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The Mutagenic and Some Physico-Chemical Properties of Organic Residues Obtained from Drinking Water by Amberlite XAD-2 and Activated Carbon Extraction

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Several types of drinking-water organic residues, obtained by solvent extractions from XAD-2 resin and activated carbon columns after the passage of drinking water (10 m³), were tested for mutagenic activity in TA-98 and TA-100 strains with and without the addition of S9. Direct-acting mutagens and some substances toxic to the tester strains were found to be present in these organic residues. Thin-layer chromatographic (TLC) fractionations, using polyamide plates, coupled with bioassay for mutagenicity showed that some of the major mutagens present in the XAD-2-extractable organic residues were of intermediate polarity and some were nonpolar. In contrast, those mutagens found in carbon-ether-extractable residues behaved on the TLC plates as compounds with a wide polarity range, with the strongest mutagenicity appearing among the acidic substances. Mutagenicity of these residues was inactivated by incubation with 4-nitrothiophenol as a nucleophile, but no change in activity was observed following reaction with *o*-phenylenediamine as a trapping reagent for mutagenic α -carboxylic compounds. In addition, it was found that mutagenicity in these organic residues was rapidly lost following heating, light irradiation or addition to alkaline solutions.

Keywords—activated carbon; drinking-water organic residue; heating; light exposure; mutagenicity; 4-nitrothiophenol; *o*-phenylenediamine; TLC fractionation; XAD-2 resin

Introduction

The findings of mutagenic and carcinogenic organic compounds in source water¹⁾ and drinking water²⁾ have caused concern as to their potential effects on human health. Mutagenicity in source water has generally been attributed to contamination by industrial waste or agricultural run-off, and to a lesser extent to naturally occurring substances. After comparison studies of raw water *vs.* finished water, Glatz *et al.*³⁾ and Maruoka and Yamanaka⁴⁾ suggested that chlorination may play a major role in the production of organic mutagenic in potable water. Subsequent laboratory experiments by Cheh *et al.*,⁵⁾ in which a drinking water treatment process was simulated, clearly demonstrated that non-volatile muta-

gens were produced by chlorine disinfection.

While the practice of chlorination is known to produce volatile halogenated organic compounds such as the trihalomethanes,^{6,7)} little is known about the potential health effects of the non-purgeable chlorinated organic compounds that are produced. Because risk estimates for regulatory use are currently dependent on toxicological testing of individual substances, one is ultimately faced with the task of identifying the substances which trigger the biological response. However, because most organic contaminants are present at microgram per liter (ppb) levels or less in drinking water, a concentration step is necessary prior to the analytical identification of individual contaminants of toxicological importance.

Drinking water concentrates for mutagenic testing have typically been prepared by liquid-liquid extraction,^{8,9,14,22)} carbon adsorption,¹¹⁾ or resin adsorption.^{3,5,10,12-22)} These methods, like other concentration procedures, fail to provide a totally representative concentrate since one or more classes of organic compounds are not recovered effectively. Consequently, determining whether the mutagenic activity in chlorinated drinking water is associated with acidic, basic, or neutral compounds is important in developing an effective concentration procedure. The work of several investigating groups^{9,10,12,22)} has suggested that acidic substances as well as neutral compounds may contribute significantly to the activity of finished drinking water.

As mentioned above, a number of research papers on isolation and separation procedures to identify mutagenic substances have been reported from North America,^{3,5,11,14,15,21)} Western Europe,^{9,10,12,13,16-19,22)} and other countries.^{8,20,29)} However, up to the present time, there has been little information on the identification of mutagenic compounds in raw water^{4,23-26)} and drinking water²⁷⁾ in Japan. In this paper, the preliminary results of Ames tests for mutagenicity in drinking-water organic residues obtained by XAD-2 and activated carbon concentration procedures are presented. In addition, results of a limited study of some of the characteristics of the organic mutagenic fraction present in the water are presented.

Experimental

Amberlite XAD-2 Resin and Activated Carbon—Rohm and Hass Amberlite XAD-2 resin and activated carbon (AMF QUNO) were cleaned in a Soxhlet apparatus with each of the following solvents, acetone, diethyl ether, and chloroform for 24 h. After evaporating the solvents completely in a vacuum desiccator for 24 h, each cleaned adsorbent was placed in a column (540 ml), which was fixed

Fig. 1. Schematic of the Apparatus for Concentration of Organic Residues from Drinking Water

to an adsorption apparatus as shown in Fig. 1. Amberlite XAD-2 and activated carbon columns were washed with 10 l of cleaned water (distilled and then filtered through a 0.45 μ m membrane filter; DMW) before sample collection. The organic solvents (acetone, chloroform, diethyl ether and methanol) used in the present study were pesticide-residue analytical grade (Cica-Merck). They were checked for mutagenicity by evaporating 100 ml to dryness and then dissolving the residue in 1 ml of dimethyl sulfoxide for Ames assays.

Collection and Preparation of Samples

Drinking water was sampled into the adsorption apparatus (Fig. 1) which was connected directly to the water tap in our laboratory. Water samples (total volume of 10 m³) were intermittently passed through the XAD-2 resin column, at a flow-rate of 700 ml/min, at room temperature, during the period from September 20 to November 9, 1985 to collect XAD-2-extractable organic residues. Drinking water (total volume of 10 m³) was also intermittently processed through the activated carbon column, under the same experimental conditions, in the period from September 16 to November 9, 1986 to collect the carbon-extractable organic residues. Each column was then washed with 2 l of distilled water and the residual water was blown from the column by dry nitrogen. The XAD-2 resin and activated carbon

Fig. 2. Experimental Design used to Concentrate and Extract Mutagenic Organic Residues from Drinking Water

were taken from the columns, and each adsorbent was separated into three portions and stored in a refrigerator at 4 °C until subsequent solvent extractions could be performed (Fig. 2).

The organic residues on each portion of the XAD-2 resin and activated carbon were Soxhlet-extracted individually with acetone,²⁸⁾ diethyl ether,²⁹⁾ and chloroform³⁰⁾ for 8 h. These extracts were filtered through a column containing 30 g of sodium sulfate (anhydrous) and evaporated to dryness by means of rotary evaporation at 40 °C. Dry concentrates were weighed and then dissolved, as rapidly as possible, in 10 ml of methanol, producing a concentration factor of 3×10^5 . Mutagenicity assays were carried out immediately because the mutagenic activity of extracts declines during storage.³⁾

Control experiments to evaluate the mutagenicity of artifacts leaching from the XAD-2 resin and activated carbon columns were also carried out as follows. A small-scale column (2 cm i.d. $\times$ 25 cm) was packed by gravity using methanol slurries of clean XAD-2 resin or activated carbon (50 ml). Each column was flushed with 100 ml of DMW to displace the methanol prior to each control experiment. After processing with 50 l of DMW, both with and without chlorination to 1 mg/l of residual chlorine, at a flow-

rate of 50 ml/min, the adsorbent was separated into three portions for subsequent solvent extractions. The same procedure and concentration of each control adsorbent and the resultant extracts were dissolved in 0.5 ml of methanol, producing a concentration factor of 3×10^4 .

Fractionations of Mutagenic Extracts by TLC

—The XEEEO and CEEEO samples extracted from drinking water both exhibited strong mutagenicity in TA-100 strain without S-9. Twenty milligrams of the XEEEO and 74 mg of the CEEEO samples were individually subjected to thin-layer chromatography (TLC) on polyamide plates (Merck), using chloroform as a developing solvent,³¹⁾ with the production of several fractions. Zone detection was performed under ultraviolet light. The separated zones were scraped off by using special recovery tubes (Wako Chemicals). The adsorbed substances were then eluted with diethyl ether (20 ml $\times$ 2) with the aid of a magnetic stirrer, in order to evaluate the amount of sample recovered from each fraction, and the mutagenicity of each fraction. The recoveries (% w/w and revertants/revertants) of individual fractions were calculated from the following equations, respectively.

Eq. (1) : [(Amount of sample recovered)/(amount

of sample applied)] $\times 100$

$$\text{Eq. (2)} : [(\text{Net revertants of sample recovered})/(\text{net revertants of sample applied})] \times 100$$

Heating and Light Exposure Experiments

—Heating experiments were conducted in 25 ml stoppered test tubes placed in a water bath. Aliquots (2 or 3 mg) of XEEO sample or CEEO sample, both of which exhibited significant mutagenicity in the Ames tests, were dissolved in 10 ml of phosphate buffer solution (pH 7) in the test tubes. After heating for 5, 10, or 20 min in the boiling water bath, samples in the test tubes were immediately cooled in an ice bath. The organic residues in the heated samples were extracted with 3 ml of diethyl ether and then redissolved in 1 ml of methanol for mutagenicity tests, using the TA-100 strain without S9. The same procedure with the exception of heating was performed as a control blank.

Irradiation of the mutagenic extracts was also conducted, utilizing a Rikokagaku Sangyo UVL-100 HA lamp. The lamp was equipped with a quartz water jacket for cooling at 20 °C and UV light (254 nm) was delivered at an intensity of approximately 50 mW/cm²/s at the surface of the water jacket. Aliquots (2 or 3 mg) of XEEO sample or CEEO sample in 10 ml of phosphate buffer solution (pH 7) were placed in a reaction vessel and exposed to the UV-light for 5, 10, or 20 min. The organic residues in the irradiated samples were extracted with 3 ml of diethyl ether and then redissolved in 1 ml of methanol for mutagenicity tests (using the TA-100 strain without S9). A dark control experiment was performed by covering the reaction vessel with aluminum foil, to establish the extent of changes in mutagenic activity of the original extracts caused by the other elements of the procedure.

Treatment with 4-Nitrothiophenol of Mutagenic Extracts—Experiments to inactivate direct-acting mutagens in XEEO and CEEO samples during reactions with the nucleophile, 4-nitrothiophenol (NTP), were conducted according to the recommendations of Agarwal *et al.*³²⁾ and Cheh and Carlson.³³⁾ The stock solution of NTP (10 mg/l) was maintained at -20 °C, as a 50% (v/v) aqueous ethanol solution acidified to pH 4.5 by the addition

of 6 N HCl solution. Concentrations were determined by dilution of the stock solution into 0.1 M sodium phosphate buffer (pH 7.4), measurement of the absorbance of this solution at 410 nm, and calculating using the reported³³⁾ molar absorptivity of 13000/M/cm. Working solutions of NTP were prepared by diluting the stock solution in 0.05 M sodium phosphate buffer (pH 7.4) to concentrations ranging from 0.64 mM to 26 mM.

Reaction of NTP with the XEEO (3 mg) or CEEO (2 mg), which contained direct-acting TA-100 mutagens, was accomplished by incubating the samples with NTP at room temperature for 30 min. Such mixtures were bioassayed for mutagenesis with strain TA-100 NR, using an ethanol solution of 1,3-dichloroacetone as the positive mutagen control for the NTP reaction, as described by Cheh and Carlson.³³⁾

Mutagenicity Assay—Tester strains TA-98 and TA-100 of the *Salmonella*/microsome mutagenicity assay system were employed essentially as described by Ames *et al.*³⁴⁾ Characteristic properties of the bacteria were verified for each fresh stock and their mutagenic properties were again verified using positive and negative controls as part of each experiment. The nitroreductase deficient strain TA-100 NR was obtained from H.S. Rosenkrantz,³⁵⁾ and utilized in the *Salmonella*/microsome mutagenicity assay as described by Cheh and Carlson.³³⁾ For testing mutagenesis requiring microsomal activation, polychlorinated biphenyl mixture-induced rat liver microsomal 9000 $\times g$ supernatant fraction (S9) was utilized. Mutagenesis assays, with (+S9) and without (-S9) microsomal activation, were conducted as previously described.³⁶⁻³⁸⁾ The criterion for detection of mutagenesis in experimental samples was a dose-dependent response exceeding the zero dose response (spontaneous control value) by at least two-fold. In some situations involving TLC fractions, where the amount of sample was limited, semiquantitative determinations of mutagenesis were made as previously described.³¹⁾ All recoveries of bioactivity from concentrated or fractionated residue organic samples were based upon an expression of mutagenesis per liter or milligram equivalent, related to the original water sample or the concentrated sample.

Results and Discussion

Ames Tests for Artifact Leaching from Activated Carbon and XAD-2 Resin

XAD resins and activated carbon are commonly used to recover and to concentrate mutagenic organic residues from raw water and drinking water. In these experiments, however,

it is important to differentiate between mutagens present in the original samples and those produced as artifacts during such concentration procedures.^{21,39,40} To examine the possibility of such artifacts existing in our clean XAD-2 resin and activated carbon columns, control experiments were carried out using a

TABLE I. Mutagenicity of Artifact Leaching from Amberlite XAD-2 Resin and Activated Carbon after Passage of Non-chlorinated and Chlorinated Water Containing 1.0 mg/l of Residual Chlorine

Adsorbent and control	Name of sample ^{a)}	Dose (l per plate) ^{b)}	Mutagenicity ratio in tester strain ^{c)}							
			Non-chlorinated				Chlorinated			
			TA-98		TA-100		TA-98		TA-100	
			- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
Amberlite XAD-2 resin	XCEO	3.0	1.2	1.1	1.2	1.1	1.0	1.3	1.2	1.2
		2.0	1.0	1.2	1.1	1.2	1.2	1.2	1.2	1.3
		1.0	1.0	1.2	1.2	1.3	1.2	0.9	1.0	1.0
		0.5	1.0	1.0	1.1	1.2	1.2	1.0	1.1	1.2
	XEE0	3.0	1.3	1.2	1.0	1.2	1.0	1.0	1.3	1.2
		2.0	1.1	1.1	1.4	1.1	1.3	1.2	1.3	1.0
		1.0	1.0	1.0	1.3	1.3	1.0	1.1	1.2	0.9
		0.5	1.0	1.1	1.3	1.0	1.2	1.1	1.0	1.0
	XAE0	3.0	1.1	1.4	1.0	1.2	1.3	1.3	1.2	1.0
		2.0	1.2	1.2	1.3	1.1	1.5	1.2	1.4	1.1
		1.0	1.2	1.1	1.2	1.1	1.4	1.0	1.4	0.9
		0.5	0.9	1.1	1.0	1.0	1.1	1.2	1.3	1.0
Activated carbon	CCEO	3.0	1.2	1.2	1.1	1.0	1.1	1.3	1.2	1.1
		2.0	1.2	1.0	1.1	1.3	1.0	1.0	1.1	1.0
		1.0	1.0	1.1	1.0	1.1	1.2	1.0	1.2	1.0
		0.5	1.0	0.9	1.0	1.1	1.1	1.0	1.1	1.0
	CEEO	3.0	0.7	1.2	1.3	1.2	0.9	1.3	0.7	1.0
		2.0	1.2	1.1	1.0	1.2	1.5	1.2	1.6	1.0
		1.0	1.0	1.1	1.2	1.1	1.4	1.2	1.5	1.0
		0.5	1.0	1.0	1.1	1.0	1.2	1.0	1.3	1.2
	CAEO	3.0	1.2	1.5	1.1	1.0	1.2	1.3	1.5	1.0
		2.0	1.4	1.2	1.3	0.9	1.4	1.0	1.5	1.0
		1.0	1.2	1.0	1.1	1.3	1.2	1.0	1.4	1.0
		0.5	1.0	1.1	1.1	0.9	1.0	1.2	1.3	1.0
Spontaneous Positive	revertants		34	37	158	203	19	26	107	114
	revertants									
	Nitrofluorene	2 μ g	282	-	-	-	128	-	-	-
	B[a]P	5 μ g	-	719	-	662	-	514	-	431
	Na-azide	1 μ g	-	-	814	-	-	-	638	-

a) Nomenclature of samples; see Fig. 2.

b) Liter equivalents per plate values refer to the volume of original water sample proportional to the fractional volume of the solvent extract on the plate.

c) These numbers show the mean value in two plates per sample.

small-scale column (50 ml) packed with the resin or carbon as described above. Results of Ames tests for each control sample eluted from the columns after processing with non-chlorinated and chlorinated DMW (50 l) are summarized in Table I. Because daily variations in revertants in the Ames assays were observed (from 12 to 40 in the TA-98 strain without S9, and 70 to 150 in TA-100 without S9), mutagenicity in these samples was expressed as a mutagenicity ratio. This was the ratio of revertants on the test plates (spontaneous+induced revertants) to those on the

negative control (spontaneous revertants).

No mutagenic effects were apparent from the XCEO and CCEO samples obtained from either the non-chlorinated or chlorinated DMW (Table I). However, weak mutagenic effects were observed when testing the XAEO, CEEEO, and CAEO samples obtained from chlorinated DMW, using both TA-98 and TA-100 in the absence of S9. But the mutagenicity ratios of these samples did not exceed negative controls by two-fold in the tested range (0.5 to 3 l

Fig. 3. Mutagenic Activity in XAD-2-Solvent-Extractable Organics (A and B) and Carbon-Solvent-Extractable Organics (C and D) from Drinking Water

Mutagenicity assays were conducted in the absence and presence (+S9) of metabolic activation. In this figure, liter equivalents per plate values refer to the volume of original water sample proportional to the fractional volume of the solvent extract on the plate. The plots in this figure show the mean value of two plates for each sample. Abbreviations for these water concentrates; see Fig. 2.

equivalents per plate). These findings indicate that small amounts of polar mutagenic artifacts are produced as a result of the reaction of free chlorine with XAD-2 resin^{21,39,40} or activated carbon. However, it seems that the contribution of these mutagenic artifacts is relatively small compared with the mutagenicity of the organic residues from the drinking water (Fig. 3).

Mutagenic Properties of Drinking Water Concentrates

Figure 3 shows the results of the Ames tests for mutagenicity of drinking water concentrates (XCEO, XEE0, XAEO, CCEO, CEE0, and CAEO), using the TA-98 and TA-100 strains both in the absence and presence of S9. Substantially greater activity was seen for these water concentrates in the TA-100 strain without S9. However, those samples obtained by acetone and diethyl ether extractions from the XAD-2 resin and activated carbon columns were toxic to the TA-98 strain without S9, but not to the TA-100 strain. Enzymatic activation with S9 reduced both the toxic and mutagenic effects of all the drinking water concentrates examined. These findings are in good agreement with those previously published concerning tests on drinking water in other locations, demonstrating that direct-acting mutagens had a higher frequency of occurrence in chlorinated drinking water.^{1-3,5,8,14,15,21,40-43}

The dry mass of the drinking water concentrates and the net revertants per sample, calculated from the initial and linear portions of the dose-response curves, are summarized in Table II. The range of mutagenicity in drinking water concentrates was between 7 and 46 net revertants/1 when tested on the TA-98 strain without S9 and from 53 to 200 net revertants/1 for TA-100 without S9, depending on the extraction methods. The results

TABLE II. Amounts and Mutagenicity of Solvent-Extractable Organics from XAD-2 Resin and Activated Carbon Columns after Passage of Drinking Water (10 m³)

Name of sample ^{a)}	Amount of sample recovered (mg/m ³)	Net revertants per m ³ equivalent			
		TA-98		TA-100	
		- S9	+ S9	- S9	+ S9
XCEO	29.7	8800	5000	83000	15000
XEE0	34.1	7000 ^{b)}	4000	53000	13000
XAEO	77.5	17500	5800	88000	23000
CCEO	66.0	46000	- ^{c)}	118000	19000
CEE0	52.5	7600	- ^{c)}	206000	17000
CAEO	93.0	10800 ^{b)}	- ^{c)}	60000	14000 ^{b)}

a) Abbreviations for samples; see Fig. 2.

b) Not significant (dose not equal or exceed double the spontaneous reversion rate).

c) Same as the spontaneous reversion rate.

obtained in similar studies performed elsewhere have ranged from 20 to 700 net revertants/1 for TA-98 and TA-100 strains.^{8,11-16,18,21,40}

Recently, Vartiainen and Liimatainen²² have reported that humus-rich chlorinated drinking water in Finland exhibits high levels of mutagenicity for the TA-100 strain without S9 (average value, 1700 net revertants/1). Concentrates were obtained using continuous solvent extraction with dichloromethane. Similarly high levels of mutagenicity (about 1300 net revertants/1 for TA-100 without S9) in chlorinated drinking water have also been reported by Cheh *et al.*,⁵ who used XAD-4 adsorption and acetone (and then dichloromethane) extraction to obtain their concentrates for testing. Although the results reported here cannot be compared directly with those described above, the low level of mutagenicity in drinking water shown in Table II can be explained in terms of the low concentration of mutagen precursors present in raw water. In addition, the levels of mutagenic activity in the carbon extracts seem to be somewhat higher than those found in XAD-2 extracts (Fig. 3 and Table II). This may be due to

the greater efficiency of activated carbon in adsorbing mutagens with a wide polarity range present in water samples, as compared with XAD-2 resin (Table II).

Mutagenicity of the Fractions Separated by Using TLC

In general, it is known that Amberlite XAD-2 resin is capable of adsorbing non-polar compounds from water samples, while activated carbon can be used for isolating compounds with a wide range of polarities, although the recovery of organic materials from these adsorbents is incomplete. To determine the approximate polarity of the mutagenic materials present in our drinking water concentrates, subsequent experiments were conducted to fractionate the XEEO and CEEO samples using polyamide plates with chloroform as a developing solvent. Because the highest response was seen in the TA-100 strain, while the addition of S9 reduced mutagenic activity, all further testing was restricted to the use of the TA-100 strain without S9. The results of the TLC fractionation of mutagenic XEEO and CEEO samples and of the Ames tests for mutagenicity in these fractions are shown in Figs. 4 and 5.

When the mutagenic XEEO sample (20 mg) was fractionated on polyamide plates, the recovery in the fractions was approximately 55% (w/w) of the amount of sample applied, while the mutagenicity recovered from the plates was 60% (revertants/revertants) of the overall activity applied (Fig. 4). These results indicate that both mutagenic and non-mutagenic substances in the XEEO sample were strongly adsorbed onto the polyamide plates, including decomposition products of the applied sample. However, about 85% (revertants/revertants) of the total mutagenicity of the sample recovered from the polyamide plates was found to be concentrated in fractions 5,

Fig. 4. Distribution of Direct-Acting TA100 Mutagens in XAD-2-Ether-Extractable Organics (XEEO) on a Polyamide Plate with Chloroform (Left) and Mutagenicity in the TLC Fractions (Right)

6, and 7. This suggests that some of the mutagenic materials present in the XEEO sample are of intermediate polarity and some are non-polar.

Figure 5 shows the results of the TLC fractionations of the mutagenic CEEO sample and of the Ames tests for mutagenicity in these fractions. When the CEEO sample (74 mg) was fractionated on polyamide plates, recovery in the fractions was approximately 43% (w/w) of the sample applied, while the mutagenicity recovered from the plates was 45% (revertants/revertants) of the overall activity applied. These results indicate that both mutagenic and non-mutagenic substances are strongly adsorbed on the polyamide plates, including their decomposition products. In this case, the mutagenic activity of the samples recovered from the plates was found to be distributed in fractions 1, 5, and 7. Among these components, fraction 1 showed the strongest mutagenic response. Its mutagenicity was approximately 35% (revertants/revertants) of the over-

Fig. 5. Distribution of Direct-Acting TA100 Mutagens in Carbon-Ether-Extractable Organics (CEEEO) on a Polyamide Plate with Chloroform (Left) and Mutagenicity in the TLC Fractions (Right)

all activity recovered from the plates. Fractions 5 and 7 also exhibited weak mutagenicity. This suggests that the major mutagenic materials present in the CEEEO are strongly acidic compounds while minor components are of intermediate polarity or are non-polar.

Inactivation of Direct-Acting TA-100 Mutagens by NTP

In order to assess the presence of mutagenic electrophiles (*e.g.*, alkyl halides and epoxides) in the XEEEO and CEEEO samples, reactions of these samples with 4-nitrothiophenol (NTP), a strong nucleophile,³³⁾ were conducted as described above. The results of these experiments for both the XEEEO and CEEEO samples are shown in Fig. 6. Bioassays were conducted with TA-100 NR, a tester strain similar to TA-100 but genetically deficient in nitroreductase activity, to avoid the production of possible mutagenic by-products from NTP. As can be seen in Fig. 6, two samples obtained by XAD-2 and carbon extraction from drinking water were inactivated by addi-

Fig. 6. Sensitivity of Direct-Acting TA100 Mutagens in XAD-2-Ether-Extractable Organics (XEEEO) and Carbon-Ether-Extractable Organics (CEEEO) to Inactivation by 4-Nitrothiophenol (NTP)

○; CEEEO, △; XEEEO, □; 1,3-dichloroacetone. Mutagenicity assays were conducted using TA 100 NR strain in the absence of metabolic activation.

tion of NTP. However, the mutagenic activity of the CEEEO sample was trapped as NTP-mutagen adducts^{32,33)} at lower concentrations of the reagent NTP than were required to inactivate the TA-100 mutagen in the XEEEO sample. These results indicate that the TA-100 mutagens in the CEEEO sample from drinking water more readily react with NTP, and suggest that they may be more potent mutagens than those in the XEEEO sample.

As an additional experiment, reactions of the XEEEO and CEEEO samples with *o*-phenylenediamine were conducted according to the methods described previously,⁴²⁾ in order to assess the presence of mutagenic α -dicarboxylic compounds (*e.g.*, chlorinated α -diketones and *o*-benzoquinones) in our drinking water concentrates. However, no change in mutagenicity was observed for both XEEEO and CEEEO samples, compared with the control samples. This leads to the conclusion that no detectable levels of α -dicarboxylic mutagens are present in our water concentrates.

Destruction of Direct-Acting TA-100 Mutagens by Heating and UV-Light Exposure

We have at present very little information concerning the physical properties of the mutagens in drinking water organic residues. Such information is very important for evaluating the loss of mutagenicity during sample preparation and sample storage in the laboratory, or when planning the elimination of mutagenicity from drinking water supplying systems. Thus, final experiments were carried out to determine the effects of heating and light irradiation on the mutagenic potential of the drinking water organic residues. The results in Table III show that the mutagenicity of both the XEE0 and CEE0 samples in a buffer solution of pH 7 was rapidly lost following heating or UV-light irradiation. After a 20 min treatment, mutagenic activity of the CEE0 sample was no longer detectable. However, approximately 20 % (revertants/revertants) of the activity still remained in the XEE0 sample following heating. Hashizume *et al.*²⁷⁾ recently reported that the direct-acting TA-100 mutagens present in a methanol extract from drinking water, obtained by using a membrane of microporus polyethylene hollow fibers (EHF), were relatively stable to heat.

A decrease in mutagenicity was also observed for control XEE0 and CEE0 samples after mixing them with phosphate buffer solutions (pH 7 and 9) and then standing them at room temperature for 20 min. The decrease in activity in the pH 9 buffer solution was approximately 2 times greater than that found in the neutral solution (Table III). This finding indicates that the direct-acting TA-100 mutagens in our water concentrates can be destroyed by some chemical factors such as solution pH, in addition to temperature and light. Earlier workers⁴¹⁻⁴³⁾ have been shown that production of mutagens from humic solutions

TABLE III. Inactivation of Direct-Acting TA100 Mutagens by Heating and UV-Irradiation

Experiment ^{a)}	Time (min)	Remaining activity ^{b)} (%)	
		XEE0	CEE0
A: Heating at 100°C	5	86	79
	10	19	10
	20	20	5
B: Irradiation	5	35	43
	10	10	13
	20	5	7
C: Control (pH 7)	10	79	81
	20	72	70
	10	73	68
	20	48	45

a) Details ; see Experimental.

b) The mutagenicity in these samples was evaluated from dose-response curves.

is greatly dependent on the chlorination pH, with a pattern of decreasing mutagenic activity with increasing pH.

Conclusion

While chlorine is used widely in water and wastewater treatment both as an oxidizing agent and a disinfectant, it is also known that water chlorination produces mutagenic species from natural organic precursors. However, there is little information on the identity of the mutagenic compounds in chlorinated drinking water. Drinking water organic residues obtained by solvent extraction following XAD-2 resin or activated carbon concentration procedures were tested for mutagenicity in tester strains TA-98 and TA-100 with and without the addition of S9.

Direct-acting mutagens and some toxicants were found to be present in these water concentrates (Fig. 3 and Table II). TLC fractionations coupled with Ames assays demonstrated that some of the major direct-acting mutagens present in the XEE0 sample were of intermediate polarity and some were nonpolar, while those found in the CEE0 sample were

compounds with a wide polarity range, with acidic substances showing the strongest mutagenicity (Figs. 4 and 5). Mutagenicity in these water concentrates was rapidly inactivated by incubation with 4-nitrothiophenol as a nucleophile, but not in the reaction with *o*-phenylenediamine as a trapping reagent for mutagenic α -dicarboxylic compounds (Fig. 6). In addition, it was found that the mutagenicity of these water concentrates was rapidly lost upon heating, UV-irradiation, or in alkaline solution (Table III).

From these results, it is concluded that further investigations should be undertaken to determine in more detail the seasonal changes in the mutagens in drinking water and to clarify the nature of the mutagens which are present.

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References

- 1) W. Pelon, B.F. Whitman and T.W. Beasley, *Environ. Sci. Technol.*, **11**, 619 (1977).
- 2) J.C. Loper, *Mutat. Res.*, **76**, 241 (1980).
- 3) B.A. Glatz, C.D. Criswell, N.D. Arguello, H.J. Svec, J.S. Fritz, S.M. Grimm and M.A. Thomson, *J. Am. Water Works Assoc.*, **70**, 465 (1978).
- 4) S. Maruoka and S. Yamanaka, *Mutat. Res.*, **79**, 381 (1980).
- 5) A.M. Cheh, J. Skochdopole, P. Koski and L. Cole, *Science*, **207**, 90 (1980).
- 6) J.J. Rook, *J. Water Treat. Exam.*, **23**, 234 (1974).
- 7) T.A. Bellar, J.J. Lichtenberg, and R.C. Kroner, *J. Am. Water Works Assoc.*, **66**, 703 (1974).
- 8) W.O.K. Grabow, P.G. Van Rossum, N.A. Grabow and R. Denkhaus, *Water Res.*, **15**, 1037 (1981).
- 9) B. Wigilius, H. Borén, G.E. Carlberg, A. Grimvall and M. Möller, *Sci. Total Environ.*, **47**, 265 (1985).
- 10) S. Monarca, R. Pasquini, and G.S. Sforzolini, *Bull. Environ. Contam. Toxicol.*, **34**, 815 (1985).
- 11) M.W. Tabor and J.C. Loper, *Intern. J. Environ. Anal. Chem.*, **8**, 197 (1980).
- 12) H.G. Kool, C.F. Van Kreijl, H.J. Van Kranen, E. De Greef, *Sci. Total Environ.*, **18**, 135 (1981).
- 13) H.G. Kool, C.F. Van Kreijl, E. De Greef and H.J. Van Kranen, *Environ. Health Perspect.*, **46**, 207 (1982).
- 14) S.M. Grimm-Kibalo, B.A. Glatz, and J.S. Fritz, *Bull. Environ. Contam. Toxicol.*, **26**, 188 (1981).
- 15) D.T. Williams, B.R. Nestman, G.L. Lebel, F.M. Benoit and R. Otson, *Chemosphere*, **11**, 263 (1982).
- 16) K. Anthanasiou and S.A. Krytopoulos, *Sci. Total Environ.*, **27**, 113 (1983).
- 17) H.J. Kool, C.F. Van Kreijl and H. Van Oers, *Toxicol. Environ. Chem.*, **7**, 111 (1984).
- 18) H.J. Kool and C.F. Van Kreijl, *Water Res.*, **18**, 1011 (1984).
- 19) H.J. Kool, J. Hrubec, C.F. Van Kreijl and G.J. Piet, *Sci. Total Environ.*, **47**, 229 (1985).
- 20) P.G. Van Rossum, *Sci. Total Environ.*, **47**, 385 (1985).
- 21) M.W. Tabor and J.C. Loper, *Intern. J. Environ. Anal. Chem.*, **19**, 281 (1985).
- 22) T. Vartiainen and A. Liimatainen, *Mutat. Res.*, **169**, 29 (1986).
- 23) M. Tomita, H. Manabe and A. Hamada, *Jpn. J. Water Pollut. Res.*, **3**, 187 (1980).
- 24) S. Maruoka and S. Yamanaka, *Mutat. Res.*, **102**, 13 (1982).
- 25) S. Maruoka and S. Yamanaka, *Environ. Sci. Technol.*, **17**, 177 (1983).
- 26) S. Maruoka, S. Yamanaka and Y. Yamamoto, *Water Res.*, **19**, 249 (1985).
- 27) T. Hashizume, T. Yokoyama, Y. Sawada, A. Kawai, I. Igami, T. Kamiki, N. Kinai and I. Tomita, *Eisei Kagaku*, **31**, 385 (1985).
- 28) G.A. Junk, J.J. Richard, M.D. Greiser, D. Witiak, J.L. Witiak, M.D. Arguello, R. Vick, H.J. Svec, J.S. Fritz and G.V. Galder, *J. Chromatogr.*, **99**, 745 (1974).
- 29) P. Van Rossum and R.G. Webb, *J. Chromatogr.*, **150**, 381 (1978).
- 30) F.M. Middleton, H.H. Pettit and A.A. Rosen, in Proceedings of the 17th Industrial Waste Con-

- ference, Purdue University, *Ext. Ser.*, **122**, 454 (1963).
- 31) S. Onodera, M. Yamashita, S. Ishikura and S. Suzuki, *J. Chromatogr.*, **360**, 137 (1986).
 - 32) S.C. Agarwal, B.L. Van Duuren, J.J. Solomon and S.A. Kline, *Environ. Sci. Technol.*, **14**, 1249 (1980).
 - 33) A.M. Cheh and R.E. Carlson, *Anal. Chem.*, **53**, 1001 (1981).
 - 34) B.M. Ames, J. McCann and E. Yamasaki, *Mutat. Res.*, **31**, 347 (1975).
 - 35) M.S. Rosenkrantz and R. Mermelstein, *Mutat. Res.*, **114**, 217 (1983).
 - 36) S. Onodera, Y. Usui, M. Fujii and S. Suzuki, *Eisei Kagaku*, **28**, 160 (1982).
 - 37) S. Onodera, R. Akutsu, M. Furuta, Y. Usui, M. Fujii, S. Maruyama and S. Suzuki, *Eisei Kagaku*, **30**, 33 (1984).
 - 38) S. Onodera, S. Maruyama, S. Ishikura and S. Suzuki, *Eisei Kagaku*, **31**, 171 (1985).
 - 39) A.G. Sweeny and A.M. Cheh, *J. Chromatogr.*, **325**, 95 (1985).
 - 40) H.P. Ringhand, J.R. Meier, F.C. Kopfler, K.M. Schenck, W.H. Kaylor and D.E. Mitchell, *Environ. Sci. Technol.*, **21**, 382 (1987).
 - 41) J.R. Meier, R.D. Lingg, and R.J. Bull, *Mutat. Res.*, **118**, 25 (1983).
 - 42) M.A. Nazar, W.H. Rapson, M.A. Brook, S. May and J. Tarhanen, *Mutat. Res.*, **89**, 45 (1981).
 - 43) K.P. Kringstad, P.O. Ljungquist, F. de Sousa and L.M. Strömberg, *Environ. Sci. Technol.*, **17**, 553 (1983).