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Comparison of Liquid-Liquid Extraction and Resin Adsorption for Concentrating Mutagens in Ames *Salmonella*/Microsome Assays on Water

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The Ames *Salmonella*/microsome mutagenicity assay (AMES *et al.* 1975) is a relatively simple, cheap and rapid test for mutagenic activity (GREIM *et al.* 1980, SOBELS 1980). Although there are uncertainties about the health implications of mutagens, the Ames test detects 85 to 93 % of known chemical carcinogens and is considered an essential part of the minimal battery of bioassays required in studies on environmental compounds which may damage chromosomal material (GREIM *et al.* 1980, SOBELS 1980). Since water may play an important role in the transmission of environmental carcinogens, which are responsible for an estimated 50 to 90 % of human cancer cases, the Ames test is being used to determine the incidence of potential carcinogens in water supplies and their removal or formation by water treatment processes (HOOPER *et al.* 1978, NESTMANN *et al.* 1979, RAPPAPORT *et al.* 1979, SAXENA & SCHWARTZ 1979, SCHWARTZ *et al.* 1979, DENKHAUS *et al.* 1980, GRABOW *et al.* 1980, LOPER 1980). In many waters, particularly drinking-water supplies, the concentration of mutagens is generally too low for direct detection by the Ames test, and a wide variety of methods are being used to concentrate mutagens from large volumes of water (HOOPER *et al.* 1978, NESTMANN *et al.* 1979, RAPPAPORT *et al.* 1979, SCHWARTZ *et al.* 1979, GRABOW *et al.* 1980, LOPER 1980, GRIMM-KIBALO *et al.* 1981).

This paper describes a comparison of mutagen recovery from different waters by dichloromethane (DCM) liquid-liquid extraction, and adsorption on Amberlite XAD resins, which are often used for the recovery of mutagens from water. The information is essential for comparing data reported in different studies, the selection of appropriate techniques, and the development of standard procedures.

MATERIALS AND METHODS

Samples. Grab samples were collected from the final product of a 4,500 m^3/d multiple-barrier wastewater reclamation plant (DENKHAUS *et al.* 1980), laboratory tap water representing a potable water supply of about 2,200 ML/d prepared mainly from surface water by the Rand Water Board for some five million consumers (GRABOW *et al.* 1980, as well as the Vaal River at points about 50 m below the barrage, 20 m below the influent of a wastewater effluent from Sasolburg and 1 km below the influent of a wastewater effluent from Vanderbijlpark (GRABOW *et al.* 1980).

Recovery of mutagens. Water samples were processed within 4 h after collection. Residual chlorine (measured by the DPD ferrous

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titrimetric method, GRABOW *et al.* 1981) was not neutralized. DCM extraction at neutral pH (ambient pH of water sample was not adjusted) was done by adding 200 g of baked (overnight at 480 °C) sodium sulphate (Merck, AR) and 520 mL of glass-distilled DCM (Merck, AR) to 20 L quantities of water, mixing by Ultra-Turrax homogenization for 5 min, overnight separation of phases at room temperature, and decanting of the water phase into glass containers for extraction at acidic and basic pH levels. Combined DCM extracts from five 20 L quantities of each sample were filtered through baked sodium sulphate, reduced to about 10 mL by rotary evaporation, and evaporated to dryness under dry nitrogen at room temperature. The pH of the remaining water phase was adjusted to 2 by adding concentrated hydrochloric acid. Dichloromethane extraction was then performed as described for neutral pH followed by the same procedure after the pH of the remaining water phase had been adjusted to 12 by means of a concentrated solution of sodium hydroxide (DENKHAUS *et al.* 1980).

Rohm and Haas Amberlite XAD resins (BDH) were washed and soxhlet extracted for 16 h with distilled acetone, and stored in acetone for loading 15 mm (i.d.)x300 mm glass columns (with teflon stopcocks) with 35 mL of resin (RAPPAPORT *et al.* 1979). Columns were washed with distilled water, and 100 L of each sample was first passed through an XAD-2 column and then through an XAD-7 column at a flow rate of 60 mL/min at 10 °C (HOOPER *et al.* 1978). Columns were then washed with three bed volumes of distilled water, residual water was blown from the columns by dry nitrogen, and adsorbents eluted by means of four bed volumes (140 mL) of glass-distilled acetone (HOOPER *et al.* 1978, RAPPAPORT *et al.* 1979). Eluates were filtered through 30 g of baked sodium sulphate and evaporated to dryness by means of rotary evaporation at 45 °C. New resins were used for each sample.

Dry concentrates from all samples (100 L) were suspended in 2 mL dimethyl sulfoxide (DMSO, Merck, AR) which represents a concentration factor of 5×10^4, except the concentrates of the tap water samples from 1980-10-06 onward which were suspended in 5 mL DMSO representing a concentration factor of 2×10^4. Mutagenicity assays were carried out immediately because the mutagenic activity of extracts declines during storage (GRABOW *et al.* 1981).

Mutagenicity assays. *Salmonella typhimurium* tester strains were kindly supplied by Prof. B.N. Ames. Mutagenicity assays by means of plate incorporation assays were done as described by AMES *et al.* (1975) and GRABOW *et al.* (1980). Each test included positive, negative and sterility controls, and the toxicity of extracts and presence of excess histidine were evaluated. Whenever possible tests were done in fivefold.

RESULTS

Results of mutagenicity assays are expressed as the mutagenicity ratio (MR) which is the ratio of revertants on test plates (spontaneous + induced revertants) to those on negative control plates

(spontaneous revertants). An MR of 2.0 or more is generally considered as a statistically significant indication of mutagenic activity, provided all controls conform to specifications (AMES *et al.* 1975). Tester strain TA98 had 15 to 50 spontaneous revertants per plate and tester strain TA100, 120 to 200. The reclaimed water had a pH of 7.0 to 8.5, 0.9 to 1.2 mg/L free chlorine and 1.1 to 1.7 mg/L total chlorine. The tap water had a pH of 7.7 to 8.0, while no residual chlorine was detected in any of the samples concerned. The Vaal River samples had a pH of 8.4 to 9.2 and no residual chlorine.

The MR values for concentrates of the four Vaal River samples (Table 1) show that DCM extraction yielded the highest mutagenic activity for two samples and XAD-2 adsorption for one. The neutral DCM extract of the 1980-08-11 sample which was toxic to TA98 when tested undiluted, had an MR of 2.0 when tested in a tenfold DMSO dilution against TA98 in the absence of S9. Tester strain TA100 (-S9) had an MR of 2.3 for the XAD-2 concentrate of 1980-09-08, and values well below 2.0 for all other concentrates.

DCM extracts had the highest MR value for seven and XAD-2 concentrates for three of the ten samples of reclaimed water (Table 2). The samples of 1980-05-13 and 1980-06-16 had MR values of 2.0 or more for DCM extracts but not for XAD concentrates. MR values of all XAD-7 concentrates as well as DCM extracts made at basic pH, were well below 2.0. The highest MR value recorded for strain TA100 in the presence of S9 was 1.6 for the neutral pH DCM extract of the 1980-06-02 sample.

DCM extracts had the highest MR value for 11 and XAD-2 concentrates for two of the 15 tap water samples (Table 3). Mutagenic activity was detected in DCM extracts of all 15 samples while XAD concentrates of the 1980-10-13 and 1980-10-20 samples failed to show mutagenic activity. The XAD-7 concentrate of the 1980-07-14 sample had an MR of 2.4 while all other XAD-7 concentrates, as well as DCM extracts at basic pH, had MR values of well below 2.0. The highest MR values recorded for tester strain TA100 (+S9) were 1.9 and 1.8 for the DCM extracts at acidic pH of the 1980-09-15 and 1980-07-07 samples, respectively.

Dose-response tests on nine tap water samples were limited to strain TA98 (-S9), DCM extracts at neutral and acidic pH and XAD-2 concentrates because these yielded the highest mutagenic activity. The results in Table 4 show that at least 14 of the 18 DCM extracts had higher MR values for DMSO dilutions than for the original extract. In the case of XAD-2 this was noted for only one (1980-10-20) of the nine concentrates. Mutagenicity assays on six of the original concentrates had MR values of less than 2.0 while assays on dilutions had MR values of 2.0 or more.

DISCUSSION

The MR values in Tables 1 to 4 show that DCM extraction recovered more mutagenic material than XAD adsorption from the great majority of samples. However, the lack of direct correlation between MR values

TABLE 1. Mutagenicity Ratios for Concentrates Prepared by Dichloromethane Extraction and Adsorption on XAD Resins from Water Samples of the Vaal River.

Date and sample	pH	MR for tester strain TA98 +/- S9 liver					
		DCM		XAD-2		XAD-7	
		98-S9	98+S9	98-S9	98+S9	98-S9	98+S9
1980-08-11 VR2	Neutral	Toxic	Toxic	21.9	18.7	9.8	8.4
	Acidic	15.0	16.3				
	Basic	4.7	2.5				
1980-08-18 VR2	Neutral	13.9	12.8	31.2	>26	5.9	8.2
	Acidic	6.0	5.8				
	Basic	1.7	1.4				
1980-08-25 VR1	Neutral	11.1	11.5	5.9	7.3	2.6	2.0
	Acidic	Toxic	7.0				
	Basic	2.9	2.3				
1980-09-08 VR3	Neutral	>29	>33	14.7	21.8	6.2	5.4
	Acidic	5.8	12.9				
	Basic	5.1	2.6				

VR1, VR2 and VR3 = Vaal River samples taken about 50 m below the barrage, 20 m below the Sasolburg influent, and 1 km below the Vanderbijlpark influent, respectively.

of DCM and XAD concentrates, and the higher MR value recorded for occasional XAD-2 concentrates, suggest that the difference in recovery efficiency may not be the same for all mutagens, and that XAD-2 adsorption may in fact be relatively efficient for some mutagens which generally occurred in low concentrations. The XAD-7 resin, which adsorbs mainly moderately polar molecules (RAPPAPORT *et al.* 1979), would not seem to serve a purpose in drinking-water analysis when used in combination with XAD-2 because it failed to recover mutagens from treated water. XAD-7 only seemed to make a contribution in the case of Vaal River water (Table 1) which contained a relatively high concentration of industrial effluents (GRABOW *et al.* 1980). However, the extent to which the XAD-7 recoveries were the result of saturation of the preceding XAD-2 column has not been established. XAD-2 was preferred to XAD-4 or Tenax GC resins for the recovery of nonpolar compounds because it has been found to have superior adsorption capabilities, even for relatively polar compounds (HOOPER *et al.* 1978).

Apart from the higher recovery efficiency, DCM extraction at different pH levels has the advantage of indicating the chemical nature of mutagens because extracts at neutral pH contain mainly neutral organic compounds including polycyclic hydrocarbons, extracts at acidic pH mainly organic acids and phenols, and extracts at basic

TABLE 2. Mutagenicity Ratios for Concentrates Prepared by Dichloromethane Extraction and Adsorption on XAD Resin from Reclaimed Water.

Date	pH	MR for tester strains TA98 and TA100 +/- S9					
		DCM			XAD-2		
		98-S9	98+S9	100-S9	98-S9	98+S9	100-S9
80-05-05	Neutral	1.1	-	0.9	1.0	-	1.3
	Acidic	-	-	-			
80-05-13	Neutral	0.9	-	1.0	1.4	-	1.0
	Acidic	2.0	-	0.9			
80-05-26	Neutral	1.1	0.9	1.0	0.8	0.7	0.9
	Acidic	1.6	1.1	-			
80-06-02	Neutral	1.4	1.6	1.1	3.0	1.9	0.8
	Acidic	2.3	0.9	0.8			
80-06-09	Neutral	1.2	1.1	1.8	3.0	2.8	1.0
	Acidic	2.9	1.9	1.1			
80-06-16	Neutral	2.3	1.2	0.7	0.8	0.8	1.0
	Acidic	Toxic	0.8	1.3			
80-06-23	Neutral	Toxic	1.7	1.0	1.4	1.1	0.9
	Acidic	1.9	1.1	1.2			
80-07-21	Neutral	-	1.3	1.6	1.2	1.4	0.9
	Acidic	1.7	1.4	0.8			
80-07-28	Neutral	2.1	1.5	2.1	2.0	1.6	2.0
	Acidic	2.6	1.6	1.8			
80-08-04	Neutral	Toxic	1.2	1.2	2.8	1.5	2.1
	Acidic	2.9	1.5	1.1			

pH mainly basic organic compounds such as amines (GRABOW *et al.* 1981). Resin columns have the advantage that large volumes of water can be processed conveniently and at relatively low cost. However, turbid water tends to clog columns, and prefiltration may result in loss of mutagens.

Tester strains TA1535, TA1537 and TA1538 were not included because they rarely if ever contribute to information obtained with the more sensitive strains TA98 and TA100 (HOOPER *et al.* 1978, RAPPAPORT *et al.* 1979, GRABOW *et al.* 1981, GRIMM-KIBALO *et al.* 1981). The finding that S9 rarely increased mutagenic activity, is in agreement with earlier observations (HOOPER *et al.* 1978, NESTMANN *et al.* 1979, SCHWARTZ *et al.* 1979, GRABOW *et al.* 1981, GRIMM-KIBALO *et al.* 1981),

TABLE 3. Mutagenicity Ratios for Concentrates Prepared by Dichloromethane Extraction and Adsorption on XAD Resin from Tap Water.

Date	pH	MR for tester strains TA98 and TA100 +/- S9					
		DCM			XAD-2		
		98-S9	98+S9	100-S9	98-S9	98+S9	100-S9
80-06-30	Neutral	2.5	1.3	1.5	3.4	2.2	1.8
	Acidic	Toxic	1.6	Toxic			
80-07-07	Neutral	3.7	2.5	1.6	2.8	1.5	2.7
	Acidic	2.6	2.0	2.5			
80-07-14	Neutral	1.9	1.2	1.1	4.2	2.4	0.8
	Acidic	2.6	2.2	1.5			
80-09-15	Neutral	2.2	1.6	1.7	3.3	2.7	1.5
	Acidic	5.8	2.4	2.5			
80-09-22	Neutral	2.8	2.7	-	-	-	1.3
	Acidic	-	-	1.3			
80-09-29	Neutral	2.0	1.8	1.4	3.2	1.4	1.0
	Acidic	3.9	1.3	1.6			
80-10-06	Neutral	2.1	1.2	1.2	2.2	2.0	1.2
	Acidic	2.8	2.0	1.3			
80-10-13	Neutral	1.6	1.2	1.3	1.9	1.4	1.3
	Acidic	2.7	1.4	0.9			
80-10-20	Neutral	2.1	1.8	1.2	1.7	1.9	1.1
	Acidic	2.9	3.4	1.2			
80-11-03	Neutral	1.6	1.4	1.0	3.4	3.2	2.1
	Acidic	5.1	3.6	2.1			
80-11-10	Neutral	2.1	1.6	1.1	2.7	2.4	1.3
	Acidic	3.3	2.9	1.2			
80-11-18	Neutral	1.7	1.7	1.2	3.2	1.8	1.1
	Acidic	3.9	2.1	1.3			
80-11-24	Neutral	1.6	1.5	0.8	4.6	2.1	1.4
	Acidic	6.6	2.8	2.1			
80-12-01	Neutral	1.7	1.7	1.3	3.3	2.9	1.4
	Acidic	5.2	3.3	1.4			
80-12-08	Neutral	Toxic	1.1	Toxic	3.6	3.1	1.4
	Acidic	3.1	5.0	1.4			

TABLE 4. Mutagenicity Ratios for Dilutions of Concentrates Prepared by Dichloromethane Extraction and Adsorption on XAD-2 Resin from Tap Water.

Concentrate and dilution		Sampling date and mutagenicity ratio for tester strain TA98 without S9 liver								
		80-10-06	80-10-13	80-10-20	80-11-03	80-11-10	80-11-18	80-11-24	80-12-01	80-12-08
DCM	10:10	2.05	1.63	2.05	1.62	2.11	1.70	1.56	1.73	Toxic
Neutral pH	8:10	2.37	2.19	2.21	4.39	2.11	2.00	2.06	2.20	3.00
	6:10	1.74	1.56	1.53	2.54	2.06	1.44	1.81	2.07	1.60
	4:10	1.47	1.31	1.21	2.08	1.72	1.13	1.63	1.73	1.60
	2:10	0.95	1.25	1.00	1.77	1.44	1.13	1.31	1.47	1.33
DCM	10:10	2.78	2.73	2.94	5.12	3.28	3.92	6.62	5.20	3.05
Acidic pH	8:10	4.83	4.40	2.67	3.82	5.72	4.54	7.85	5.53	4.53
	6:10	2.39	2.33	1.83	3.12	3.67	2.85	5.23	3.40	3.68
	4:10	2.00	2.27	1.78	2.12	3.33	2.77	3.69	2.33	2.74
	2:10	1.17	2.07	1.39	2.06	2.11	2.15	2.85	2.27	2.11
XAD-2	10:10	2.22	1.95	1.72	3.35	2.67	3.15	4.62	3.27	3.63
Neutral pH	8:10	2.00	1.63	2.00	2.35	1.94	2.39	2.62	1.60	3.05
	6:10	1.94	1.58	1.61	2.18	1.78	2.31	2.62	1.40	2.53
	4:10	1.61	1.21	1.28	1.71	1.50	2.00	1.77	1.33	1.58
	2:10	1.33	0.95	1.11	1.65	0.89	1.54	1.54	1.13	1.32

and indicates that this expensive and cumbersome part may be eliminated from tests on many waters. DCM extracts at basic pH may likewise be eliminated from tests on drinking-water supplies. The results in Table 4 confirm the importance of testing dilutions of concentrates for mutagenic activity (AMES *et al.* 1975, HOOPER *et al.* 1978) because original concentrates may often fail to show mutagenic activity even though they may not contain detectable concentrations of toxicants or histidine.

Although this study shows that DCM liquid-liquid extraction is superior to XAD adsorption for concentrating mutagens from a variety of waters, it should be kept in mind that both methods have limitations, particularly with regard to the recovery of volatile mutagens, highly polar organic mutagens and inorganic mutagens (HOOPER *et al.* 1978, GRABOW *et al.* 1980, JANARDAN *et al.* 1980).

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