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Haskell Laboratory for Toxicology and Industrial Medicine
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HASKELL LABORATORY REPORT NO. 1072-80 [redacted]

Haskell No.: 13,783

Test Material: [redacted]

Other Code: [redacted]

(see page 4 for composition)

Submitted by: [redacted]
Polymer Products
Washington Works

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MUTAGENICITY EVALUATION IN SALMONELLA/TYPHIMURIUM

INTRODUCTION

The purpose of this test is to determine whether the test sample causes mutations in Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100. These strains cannot produce histidine because of mutations in the genes coding for enzymes involved in the synthesis of this essential amino acid. Individual bacteria acquiring a specific mutation in these genes regain the ability to synthesize histidine. These revertants form visible colonies in agar. A comparison of the number of revertants observed in the presence and absence of test sample indicates whether the sample induces mutations in these strains of bacteria.

METHODS

Plate Incorporation Assay - Solids and Nonvolatile Liquids

The assay was performed in the presence and the absence of a rat-liver homogenate activation system similar to the method described by Ames, et al. (Mutation Res. 31:347-364, 1975). Treatments without activation were conducted by adding 0.1 ml of the solvent or a solution of the test sample and 0.1 ml of an overnight culture containing approximately 10^8 bacteria to 2 ml of top agar (0.6% agar, 0.6% NaCl, 0.05 mM L-histidine, 0.05 mM biotin). These components were mixed and poured on the surface of a plate containing 20 ml of Davis minimal agar. Treatments with activation were conducted by adding 0.5 ml of S-9 mix to the bacteria/test sample/top agar and pouring the mixture onto a minimal agar plate. The S-9 mix contained per ml: 0.3 ml of S-9 diluted with 0.15 M KCL (1.6 mg of protein), 8 micromoles $MgCl_2$, 33 micromoles KCl, 5 micromoles glucose-6-phosphate, 4 micromoles NADP and 100 micromoles sodium phosphate (from a 0.2 M stock sodium phosphate solution at pH 7.4).

The S-9 was the 9,000 x g supernatant of liver homogenate (1 gm wet liver:3 ml 0.15 M KCl). The livers were obtained from 8 to 9 week old male Charles River CD rats given 500 mg of Aroclor 1254/kg five days before sacrifice. The revertant colonies were counted after the plates were incubated at 37°C for 48 hours.

Positive (known mutagens) and negative (solvent) controls were included in all assays.

The cytotoxicity of the test sample in the presence and absence of an activation system, as measured in strain TA 1535, was the basis for selecting concentrations for the mutagenesis experiments. The protocol used to determine the cytotoxicity was identical to the mutagenesis protocol except that 10^9 rather than 10^8 bacteria were used per plate and excess histidine was present. Concentrations of test sample that were nontoxic and, if possible, slightly toxic were selected for the mutagenesis assay.

The concentrations of test sample per ml of treatment medium per plate were calculated with the following assumptions: liquids - the solvent and test sample volumes were additive; solids - the addition of test sample did not change the volume of solvent.

Spot Test in a Closed System

The plate incorporation assay may not be the best method to test liquid samples that vaporize appreciably when this protocol is followed. A spot test in a closed system is performed on all nonaqueous liquids to aid in identifying samples with volatile mutagenic components. These samples are tested by different protocols.

Experiments without activation were performed by adding 10^8 bacteria (in 0.1 ml) to 2.0 ml of standard mutagenesis top agar, mixing immediately, and pouring on a Davis minimal agar plate. Experiments with activation were performed in the same manner except that 0.5 ml of S-9 mix was also added before mixing. All components used for this assay were identical to the ones used in the plate incorporation assay.

A sterile disk saturated with test compound was placed in the center of one of two replicate plates. The plate with the disk was inverted and the other plate was stacked on top of it. Both plates were sealed in a plastic bag and incubated for 48 hours at 37°C.

Use of Data:

1) A Plate Incorporation Assay - Solids and Nonvolatile Liquids is performed if the spot test is negative.
2) If the spot test is positive, a limited Plate Incorporation Assay - Solids and Nonvolatile Liquids is performed with only the strains and conditions in which mutagenic activity was suggested in the spot test. A complete assay is performed if mutagenic activity is observed in the limited assay.
3) If the limited assay is negative, the test sample is assayed according to the plate assay protocols for testing gases and volatile liquids.

Statistical Analysis

The data points (number of revertants/plate, x) were transformed prior to analysis using the power transformation $y = x^{0.2}$. Two analyses were performed. In the first analysis the response observed at each concentration was compared to the control by a t-test of significance to determine which dose levels produced significant increases in mutation frequency. In the second analysis the significance of the dose-response relationship was tested. Linear, quadratic, and higher order dose-response effects were tested in an F-test of significance. In addition, an analysis was conducted to determine whether the dose-response relationship was different in different trials.

The following guidelines are used to classify a test sample as a mutagen or nonmutagen in the assay.

A test sample is classified as a nonmutagen when:

A. The probability is greater than 0.05 that the numbers of revertants at each of the test sample concentrations studied are not greater than the number of revertants in the solvent control.

AND

B. The probability is greater than 0.05 that there is not a positive correlation between the numbers of revertants and increasing concentrations of the test sample.

A test sample is classified as a mutagen when:

A. The probability is less than 0.01 that the numbers of revertants at one or more of the test sample concentrations studied are not greater than the number of revertants in the solvent control.

AND

B. The probability is less than 0.01 that there is not a positive correlation between the number of revertants and increasing concentrations of the test sample.

Test samples that cannot be classified by the above criteria are dealt with on a case-by-case basis.

A detailed description of the statistical methods is available (J. D. Irr and R. D. Snee. 1979. Statistical Evaluation of Mutagenicity in the CHO/HGPRT System. In Banbury Report 2-Mammalian Cell Mutagenesis: The Maturation of Test Systems (ed. A. W. Hsie, J. P. O'Neill, and V. K. McElheny), p. 263. Cold Spring Harbor Laboratory, New York.

ABBREVIATIONS AND DEFINITIONS

Positive Controls: 2AA, 2-aminoanthracene (DMSO)*; 9AAc, 9-aminoacridine (DMSO)*; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine (DMSO)*; 2NF, 2-nitrofluorene (DMSO)*. *solvent (DMSO = dimethylsulfoxide).

Solvent for Test Chemical: ethanol. Solvent was present where the concentration of test chemical is listed as "0".

Symbols:

(T) Toxicity indicated by decreased density of the background lawn

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RESULTS

In the initial cytotoxicity experiment with strain TA 1535, [redacted] was tested at concentrations up to 10,000 micrograms/plate. No toxicity was observed. Higher concentrations were not tested in the mutagenesis experiments.

A spot test in a closed system was performed and no indication of mutagenic activity was observed.

The mutagenesis data are listed in Tables I, II, III and IV for strains TA 1535, TA 1537, TA 98 and TA 100, respectively.

Statistical analysis of the revertant frequencies obtained in the presence of test chemical showed no significant increases over control frequencies and no significant positive linear dose responses in all strains in either the presence or absence of the activation system. Therefore, the test chemical was not mutagenic.

CONCLUSIONS

[redacted] was tested in Salmonella typhimurium strains TA 1535, TA 1537, TA 98 and TA 100 in the presence and absence of the activation system according to the protocol described in METHODS. This test sample was not mutagenic for these strains of bacteria.

Composition: [redacted]

Contaminants: [redacted]

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TABLE I

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA 1535

WITHOUT ACTIVATION

Compound	Concentration (ug/plate)	Revertants Per Plate (Duplicate Plates) Trial 1	Trial 2
13,783	0	30, 38	22, 21
"	100	31, 36	34, 26
"	500	19, 29	24, 20
"	1000	37, 31	26, 23
"	5000	40, 37	25, 26
"	10,000	40, 35	28, 22
MNNG	4	2694, 3019	2243, 3094

WITH ACTIVATION

Compound	Concentration (ug/plate)	Revertants Per Plate (Duplicate Plates) Trial 1	Trial 2
13,783	0	73, 37	35, 29
"	100	44, 45	41, 22
"	500	44, 54	27, 27
"	1000	46, 45	35, 28
"	5000	46, 54	31, 32
"	10,000	38, 56	25, 40
2AA	10	510, 567	798, 747

H-13,783 = [redacted]

TABLE II

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA 1537

WITHOUT ACTIVATION

Compound	Concentration (ug/plate)	Revertants Per Plate (Duplicate Plates) Trial 1	Trial 2
13,783	0	17, 7	10, 13
"	100	10, 7	14, 8
"	500	2, 4	10, 8
"	1000	7, 5	5, 7
"	5000	8, 11	6, 7
"	10,000	15, 11	5, 7
9AAc	50	1081, 944	1283, 1591

WITH ACTIVATION

Compound	Concentration (ug/plate)	Revertants Per Plate (Duplicate Plates) Trial 1	Trial 2
13,783	0	11, 4	11, 8
"	100	4, 9	8, 8
"	500	12, 5	15, 6
"	1000	16, 8	8, 5
"	5000	11, 9	9, 12
"	10,000	11, 8	9, 12
2AA	10	440, 728	583, 821

H-13,783 = [redacted]

TABLE III

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA 98

WITHOUT ACTIVATION

Compound	Concentration (ug/plate)	Revertants Per Plate (Duplicate Plates) Trial 1	Trial 2
13,783	0	41, 35	25, 24
"	100	37, 37	26, 33
"	500	32, 32	38, 20
"	1000	33, 30	22, 23
"	5000	28, 21	24, 26
"	10,000	23, 24	21, 21
2NF	25	2816, 2647	2895, 2820

WITH ACTIVATION

Compound	Concentration (ug/plate)	Revertants Per Plate (Duplicate Plates) Trial 1	Trial 2
13,783	0	46, 43	51, 42
"	100	48, 35	53, 50
"	500	36, 51	44, 43
"	1000	48, 37	41, 39
"	5000	30, 43	48, 37
"	10,000	42, 63	45, 44
2AA	10	3619, 3648	1838, 2395

H-13,783 = [redacted]

TABLE IV

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA 100

WITHOUT ACTIVATION

Compound	Concentration (µg/plate)	Revertants Per Plate (Duplicate Plates) Trial 1	Trial 2
13,783	0	121, 116	114, 103
"	100	146, 161	101, 170
"	500	103, 121	88, 100
"	1000	115, 156	85, 117
"	5000	115, 100	92, 90
"	10,000	120, 121	49(T), 86(T)
MNNG	4	2493, 2564	3982, 3501

WITH ACTIVATION

Compound	Concentration (µg/plate)	Revertants Per Plate (Duplicate Plates) Trial 1	Trial 2
13,783	0	135, 158	163, 154
"	100	144, 147	147, 168
"	500	145, 171	156, 152
"	1000	132, 157	156, 149
"	5000	140, 155	129, 132
"	10,000	143, 168	144, 131
2AA	5	3125, 3169	2491, 2565

H-13,783 = [redacted]