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INITIAL SUBMISSION: MUTAGENIC ACTIVITY OF METHANE, BROMOTRIFLUORO- IN THE SALMONELLA/MICROSOME ASSAY		
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METHANE, BROMOTRIFLUORO-		

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ATTACHMENT V

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

HASKELL LABORATORY REPORT NO. 980-76 MR NO. 2365-001

Material Tested: Methane, Bromotrifluoro- Haskell No. 10,261
Other Code: Halon 1301

MUTAGENIC ACTIVITY OF METHANE, BROMOTRIFLUORO- IN THE SALMONELLA/MICROSOME ASSAY

Materials and Methods: Five histidine auxotrophs of Salmonella typhimurium were used in the 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 frameshift mutations.

The tests are performed in the presence and absence of a rat-liver homogenate activation system (S·9 mix). In the absence of metabolic activation, approximately 10^8 bacteria are added to 2 ml top agar (0.6% agar, 0.6% NaCl, 0.05 mM L-histidine, 0.05 mM biotin). The solution is mixed and poured on the surface of a Davis minimal agar plate prior to exposure to the test gas. The metabolically activated system involves the addition of 0.5 ml of S·9 mixture to the bacteria-top agar mixture. The S·9 mix contains per ml: 0.3 ml of the 9000 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 is added directly to the top agar immediately before it is poured over the minimal agar plate.

The minimal plates with the bacteria overlay were exposed to the test gas in 9-liter glass chambers. The chambers, with Teflon® stopcocks and Viton® o-ring gaskets, were specially designed for this purpose. The open petri plates were held in the chamber by stainless steel racks, ten plates per chamber. The test gas was mixed with air from the compressed air line and introduced into the chambers through a flow-meter system.

After the gas-air mixture had flowed through the exposure chamber for 5 volume changes, the chambers were closed and placed in a 37°C incubator for either 6, 48, or 72 hours. At the end of the exposure period the chambers were flushed with air for several minutes and the plates removed. The 6 hour exposure plates were returned to the incubator to complete their 72 hour incubation period. The 48 hour and 72 hour exposure plates were counted immediately after removal from the chambers.

The concentration of the test gas in the exposure chambers was measured using a "Varian Aerograph" Model 600-D gas chromatograph, the measured values agreed well with the values indicated from the flow-meter system. The concentrations of test gas reported in the appended tables are

Exposure levels of up to 40% were tested in the activated and nonactivated assays.

Results: Tables I, II, and III.

Summary: Methane, Bromotrifluoro- was tested in Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 at levels of 10%, 20%, 30% and 40%. The test gas was not mutagenic in the microbial assay either in the presence or absence of a liver microsomal system, i.e., it did not significantly increase the spontaneous mutation frequency.

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Date: January 7, 1977

TABLE I

MUTAGENIC ACTIVITY OF METHANE, BROMOTRIFLUORO- IN SALMONELLA TYPHIMURIUM STRAINS TA 1535 AND TA 100, AT 6 AND 48 HOUR EXPOSURE PERIODS, WITH AND WITHOUT METABOLIC ACTIVATION

Gas Added		Histidine$^+$ Revertants Per Plate*			
		6 Hour Exposure Period		48 Hour Exposure Period	
		TA 1535	TA 100	TA 1535	TA 100
0%	Methane, Bromotrifluoro-				
	with activation	44	148	49	162
	without activation	20	136	29	116
10%	Methane, Bromotrifluoro-				
	with activation	40	130	48	139
	without activation	16	127	26	129
20%	Methane, Bromotrifluoro-				
	with activation	37	143	51	138
	without activation	24	110	20	117
15%	Methane, Chlorofluoro (positive control)				
	with activation	1143	2522	-	-
	without activation	846	1363	-	-

* Average number of revertants from 3 plates

TABLE II

MUTAGENIC ACTIVITY OF METHANE, BROMOTRIFLUORO- IN SALMONELLA TYPHIMURIUM STRAINS TA 1535, TA 1537, TA 1538, TA 98 AND TA 100 AT 48 AND 72 HOUR EXPOSURE PERIODS WITH AND WITHOUT METABOLIC ACTIVATION

	Histidine$^+$ Revertants Per Plate*				
Gas Added	TA 1535	TA 1537	TA 1538	TA 98	TA 100
48 Hour Exposure					
Methane, Bromotrifluoro-					
0% with activation	16	8	15	16	81
without activation	8	10	9	18	63
20% with activation	34	14	18	38	131
without activation	23	11	6	25	83
30% with activation	42	10	13	30	161
without activation	18	13	3	35	92
40% with activation	42	15	8	25	90
without activation	16	8	4	18	89
72 Hour Exposure					
Methane, Bromotrifluoro-					
0% with activation	59	6	23	28	150
without activation	24	11	7	14	98
20% with activation	55	7	20	34	110
without activation	26	10	7	27	148
6 Hour Exposure					
Methane, Chlorofluoro-					
15% with activation	1375	-	-	-	1924
without activation	769	-	-	-	1284

* Average number of revertants from 2 plates

TABLE III

MUTAGENIC ACTIVITY OF METHANE, BROMOTRIFLUORO- IN SALMONELLA TYPHIMURIU
STRAIN TA 1535 AT 48 HOUR EXPOSURE PERIODS WITH AND WITHOUT METABOLIC ACTIVAT

Gas Added	Histidine$^+$ Revertants Per Plate* TA 1535	
Methane, Bromotrifluoro-		
0% with activation	15	(43)**
without activation	12	(19)
20% with activation	16	(24)
without activation	13	(19)
30% with activation	16	(63)
without activation	12	(16)
Methane, Chlorofluoro-		
10% with activation	3330	(3608)
without activation	2537	(2711)

* Average number of revertants from 10 plates.

** Values in parentheses are from the 48 hour exposure plates which were reincuba
additional 24 hours to give 48 hour exposure with a total of 72 hour incubatio