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Short communication

A COMPARISON OF THE MUTAGENIC PROPERTIES OF VINYL CHLORIDE AND METHYL CHLORIDE *

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In developing a screening program for environmental gases using the Ames assay [1], we have found that vinyl chloride (C_2H_3Cl) and methyl chloride (CH_3Cl) are highly mutagenic. Both gases are industrial chemicals; vinyl chloride in the manufacture of polyvinyl chloride (PVC) and methyl chloride as a refrigerant. Chopra and Sherman [3] have shown the presence of methyl chloride in tobacco smoke, thus suggesting that a large portion of our population may be exposed to this gas.

The *Salmonella typhimurium* tester strain TA 1535 which can detect mutagens causing base pair substitutions, was obtained from Dr. Bruce Ames. The strain was grown in nutrient broth, shaken for 14 h at 37°C. and 0.2 ml was then added to molten top agar, with and without 0.5 ml of a 1 : 2 dilution of Aroclor 1254-stimulated liver homogenate [9]. Quintuplicate petri plates containing Vogel Bonner E medium [8] were overlayed with this mixture and incubated inside an anaerobic jar in the presence of the test gas. After 72 h at 37°C the revertant colonies were counted using a hand-held tally.

To obtain a given percentage of test gas, the anaerobic jar was evacuated to a known pressure. Atmospheric pressure was assumed to be 760 mm of mercury so that a pressure change of 7.6 mm approximated 1% of the jar volume. Gas was added until the desired pressure change was observed, and the jar was then allowed to fill with room air. The head space of the jar was analyzed after 24–48 h by gas chromatography.

A gas-tight syringe was used to transfer 5.0 ml of the jar's atmosphere to a sealed 120 ml serum bottle. One ml was then removed from the serum bottle and injected into a Shimadzu GC-4BM gas chromatograph equipped with a flame ionization detector and a 3 foot × 3 mm I.D. glass column packed with 80–100 mesh Porapak Q. The column operating temperatures were 75°C for methyl chloride and 100°C for vinyl chloride. The helium carrier gas-flow rate

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TABLE I

MUTAGENESIS TEST RESULTS OF VINYL CHLORIDE AND METHYL CHLORIDE

Gas	Concentration (%)	Mean values [a] and standard deviation for the number of revertant colonies for strain TA 1535	
		S9 Absent	S9 Added
O	—	28.9 ± 6.24	14.9 ± 3.70
C_2H_3Cl	0.4	53.2 ± 7.60	116.8 ± 46.57
	1.2	70.0 ± 12.83	77.8 ± 2.95
	4.8	121.4 ± 30.68	158.4 ± 34.53
	9.4	166.2 ± 61.40	216.2 ± 64.57
	15.4	233.8 ± 96.94	302.4 ± 63.08
CH_3Cl	0.5	31.6 ± 5.55	46.6 ± 8.26
	0.8	53.6 ± 4.04	79.4 ± 9.71
	3.8	239.2 ± 43.79	268.8 ± 40.38
	8.7	928.0 ± 179.24	1046.4 ± 144.27
	13.3	1600.0 ± 329.46	1939.2 ± 558.56
	20.7	1558.4 ± 159.44	2038.4 ± 416.10
		$P < 0.01$	

[a] Of five replicates.

was 60 ml/min. The retention time for methyl chloride was 6.5 minutes and for vinyl chloride 5.8 min. The relationship of detector response to gas concentration was a straight line passing through the origin. The concentrations used (see Table I) fell on this straight line. The vinyl chloride and methyl chloride standards (obtained from MG Scientific, Kearny, N.J.) showed only one peak when injected into the gas chromatograph and were assumed to be pure.

Table I shows the mean values for the number of revertant colonies expressed using the standard Ames assay with various gas concentrations. With the exception of 0.5% CH_3Cl all figures are statistically significant at $P < 0.01$ when compared to the gasless control [4,5]. Metabolic activation is not required to detect mutagenesis since there is no significant difference between the number of revertant colonies with or without added rat liver homogenate.

A level of 23% methyl chloride was toxic to the bacteria but an inhibitory level of vinyl chloride was not reached during these studies.

Because of the similar properties of these gases and because vinyl chloride is a proven mutagen/carcinogen [2,6,7], it is strongly suggested that methyl chloride be considered and investigated as a possible carcinogen.

The authors wish to thank Drs. M.I. Kelsey, S.J. Silverman and M.W. Slein for their encouragement, Corinthia Brown for excellent technical help, and Charles W. Riggs and Pete Bostian for the statistical analysis.

References

1 Ames, Bruce N., William E. Durston, Edith Yamasaki and Frank D. Lee, Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection, Proc. Natl. Acad. Sci., U.S.A., 70 (1973) 2281—2285.

2 Bartsch, H., C. Malaveille and R. Montesano, Human, rat and mouse liver-mediated mutagenicity of vinyl chloride in *S. typhimurium* strains, Int. J. Cancer, 15 (1975) 429—437.

3 Chopra, N.M. and Larry R. Sherman, Systematic studies on the breakdown of *p,p'*-DDT in tobacco smokes. Investigations into the presence of methyl chloride, dichloromethane, and chloroform in tobacco smoke, Anal. Chem., 44 (1972) 1036—1038.
4 Dunnett, Charles W., A multiple comparison procedure for comparing several treatments with a control, J. Amer. Stat. Assoc. 50 (1955) 1096—1121.
5 Dunnett, Charles W., New tables for multiple comparison with a control, Biometrics 20 (1964) 482—491.
6 Malaveille, C., H. Bartsch, A. Barbin, A.M. Camus, R. Montesano, Mutagenicity of vinyl chloride, chloroethyleneoxide, chloroacetaldehyde, and chloroethanol, Biochem. Biophys. Res. Comm., 63 (1975) 363—370.
7 Maltoni, C., G. Lafemine, P. Chieco and D. Carretti, Vinyl chloride carcinogenesis: current results and perspectives, Mod. Lav. 65 (1974) 421—444.
8 Vogel, Henry J. and David M. Bonner, Acetylornithinase of *Escherichia coli*: Partial purification and some properties, J. Biol. Chem., 218 (1956) 97—106.
9 Yamasaki, E. and B.N. Ames, P C B Induction of rat liver enzymes for carcinogen evaluation, Toxicol. Appl. Pharmacol., 29 (1974) 157—180.