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

HASKELL LABORATORY REPORT NO. 622-77 [redacted]

aterial Tested: [redacted] Haskell No. 11,308

aterial Submitted By: [redacted], Organic Chemicals Dept., Jackson Laboratory Sample Ready for Testing: 6-15-77

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

aterials and Methods: Five histidine-requiring strains of *Salmonella typhimurium* were used in the mutagen assays. trains TA 1535 and TA 100 are used to detect base-pair substitution mutations, whereas strains TA 1537, TA 1538 and A 98 are used to detect frame-shift mutations. (Ames, McCann and Yamasaki, Mutation Research 31 (1975) 347-364).

The tests were performed in the presence and absence of a rat-liver homogenate activation system (S-9). In the sence of metabolic activation, 0.1 ml of a solution of the test compound and approximately 10^8 bacteria were added to ml of top agar (0.6% agar, 0.6% NaCl, 0.05 mM L-histidine, 0.05 mM biotin). The solution was mixed and poured on the rface of a Davis minimal agar plate. The metabolic activation system involved the addition of 0.5 ml of S-9 mixture the chemical-top agar solution. The S-9 mix contains per ml: 0.3 ml of the 9,000 X g supernatant of homogenized ra iver, 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 100 M sodium phosphate (pH 7.4). This mixture is added directly to the top agar immediately before it was poured over the minimal agar plate.

Prior to testing for mutagenicity, the compound is tested for toxicity. Routinely, TA 1535 is used to determine ie general toxicity range for all strains.

Appropriate controls were included for each strain. In the nonactivated system these controls consisted of a gative, or solvent control, and positive controls. A second negative control (-S-9 control) is included in the activated say to measure any activity of the compound in the absence of the S-9 activator mixture.

All plates were incubated at 37°C for 48 hours.

Results: Tables I and II.

Summary: [redacted] was tested in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 in concentrations up to 10,000 µg per petri plate. The compound was not mutagenic in the microbial assays either in the presence or absence of a liver microsomal system, i.e., it did not induce a significant increase over the spontaneous mutation frequency.

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Date: August 19, 1977

[redacted]

TABLE 1

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

Compound Added			Histidine+ Revertants Per Plate**				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
H_2O			20	20	17	24	163
11,308		µg/plate					
-S-9*	10,000	"	7	10	6	16	63
	2000	"	20	17	20	29	153
	4000	"	16	13	16	32	119
	6000	"	11	14	19	28	106
	8000	"	12	20	18	24	102
	10,000	"	11	16	19	28	93
2AA	5	"					2489
	10	"	369		1870	2038	
	100	"		235			

H_2O = Distilled water (solvent control)

11,308 = [redacted]

2AA = 2-Aminoanthracene (positive control)

* = Test plate without S-9 and activators

** = Average of two plates

TABLE II

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

Compound Added			Histidine+ Revertants Per Plate*				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
H_2O			15	22	10	22	140
11,308		µg/plate					
	2000	"	17	19	12	16	133
	4000	"	15	15	12	22	100
	6000	"	10	7	11	22	107
	8000	"	10	12	8	13	88
	10,000	"	5	13	5	15	83
MNNG	2	"	3086				3059
9AAc	50	"		1384			
2NF	25	"			1651	2350	

H_2O = Distilled water (solvent control)

11,308 = [redacted]

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

9AAc = 9-Aminoacridine (positive control)

2NF = 2-Nitrofluorene (positive control)

* = Average of two plates