate was formed on the plate at 5000 and 10000 µg per plate.

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