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Mutagenicity Assessment of Different Drinking Water Supplies before and after Treatments

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In recent years increasing concern about the introduction of mutagens/carcinogens into drinking water has resulted in a number of researches on the application of short-term mutagenicity tests to assess the potential effects of these aquatic micropollutants on the public health (Dolara et al. 1981;Grabow et al. 1980; Grabow et al. 1981;Kool et al. 1982;Monarca et al. 1983;Nestmann et al. 1979;van Rossum et al. 1982). The Salmonella/microsome assay (Ames et al. 1975) has received considerable attention because it is a simple and reliable test for routine screening of potential mutagens in the environment (Purchase 1982).

Drinking water treatments have been recently studied by this or similar tests to evaluate their ability to remove mutagens from raw water or to produce new mutagens during the treatment (Denkhaus et al. 1980; Dolara et al. 1981;Kool et al. 1982;Monarca et al. 1983).However,chlorination,which is widely used to disinfect drinking water,is known to generate chlorinated organics with mutagenic/carcinogenic properties (Cheh et al. 1980;Fleischacker and Randtke 1983; Maruoka and Yamanaka 1980;Zoeteman et al. 1982).Moreover ,distribution systems can become an additional source of mutagens in drinking water(Monarca and Meier in press;Schwartz et al. 1979). Since drinking water pollutants are usually at trace level,preliminary efficient concentration procedures are needed to evaluate correctly the mutagenic activity of drinking water. For this purpose, adsorption on macroreticular resins and liquid-liquid extraction are at present the most used techniques (Dutka et al. 1981;Jolley 1981; Junk et al. 1974;Kfir et al. 1982;Kool et al. 1981; Webb 1978).

In this study, three qualitatively different raw water supplies together with their correspondent chlorinated drinking waters were investigated for their mutagenic properties using the Salmonella/microsome assay. A drinking water supply distributed through two different types of pipelines was chosen in order to study the effect of the distribution systems on water mutagenicity. A sequential liquid-liquid extraction at three different pHs and an in situ XAD-2 adsorption technique were used to prepare extracts and adsorbates from both untreated and treated water samples to compare mutagen recovery by these widely used methods and with a view to selecting a routine screening technique for evaluating the effect of treatments on drinking water mutagenicity.

MATERIALS AND METHODS

Three water supplies, assumed to be qualitatively different, were chosen for samplings. 1. Lake water, polluted mainly by municipal and agricultural wastes and used as drinking water source for about 15,000 inhabitants. The treatment for this is prechlorination, flocculation, rapid sand filtration and postchlorination. 2. River water, polluted mainly by municipal and industrial wastes, and used as drinking water source for about 30,000 inhabitants. The treatment consists of prechlorination, flocculation, slow sand filtration and postchlorination. 3. Spring water, assumed to be unpolluted by man-made organics, serving about 50,000 inhabitants. Only the chlorination step is used for this. Then the chlorinated water is distributed through two different pipelines, one cast iron pipeline and one steel pipeline coated with coal tar paints. This paint is known to contain mutagenic/carcinogenic compounds which could leach into water (Monarca and Meier in press). The two pipelines go parallel for about 14 Km and then only one pipeline distributes both waters.

The samples under study were the raw waters before treatment and the drinking waters after treatment. Spring water was sampled before chlorination (raw water) and after 14 Km of distribution through the cast iron pipeline (drinking water No.1) and steel pipeline coated with coal tar paint (drinking water No.2).

Raw water samples were collected in glass containers, shipped to the laboratory and processed within a few hours. 50 litres were adsorbed on two XAD-2 resin columns and 40 litres were concentrated by a sequential liquid-liquid extraction procedure as follows.

Drinking waters were sampled in two different ways : 100 litres were adsorbed in situ on four XAD-2 resin columns connected in parallel to the tap (Nestmann et al. 1979) and 40 litres were collected in glass containers and shipped to the laboratory for the sequential liquid-liquid extraction.

XAD-2 adsorption was performed using glass columns (30x2.5 cm) with Teflon stopcock and Teflon-lined screw fittings for resisting tap water pressure. 15 g of purified XAD-2 resin (Junk et al. 1974) were placed in each column as a methanol slurry. Purified glass wool was placed over the resin layer. Methanol was drained off and the column washed with 200 ml distilled water. The flow was adjusted to about 80 ml/min by tap water pressure for in situ adsorption of treated water or by peristaltic pump for laboratory adsorption of raw water. 25 litres of each water sample was allowed to pass through each column. The adsorbed organics were then eluted with 200 ml bidistilled acetone. Eluates were evaporated to small volume at 40°C under vacuum in a rotary evaporator and finally to dryness under a nitrogen stream. Residues were weighed and dissolved in dimethylsulfoxide (DMSO) to obtain the proper concentration. Blanks with distilled water were prepared as negative controls.

Liquid-liquid extractions were performed sequentially at three different pHs, according to the Denkhaus et al. (1980) method with some modifications. A 40 litres water sample (raw water was previously filtered) was extracted at pH 7 with 2 litres distilled dichloromethane by an Ultra Turrax homogenizer for 15 min. After separation of the two layers, the dichloromethane phase (neutral extract) was separated from the water phase; this was adjusted to pH 2 with H_2SO_4 6 N and reextracted with 2 litres dichloromethane (acidic extract) as described above. The water phase was finally adjusted to pH 11 with NaOH 6 N and again extracted with dichloromethane (basic extract). The three extracts obtained

were dried with anhydrous sodium sulphate and then by rotary evaporator. The residues were weighed at constant weight and the proper volume of DMSO added for mutagenicity assays. Solvent blanks were prepared as negative controls.

The Salmonella/microsome assay (Ames et al. 1975) was performed on extracts and adsorbates of the water samples. Tests on unconcentrated water samples incorporated in the top agar (up to 1 ml/plate) were also performed. The tests were only carried out with two strains of Salmonella typhimurium, TA98 and TA100, which have been found to be the most sensitive to compounds present in the aquatic environment (Grabow et al. 1980; Monarca et al. 1983). Aroclor-induced male Sprague-Dawley rats were used for preparation of liver post-mitocondrial supernatant (S9), according to Ames et al. (1975). Solutions of adsorbates and extracts in DMSO at different concentrations were prepared and tested in broad concentration range (at least a 3-log range). Narrower concentration ranges were tested for obtaining dose-response curves. The standard plate method (Ames et al. 1975) was followed. Sample volumes of 100 µl/plate were tested in DMSO. The observation of dose related responses and 2-fold increases in the number of induced revertants/plate over spontaneous revertants/plate values were the criteria for positive results. Specific values for mutagenicity, as net revertants/mg or net revertants/litre of water, were calculated by least-squares regression analysis of the linear portion of the dose-response curves (Monarca et al. 1983).

RESULTS AND DISCUSSION

Table 1 shows the concentrations of adsorbates and extracts recovered in the raw and drinking waters under study. The concentrations of adsorbates were higher in the raw waters. Total extracts were similar for lake water source, higher in raw water of river source and higher in drinking water No.2 derived from spring water. Acidic extracts constituted the majority of the three extracts for treated and untreated surface waters, while neutral extracts were higher only for drinking water No.2 from spring water. Table 2 gives the results of the Ames test without metabolic activation and with TA98 and TA100 strains. Adsorbates were constantly negative for all

Table 1. Concentrations (mg/l) of adsorbates and extracts obtained from raw and drinking waters

Fractions	Concentrations of adsorbates and extracts (mg/l)[a]						
	Lake water source		River water source		Spring water source		
	Raw water	Drinking water	Raw water	Drinking water	Raw water	Drinking water No.1[b]	Drinking water No.2[c]
XAD-2 adsorbates	0.88	0.50	1.30	0.70	0.62	0.15	0.29
Total dichloromethane extracts	2.45	2.42	0.54	0.21	0.34	0.23	1.16
Neutral extract	0.07	0.62	0.00	0.01	0.05	0.05	0.84
Acidic extract	2.04	1.10	0.48	0.20	0.14	0.13	0.12
Basic extract	0.34	0.70	0.06	0.00	0.15	0.05	0.20

[a] Solvent residue weights subtracted

[b] Sampled after about 14 Km of distribution in cast iron pipeline

[c] Sampled after 14 Km of distribution in steel pipeline coated with coal tar paint

Table 2. Mutagenicity of raw and drinking waters without metabolic activation

	Mutagenicity without metabolic activation (-S9)[a]									
			Dichloromethane extracts (net rev/mg)[b]							
	Adsorbates (net rev/mg)		Neutral		Acidic		Basic		Specific mutagenicity (net rev/litre)	
	TA98	TA100	TA98	TA100	TA98	TA100	TA98	TA100	TA98	TA100
Lake water source										
Raw water	-	-	-	-	-	-	-	-	-	-
Drinking water	-	-	-	-	-	150	-	90		228
River water source										
Raw water	-	-	-	-	-	-	-	-	-	-
Drinking water	-	-	61	-	36	392	-	-	8	188
Spring water source										
Raw water	-	-	-	-	-	-	-	-	-	-
Drinking water No.1	-	-	-	-	-	-	-	-	-	-
Drinking water No.2	-	-	-	-	-	-	-	-	-	-

[a] Spontaneous revertants/plate: TA98 -S9 = 8 ± 2; TA100 -S9 = 58 ± 12

[b] Calculated by least-squares regression analysis

[c] Calculated multiplying net rev/mg values by each correspondent mg/litre value (Table 1)

- = Negative results

the samples. Extracts were mutagenic only for surface waters and only after treatment (drinking waters). Acidic extracts gave the highest responses with TA100 strain and constituted about 70% of the total mutagenicity per litre of lake drinking water and 100% for river drinking water. The TA98 strain revealed the presence of direct mutagens in river drinking water.

Tests performed with the two strains and with metabolic activation (+S9) were always negative for all the samples. Direct tests of water samples by incorporating up to 1 ml/plate of water in the top agar gave negative responses.

From the above results it can be seen that the in situ resin adsorption technique was unable to recover mutagens from all the water samples, while the liquid-liquid extraction technique gave extracts with mutagenic properties without metabolic activation for the two surface waters only after treatment. The appearance of direct-acting mutagens after treatment of surface waters could be due to chlorination steps (especially pre-chlorination) which allowed the chlorine to react with organic substances producing these potentially harmful compounds (Fleischacker and Randtke 1983). The formation of chlorinated organics could be minimized for instance by avoiding pre-chlorination, chlorine concentrations higher than are necessary or by using alternative disinfectants.

The liquid-liquid extraction technique showed higher recovery efficiency of mutagens from water samples. This was also observed by Grabow et al. (1981). Moreover, extraction at different pHs gave useful indications about the chemical class of mutagens. Since acidic extracts contributed for the major percentage to the total mutagenicity, we suggest, for routine screening of mutagens in drinking water, using the liquid-liquid extraction only at pH 2 and testing this extract by Ames test with TA98 and TA100 strains.

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