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E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine

HASKELL LABORATORY REPORT NO. 961-77 [redacted]

Material Tested: [redacted] Haskell No. 11,479
Other Code: [redacted]

Material Submitted by: [redacted] Seaford Textile Fibers Department, Sample Ready for Testing: 8-3-77

MUTAGENIC ACTIVITY OF [redacted]
IN THE *SALMONELLA*/MICROSOME ASSAY

Materials and Methods: The ability of the test sample to revert five strains of *Salmonella typhimurium* from histidine dependence to histidine independence is determined. Strains TA 1535 and TA 100 are used to detect base-pair substitution mutations, whereas TA 1537, TA 1538, and TA 98 are used to detect frame-shift mutations.[1]

The assay is performed in the presence and the absence of a rat-liver homogenate activation system. In the absence of an activation system, 0.1 ml of a solution of the test sample and approximately 10^8 bacteria in 0.1 ml are added to 2 ml of top agar (0.6% agar, 0.6% NaCl, 0.05 mM L-histidine, 0.05 mM biotin). These components are mixed and poured on the surface of a plate containing 20 ml of Davis minimal agar. To treat in the presence of an activation system, 0.5 ml of S-9 mix is added to the bacteria-test sample-top agar mixture. S-9 is the 9,000 x g supernatant of liver homogenate from rats given 500 mg of Arocior *1254*/kg five days before sacrifice. The S-9 mix contains per ml: 0.3 ml of S-9, 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 100 mM sodium phosphate (pH 7.4). The S-9 mix is added to the bacteria, test sample and top agar. These components are mixed and immediately poured over the minimal agar plate. The revertant colonies are counted after the plates are incubated at 37° for 48 hr.[1]

Positive (known mutagens) and negative (solvent) controls are included in each assay. In assays in which an activatic system is present, an additional negative control (i.e., no S-9 mix) is included.

[1]Ames, McCann and Yamasaki, Mutation Res. 31: 347-364, 1975.

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The cytotoxicity of the test sample in the presence and absence of an activation system, as measured in strain TA 1535, is the basis for selecting concentrations to be used in the mutagenesis experiment. The protocol used to determine the cytotoxicity is identical to the mutagenesis protocol except that 10^3 rather than 10^8 bacteria are used per plate and a nonlimiting concentration of histidine is present. Concentrations of test sample that are nontoxic and, if possible, slightly toxic are selected for the mutagenesis assay.

The data is handled as follows: Data from replicate plates within a single experiment are averaged. The average of these values from different experiments is determined. The highest average number of revertants that is obtained is expressed as a multiple of the control value for the sensitive strain(s). When a test sample is active, the average numbers of revertants observed before activity plateaus or decreases at the various concentrations tested are submitted to linear regression analysis. The slope of the line thus obtained is used to determine the number of revertants/nmole or µg of test sample.

Results: On the basis of the initial cytotoxicity experiment with TA 1535, nontoxic and slightly toxic concentrations of [redacted] were chosen for the nonactivated part of the mutagenesis experiment, Trial 1. No evidence of toxicity was demonstrated in the presence of an activation system in the cytotoxicity experiment at the highest concentration tested (10,000 µg/plate). An additional high concentration (15,000 µg/plate) was included in the activated part of the mutagenesis experiment, Trial 1. A subsequent cytotoxicity experiment demonstrated that this concentration was in the range approaching toxicity. In an attempt to more closely approach the toxic range in the nonactivated part of the test, additional higher concentrations were included in the mutagenesis experiment, Trial 2.

The mutagenesis data are listed in Tables I and II for Trial 1 and Tables III and IV for Trial 2.

Summary: [redacted] was tested in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100. This chemical is not mutagenic for these strains of bacteria in the presence or absence of an activation system in experiments performed by the described protocol. A chemical is classified as nonmutagenic if the reversion frequency is less than two times the spontaneous frequency, and if less than 0.02 revertants/nmole are observed.

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TABLE 1

MUTAGENIC ACTIVITY OF [redacted] IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 1538, TA 98 AND TA 100 WITH METABOLIC ACTIVATION

Trial 1

Compound Added			Histidine+ Revertants Per Plate**				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
H_2O			19	8	19	40	142
11,479		µg/plate					
-S-9*	2000	"	15	9	16	19	144
	2000	"	18	15	20	30	165
	4000	"	24	16	20	25	143
	6000	"	27	14	14	26	157
	8000	"	23	13	17	25	158
	10000	"	21	13	17	32	135
	15000	"	21	15	15	26	163
2AA	5	"					2146
	10	"	386		2225	2097	
	100	"		320			

H_2O = Distilled water (solvent control)

11,479 = [redacted]

2AA = 2-Aminoanthracene (positive control)

* = Test plate without S-9 and activators

** = Average of two plates

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TABLE II

MUTAGENIC ACTIVITY OF [redacted] IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 1538, TA 98 AND TA 100 WITHOUT METABOLIC ACTIVATION

Trial I

Compound Added		Histidine+ Revertants Per Plate*				
		TA 1535	TA 1537	TA 1538	TA 98	TA.100
H_2O		23	12	12	33	153
11,479	µg/plate					
	400 "	20	8	14	18	157
	800 "	18	11	13	25	154
	1200 "	22	12	12	21	134
	1600 "	22	10	9	21	144
	2000 "	22	9	9	23	142
MNNG	2 "	3269				2695
9AAc	50 "		1544			
2NF	25 "			1453	2916	

H_2O = Distilled water (solvent control)

11,479 = [redacted]

MNNG = N-Methyl-N'-nitro-N-nitrosoguanidine (positive control)

9AAc = 9-Aminoacridine (positive control)

2NF = 2-Nitrofluorene (positive control)

* = Average of two plates

TABLE III

MUTAGENIC ACTIVITY OF [redacted] IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 1538, TA 98 AND TA 100 WITH METABOLIC ACTIVATION

Trial 2

Compound Added			Histidine+ Revertants Per Plate**				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
H_2O			24	13	37	39	162
11,479		µg/plate					
-S-9*	4000	"	13	12	39	24	146
	2000	"	15	10	37	36	130
	4000	"	17	6	31	35	130
	6000	"	21	10	28	34	115
	8000	"	15	8	39	46	122
	10000	"	23	6	31	38	129
	15000	"	16	4	35	40	163
2AA	5	"					1517
	10	"	116		1250	1166	
	100	"		261			

H_2O = Distilled water (solvent control)

11,479 = [redacted]

2AA = 2-Aminoanthracene (positive control)

* = Test plate without S-9 and activators.

** = Average of two plates

TABLE IV

MUTAGENIC ACTIVITY OF [redacted] IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 1538, TA 98 AND TA 100 WITHOUT METABOLIC ACTIVATION

Trial 2

Compound Added			Histidine+ Revertants Per Plate*				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
H_2O			29	14	23	19	127
11,479		µg/plate					
	800	"	37	12	21	28	148
	1600	"	37	13	24	24	155
	2400	"	34	12	16	26	123
	3200	"	29	9	20	26	95
	4000	"	30	5	22	22	66
MNNG	2	"	2352				1086
9AAc	50	"		522			
2NF	25	"			1332	1614	

H_2O = Distilled water (solvent control)

11,479 = [redacted]

MNNG = N-Methyl-N'-nitro-N-nitrosoguanidine (positive control)

9AAc = 9-Aminoacridine (positive control)

2NF = 2-Nitrofluorene (positive control)

* = Average of two plates