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Mutagenic Activity of the Liquid Waste from the Production of Acetonitrile

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Industrial activities produce a large volume of complex wastes which must be disposed of in an economical and yet environmentally sound manner. The USEPA (1978) initially recommended a battery of three bioassays to evaluate the genotoxic potential of waste materials. However, regulations later published by the USEPA (1980) deleted bioassays from their criteria for characterizing hazardous wastes because it believed that there were no widely available and uncomplicated methods available for evaluating genotoxic potential. In addition, the USEPA (1980) did not feel that bioassays could be used to define the threshold at which wastes exhibiting genetic toxicity would present a substantial hazard. Identifying the potential of a waste to induce genetic damage was instead left to listing mechanisms. Utilizing listing mechanisms to identify these criteria may result in the delisting of many wastes because they do not contain significant quantities of hazardous constituents. Furthermore, listing the components of a complex mixture fails to account for the synergistic, antagonistic, or additive effects of a mixture's components. To provide a more accurate evaluation of hazardous waste characteristics, research is needed to test protocols and to provide information which can be used to provide the basis of a semi-quantitative risk assessment. This paper discusses the mutagenic and DNA-damaging potential of a waste stream from the production of acetonitrile. Annual production of this chemical in the United States in 1980 was 11.4×10^6 kg (Conant, 1981).

MATERIALS AND METHODS

The waste used in this study was collected at a petrochemical plant as the bottom stream from the acetonitrile purification column. An analysis of this waste indicated that it contained 3.46% acetonitrile, 0.09% R-OH, 0.92% acetamide, 0.5% heavy ends, and approximately 95% water. The samples were collected in amber glass bottles and stored at $4^{\circ}C$ until processed. The organic fraction of the waste was extracted with dichloromethane and separated into acid, base, and neutral fractions using the procedures of Brown et al. (1982). All waste fractions were dissolved in dimethyl sulfoxide (Sigma Chemical Corp., St. Louis, MO) for testing in the bioassays.

The mutagenic potential of the acid, base, and neutral fraction of the acetonitrile waste was measured with the Salmonella/microsome assay of Ames et al. (1975). The two Salmonella strains TA98 (a frameshift mutant) and TA100 (a base-pair substitution mutant) were supplied by Dr. B.N. Ames (University of California, Berkeley, CA). The methods used were the same as Ames et al. (1975) except that overnight cultures were prepared by inoculation into 10 mL of Oxoid Nutrient Broth No. 2 (KC Biological, Lenexa, KS). Extracts were tested on duplicate plates in two independent experiments in the standard plate incorporation assay at a minimum of five dose levels with and without enzyme activation (0.3 mL rat liver/mL S·9 mix). Aroclor 1254 induced rat liver was obtained from Litton Bionetics (Charleston, SC). Positive controls included 2 µg/plate N-methyl-N'-nitro-N-nitrosoguanidine (Sigma) for TA100, 25 µg/plate 2-nitrofluorene (Aldrich Chemical Co., Milwaukee, WI) for TA98, and 10 µg/plate 2-aminoanthracene (Sigma) which was used to verify the functioning of the metabolic activation system. All reagents and extracts were tested for sterility. Dimethyl sulfoxide was used as a negative control.

Six strains of B. subtilis deficient in different recombination (Rec$^-$) and/or excision (Exc$^-$) repair were used to test for lethal DNA damage. These included the Rec$^-$ strains recA8, recB2, recE4, mc-1; Exc$^-$ strain hcr 9; and Rec$^-$/Exc$^-$ fh2006 7. All of these strains are isogenic with B. subtilis strain 168 which has all repair intact. These strains were supplied by Dr. I.C. Felkner of Clements Assoc., Washington, D.C. Overnight cultures were grown in brain-heart infusion broth (Difco, Detroit, MI) incubated at 37°. Each strain was streaked radially on a nutrient agar plate to a centrally placed sensitivity disc containing 100 µl of the test chemical. After incubation at 37° for 18 hours, the distance of growth inhibition was measured in millimeters (Kada et al., 1978). A response was considered positive if the distance of growth inhibition was more than 2.5 mm greater in one of the repair deficient strains than in the repair proficient strain 168. Mitomycin C (Sigma), methylmethanesulphonate (Aldrich) and sensitivity to ultraviolet light were used as appropriate positive controls. Quadruplicate plates were run at each dose level for all samples.

RESULTS AND DISCUSSION

The results of the Salmonella/microsome mutagenicity assay are presented in Figure 1 and Table 1. These results indicate that mutagenic activity could be detected in all three waste fractions and that primarily indirect acting mutagens were detected. The dose-response curves for all fractions were non-linear indicating that the fractions are composed of constituents with non-equivalent kinetics of mutation induction. The base and neutral fraction induced a two-fold increase in revertant colonies in both strain TA98 and TA100, while the acid fraction induced a significant increase in strain TA98 only.

Table 1. Mutagenic activity of liquid stream from acetonitrile purilific column as measured with S. typhimurium strain TA98 and TA100 with and without metabolic activation.

Sample	Dose/plate (mg)	Strain			
		TA98		TA100	
		+S-9	-S-9	+S-9	-S-9
		---------Total His^+ revertants/plate - Mean ± S. D.----------			
Acid	5.0	162 ± 31	69 ± 3	197 ± 31	188 ± 58
	2.5	122 ± 2	36 ± 5	NT	149 ± 1
	1.0	45 ± 13	32 ± 5	145 ± 14	85 ± 11
	0.5	30 ± 5	36 ± 7	155 ± 3	149 ± 6
	0.1	30 ± 14	15 ± 4	140 ± 18	80 ± 9
	0.01	20 ± 4	17 ± 4	140 ± 24	80 ± 5
Base	5.0	328 ± 5	77 ± 12	366 ± 76	109 ± 5
	1.0	97 ± 32	36 ± 4	173 ± 4	112 ± 17
	0.5	62 ± 1	28 ± 2	143 ± 15	182 ± 12
	0.1	35 ± 15	20 ± 1	120 ± 18	92 ± 14
	0.01	20 ± 2	17 ± 0	140 ± 8	87 ± 15
Neutral	10.0	126 ± 10	67 ± 1	326 ± 47	131 ± 21
	5.0	106 ± 21	55 ± 2	289 ± 15	NT
	1.0	55 ± 5	27 ± 8	87 ± 34	102 ± 35
	0.1	36 ± 19	13 ± 1	97 ± 39	93 ± 20
	0.01	21 ± 3	15 ± 3	79 ± 25	85 ± 4
DMSO	100 µl	22 ± 5	19 ± 3	117 ± 21	103 ± 16

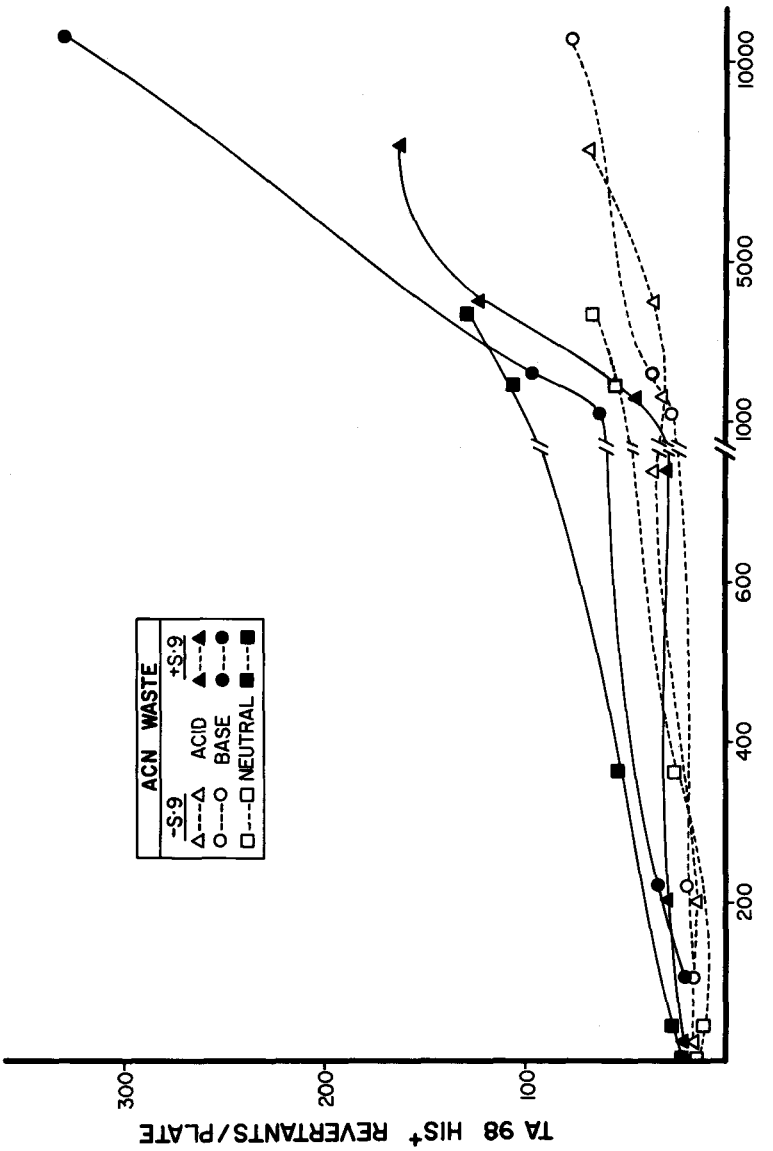

Figure 1. Mutagenicity, as measured with S. typhimurium strain TA98, of fractions of acetonitrile waste.

The basic fraction of the waste induced the greatest number of revertant colonies in both strains. At an exposure level equivalent to almost 11 g of the original waste material (5.0 mg/plate of extract), the basic fraction induced 328 and 366 revertant colonies in strain TA98 and TA100, respectively. In the absence of metabolic activation, a doubling of revertant colonies was induced by all three fractions in TA98 at dose levels greater than 5 mg/plate. The addition of metabolic activation to the assay system resulted in an increase of at least twice the number of revertant colonies that was obtained without metabolic activation.

One of the known constituents of this waste, acetamide, induced less than 14 revertant colonies/mg at a dose level of 5 mg (McCann et al., 1975). At the same dose level, the acid, base, and neutral fraction induced 31, 71, and 21 net revertants/mg, respectively. Although the acetonitrile waste contains the experimental carcinogens acetamide (Sax, 1979), our data indicate that it also contained small concentrations of other mutagenic agents or agents which promoted the activity of acetamide in the various waste fractions.

An evaluation of the acid, base, and neutral fraction of the acetonitrile waste using the B. subtilis DNA repair assay (Table 2) indicates that none of the waste fractions induced increased lethal damage in the repair deficient strains. Two limitations of the DNA repair spot test are limited sensitivity to compounds which are indirect acting and limited sensitivity to compounds which are insoluble in water. The negative response in the DNA repair assay may have been due to the absense of metabolic activation in this test since metabolic activation was required to obtain the maximum response in the Salmonella assay.

The determination of the mutagenic potential of the fractions of the actonitrile waste indicates that they contain primarily indirect acting mutagens and that the base fraction was the most active. The basic fraction induced 306 net revertants in the Salmonella assay at a dose level equivalent to approximately 11 g of waste. By comparison, the basic N-heterocyclic compound 10-azobenzo[a]pyrene induced 130,000 revertants/mg in an evaluation by Ho et al. (1981). Thus, the mutagenic potential of the components of the basic fraction appears to be much lower than that of the substituted polycyclic aromatic hydrocarbon. While these results apply only to the waste material studied, they do indicate that wastes of a similar composition may also contain mutagenic materials.

These results demonstrate the need for a biological testing protocol to evaluate the hazardous characteristics of a waste. The use of chemical analysis alone has the disadvantage of failing to account for the potential interactions of waste components. Biological analysis has the disadvantages of being unable to identify mutagenic constituents and the results can be

Table 2. Comparison of Lethal Effects of Waste Fractions on DNA Repair Deficient and Proficient Strains of B. subtilis.

Sample	Dose/pt	Inhibition radius (mm)						
		168 wt RP[1]	fh2006·7	recE4	mc-1	hcr·9	recA8	recB2
			repair deficient					
Acid	1 mg	0	1	0	1	0	0	0
Base	1 mg	0	1	1	0	2	1	1
Neutral	10 mg	0	0	0	1	0	0	0
MMS[2]	2 µl	14	26	25	20	21	21	18
Mit·C[3]	10 µl	6	11	11	12	13	13	13
DMSO[4]	100 µl	0	0	0	0	0	0	0

1 - Repair producient
2 - Methyl methane sulfonate
3 - Mitomycin C
4 - Dimethylsulfoxide

influenced by the methods of sample preparation, the selection of solvent, or the enzyme induction system. Thus, it appears that the most reliable mutagenic assessment technique would employ both chemical and biological analysis.

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REFERENCES

Ames BN, McCann J, Yamasaki E (1975) Methods for detecting carcinogens and mutagens with the salmonella/mammalian - microsome mutagenicity test. Mut Res 31:347-364.

Brown KW, Donnelly KC, Scott BR (1982) The fate of mutagenic compounds when hazardous wastes are land treated. In: Land Disposal of Hazardous Waste Proceed. 8th Annual Res. Symp. EPA-600/9-82-002.

Conant KJ III (1981) Miscellaneous cyclic and a cyclic chemicals. In: Synthetic Organic Chemicals: United States Production and Sales. U. S. International Trade Commission, Publ. No. 1183, Washington, D.C. p 259.

Ho C-H, Clark MR, Guerin MR, Barkenbus BD, Rao TK, Epler JL (1981) Analytical and biological analyses of test materials from the synthetic fuel technologies. IV. Studies of chemical structure-mutagenic activity relationships of aromatic nitrogen compounds relevant to synfuels. Mut Res 85:335-345.

Kada T, Hirano K, Shirasu Y (1978) Screening of environmental chemical mutagens by the REC assay systems with Bacillus substilis. In: Hollaender A (ed) Chemical mutagens. p 472.

McCann J, Ames BN (1975) Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc Nat Acad Sci 72:5135-5139.

Sax NI (1979) Dangerous Properties of Industrial Materials. Van Nostrand Reinhold Co, New York.

USEPA, Hazardous Waste Guidelines and Regulations: Identification and Listing: Proposed Rules, Vol. 40, CFR (Dec. 18, 1978).

USEPA, Hazardous Waste Management System, Vol. 45, CFR (May 19, 1980).

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