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

HASKELL LABORATORY REPORT NO. 643-77 [redacted]

Material Tested: [redacted] Haskell No. 11,336

Material Submitted By: [redacted] Organic Chemicals Dept., Jackson Laboratory Sample Ready for Testing: 6-23-77

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

Materials and Methods: Five histidine-requiring strains of *Salmonella typhimurium* were used in the mutagen assays. Strains TA 1535 and TA 100 are used to detect base-pair substitution mutations, whereas strains TA 1537, TA 1538 and TA 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 absence of metabolic activation, 0.1 ml of a solution of the test compound and approximately 10^8 bacteria were added to 2 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 surface of a Davis minimal agar plate. The metabolic activation system involved the addition of 0.5 ml of S-9 mixture to the chemical-top agar solution. The S-9 mix contains per ml: 0.3 ml of the 9,000 X g supernatant of homogenized rat liver, 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 100 mM sodium phosphate (pH 7.4). This mixture was 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. TA 1535 is routinely used to determine the general toxicity range for all strains.

Appropriate controls were included for each strain. In the nonactivated system these controls consisted of a negative, or solvent control, and positive controls. A second negative control (-S-9 control) is included in the activated assay 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 I

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			16	16	26	31	185
11,336		µg/plate					
-S-9*	10,000	"	30	11	7	21	177
	2,000	"	17	11	22	27	185
	4,000	"	18	8	23	29	151
	6,000	"	16	7	23	18	178
	8,000	"	17	10	26	35	184
	10,000	"	16	12	22	38	170
2AA	5	"					1966
	10	"	414		2015	1927	
	100	"		520			

H_2O = Distilled water (solvent control)

11,336 = [redacted]

2AA = 2-Aminoanthracene (positive control)

* = Test plate without S-9 and activators

** = Average of two plates

Company Sanitized. Does not contain TSCA CBI

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			23	18	10	26	167
11,336		µg/plate					
	2,000	"	26	11	16	25	173
	4,000	"	27	14	18	30	202
	6,000	"	31	10	9	28	182
	8,000	"	22	8	11	28	200
	10,000	"	26	12	11	30	184
MNNG	2	"	1897				1846
9AAc	50	"		1213			
2NF	25	"			1950	2348	

H_2O = Distilled water (solvent control)

11,336 = [redacted]

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

9AAc = 9-Aminoacridine (positive control)

2NF = 2-Nitrofluorene (positive control)

* = Average of two plates