Original Article

Mutagenic and Estrogenic Effects of Organic Compounds in Water Treated by Different Processes: A Pilot Study

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Abstract

Objective In this study, a pilot-scale investigation was conducted to examine and compare the biotoxicity of the organic compounds in effluents from five treatment processes (P1-P5) where each process was combination of preoxidation (O_3), coagulation, sedimentation, sand filtration, ozonation, granular activated carbon, biological activated carbon and chlorination (NaClO).

Methods Organic compounds were extracted by XAD-2 resins and eluted with acetone and dichlormethane (DCM). The eluents were evaporated and redissolved with DMSO or DCM. The mutagenicity and estrogenicity of the extracts were assayed with the Ames test and yeast estrogen screen (YES assay), respectively. The organic compounds were detected by GC-MS.

Results The results indicated that the mutation ratio (MR) of organic compounds in source water was higher than that for treated water. GC-MS showed that more than 48 organic compounds were identified in all samples and that treated water had significantly fewer types and concentrations of organic compounds than source water.

Conclusion To different extents, all water treatment processes could reduce both the mutagenicity and estrogenicity, relative to source water. P2, P3, and P5 reduced mutagenicity more effectively, while P1 reduced estrogenicity, most effectively. Water treatment processes in this pilot plant had weak abilities to remove Di-n-butyl phthalate or 1, 2-Benzene dicarboxylic acid.

Key words: Water treatment processes; Organic compounds; Yeast estrogen screen (YES assay); Ames test; GC-MS

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INTRODUCTION

Disinfection by-products (DBPs) of chlorination have drawn special attentions since the 1970s due to their potentially mutagenic or carcinogenic effects. In 1974, reports of DBPs forming during water treatment were presented by Rook and others[1-2]. Mutagenic DBPs may be formed when chlorine reacts with organic matter or pollutants in source water. Since those earliest reports, a number of studies about DBPs and their adverse effects have been performed[3-5], and methods to decrease organic compounds in source water and consumption of chlorine and DBPs in treated water have been carefully considered. In order to decrease mutagenic compounds in drinking water as much as possible and still ensure biosafety, advanced treatments such as ozone oxidization, granular active carbon (GAC) and/or biological activated carbon

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(BAC) have been combined with conventional drinking water treatment (DWT), which usually consists of coagulation, sedimentation, sand filtration, and chlorination. Combinations of these advanced and conventional treatment steps may lead to a better way to control mutagenic organic compounds formed during water treatment processes[6-8].

Another potential threat to public health is the estrogenic activity of some organic compounds in water. The so-called environmental estrogens (EEs) include insecticides, herbicides, industrial chemicals, and compounds associated with plastics (bisphenol A, phthalates)[9-10]. Currently, various types of EEs can be detected in a wide range of natural and engineered environments all over the world, including surface water, ground water, wastewater, seawater, and sediments[11-14]. Studies have revealed correlations between exposure to EEs and the health of humans and wildlife, including many complicated adverse effects based on toxicological tests[15-17]. Hence, control of the types and amounts of EEs in drinking water is as important as the control of mutagens.

At present, pollution of the source water is a serious and ever-growing problem in China, especially in the most rapidly developing areas, such as the Zhujiang River delta in Southern China. Recently, the water quality in this area has drawn considerable attention of the local people because of concerns over industrial pollution.

In this study, we constructed a pilot plant near the Beijiang River, a main branch of the Zhujiang River system. We extracted organic compounds from the source water and effluents from the pilot plant under five combination processes, detected and evaluated mutagenic and estrogenic effects, and analyzed chemical component changes with GC-MS. This work offers a new approach for selecting a water treatment process, through comparison of biological toxicity of organic compounds in effluents. Furthermore, this work also helped to evaluate the quality of source water from the Beijiang River.

MATERIALS AND METHODS

Reagents

Amberlite XAD-2 resins, 17β-estradiol (E2), dimethyl sulfoxide (DMSO), and chlorophenol red-β-D-galactopyranoside (CPRG) were purchased from Sigma (St. Louis, MO USA). Acetone and dichlormethane (DCM) were analytically pure and obtained from Shanghai Chemicals Company (Shanghai, China). The S-9 fraction prepared from rat liver was induced by Aroclor 1254 and purchased from Sigma (St. Louis, MO USA).

Water Treatment Process of the Pilot Plant

The pilot plant was operated with a total flux of 6 m^3/h, and consisted of conventional DWT and advanced treatment processes, including peroxidation (O_3), coagulation and sedimentation, sand filtration, ozonation, GAC adsorption, BAC adsorption, and chlorination (NaClO). A flow chart describing the five water treatment processes is shown in Figure 1.

The detailed parameters of each water treatment unit and process are shown in Table 1. The difference between P4/P5 and P2 is that the GAC in P4/P5 was back-flushed by air [intensity 12-14 L/(m^2·s), time 3-5 min] and water [intensity 8 L/(m^2·s), time 5-7 min], while P2 was not back-flushed. The operation time of BAC and GAC columns was no more than three months. Table 2 presents the water quality parameters of the source water during the experimental time.

Samples Preparation

Water samples were collected in stainless steel tanks and treated *in situ*. The volume of each sample was 100 L. The solid phase extraction (SPE) step was performed as described by Shen, et al. with some modification[18]. In brief, water samples were filtered through glass fiber filters (pore size=1 μm) to filter out suspended matter and the filtrate was applied onto XAD-2 resin columns. The resins were blown dry under gentle nitrogen flow and eluted with acetone and DCM. The acetone and DCM eluates were blown dry with nitrogen, dissolved and diluted with DMSO (for the Ames test) or ethanol (for the YES assay) to various concentrations. The samples were stored at −80 °C for further experiments. Deionized water was extracted with the same steps as a control.

Ames Test

The Ames test for mutagenic potential utilizes mutant histidine-dependent strains of *Salmonella typhimurium*, which may revert back to histidine independence and grow as viable colonies following contact with a suitable mutagen. *Salmonella* strains TA97, TA98, TA100, and TA102 were kindly gifted by

the Ames laboratory, and the test was conducted according to the standard methods described by Maron and Ames[19]. Preliminary tests demonstrated that TA98 and TA100 were more sensitive than other strains for the detection of mutagens in water samples, so only these two strains were used in this study. The *Salmonella* strain TA98, used in the test was to detect frameshift mutants for the strain is more sensitive to the frameshift mutants, while TA100 was used to detect the base-replacement mutants. The dose set (0.5 L, 1 L, 2 L, 4 L/plate) of each water extract was plated in triplicates with 0.1 mL bacterial culture. Top agar (2.5 mL) containing trace level of growth factors (with histidine and biotin) was added and the contents were poured onto minimal glucose agar plates.

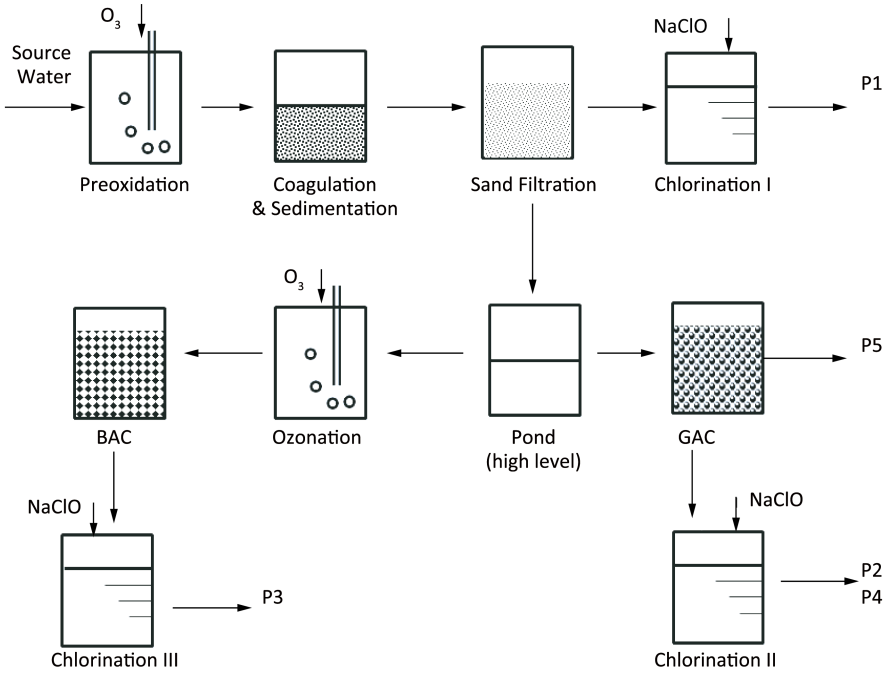

Figure 1. Process chart of the water treatments. P1: peroxidation-coagulation and sedimentation-sand filtration-chlorination (NaClO) I; P2: peroxidation-coagulation and sedimentation-sand filtration-GAC-chlorination (NaClO) II; P3: peroxidation-coagulation and sedimentation-sand filtration-Ozonation-BAC-chlorination (NaClO) III; P4: peroxidation-coagulation and sedimentation-sand filtration-GAC-chlorination (NaClO) II; P5: peroxidation-coagulation and sedimentation-sand filtration-GAC.

Table 1. Process Parameters of Relevant Water Treatment in the Pilot Plant

Process	Materials Used	Contact Time	Flux	Remark
Pre-O_3	O_3	3-5 min	6 m^3/h	Φ=350 mm; H=5.6 m; 1-1.5 mg/L
Mix	Mixture machine	30-45 s	6 m^3/h	S=0.3 × 0.3 m^2
Coagulation	Aluminum Sulfate	20 min	6 m^3/h	
Sedimentation	Chute tank	1.5 mm/s	1.5 mm/s	Length: 1000 m; tilt angle: 60° tangent circles d=25 mm
Sand filtration	Quartz sand		6 m^3/h	H=1500 mm; d=1.1-1.3 mm, S=0.87×0.87 mm^2
GAC	granular activated carbon	9-10 m/h	2.5 m^3/h	d=1.5 mm; H=2 m, S=0.5×0.5 m^2
BAC	biological activated carbon	9-10 m/h	2.5 m^3/h	d=1.5 mm; H=2 m, S=0.5×0.5 m^2
After-O_3	O_3	10 min	2.5 m^3/h	
Chlorination I	NaClO	30 min	1.0 m^3/h	RC[a]: 0.6-0.7 mg/L
Chlorination II	NaClO	30 min	2.5 m^3/h	RC[a]: 0.6-0.7 mg/L

Note. [a] residual chlorine, detected after 30 min disinfection.

Negative and positive controls were included in each test. The negative controls only had bacteria and DMSO but no test samples. All water extracts were also tested in the presence of 0.5 mL of S9 microsomal fraction mixture per plate. All determinations were performed in triplicate and incubated at 37 °C for 48-72 h. The mutation ratio (MR) was calculated as the number of histidine-positive (His+) revertants induced by the sample divided by the number of spontaneous His+ revertants in the negative control[20]. Generally, a twofold increase in the MR in a test sample was considered a positive mutagenic response.

Screening for Estrogenic Activity

Recombinant yeast cells were kindly provided by JP Sumpter (Brunel University, Uxbridge, UK) and estrogenic activity assays were conducted as previously described[21]. Yeast cells were stably transfected with the human estrogen receptor gene (hERα) and an expression plasmid carrying a reporter gene encoding β-galactosidase under the control of estrogen-responsive element (ERE). Binding of the receptor-ligand complex to ERE results in the expression of β-galactosidase, which metabolizes the chromogenic substrate β-D-galactopyranoside (CPRG), generating chlorophenol red with an absorption maximum at 540 nm. Standard solutions and sample extracts (including control extracts of deionized water) were produced in ethanol and 100 µL aliquots of each dilution series were dispensed into 96-well microtiter plates. Before adding 200 µL of the growth medium containing CPRG, the plates were allowed to evaporate to dryness at room temperature. After addition of the medium, the plates were incubated at 32 °C for 72 h and shaken at 80 rpm. Each sample was tested in triplicate. Absorbance of the medium was measured at 540 nm in a microtiter plate reader. Data were processed as described by Routledge and Sumpter, compared with E2 induction rates[21]. Estrogenic activity was computed using the EC25-E2 (effective concentration equivalent to 25% of positive controls maximum effect level). The calculation of estrogenic activity was performed as described by Rastall A C[22] with some modifications. In brief, 25% induction of the maximal E2-induced activity was defined as the effective 25th percentile concentration (EC25-E2) and was used to compare the estrogenic potency of samples.

Table 2. Quality of Source Water During Experimental Time

Water Quality Parameter	Range	Mean
Water temperature (^{o}C)	27.5-32.0	29.34
Turbidity (NTU)	4.48-31.4	11.68
Color (Degree)	<5	<5
pH	6.85-8.54	7.29
COD_{Mn} (mg/L)	1.14-2.14	1.63
NH_3-N (mg/L)	0.09-0.54	0.24
NO_2-N (mg/L)	0.001-0.104	0.037
NO_3-N (mg/L)	0.82-1.67	1.265
TOC (mg/L)	1.65-8.56	4.36
$CHCl_3$ (µg/L)	<0.02	<0.02
Petroleums (mg/L)	<0.05	<0.05
Coliform bacillus (/L)	>24,000	>24,000
Methanal (mg/L)	<0.05	<0.05

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

GC-MS analysis of samples was conducted by HP6890 gas chromatography equipped with DB-17 GC column (30 m × 0.25 mm × 0.15 µm) and detected by HP5973 mass selective detector (all from Agilent, USA). Helium was used as the carrier gas at 1 mL/min, injector temperature was set to 250 °C and 1.0 µL of the respective sample was injected per analysis using an auto injection system. All samples were separated with a temperature program of 50 °C for 2 min, followed by an increase to 130 °C at a rate of 20 °C/min, and then to 300 °C at a rate of 30 °C/min, with a final hold for 5 min.

Statistical Analysis

Statistical analyses were performing using SPSS for Windows version 12.0 (SPSS Inc, Chicago, IL, USA). Comparisons between the group with and without S9 were analyzed using a paired sample *t*-test. A one-way ANOVA was applied to determine the differences between processes. All reported *P* values are 2-sided and $P<0.05$ was considered to be statistically significant.

RESULTS

Ames Test

The reversing mutation frequencies caused by organic compounds in the water samples, with and

without the presence of eukaryotic metabolic activation system (±S9), are listed in Tables 3 and 4 for *S. typhimurium* TA98 and TA100, respectively. In TA98, the source water and effluents from P1 and P4 displayed mutagenic potential with or without S9 activation. Comparisons at the dose of 4 L/plate showed a consistent pattern of mutagenic potency in positive groups, with decreasing activity in the order: source water (3.20) > effluent P4 (2.71) > effluent P1 (2.50). There was no clear mutagenic activity observed in effluents from P2, P3, and P5. No significant mutagenic potentials were observed in the TA100 strain, as all the MR values were below 2 (Table 4).

Statistical Analysis of Mutagenicity between Water Treatment Processes

To determine the difference between the effects observed with and without the addition of S9 metabolic activation mixture, a paired sample *t*-test was applied (Table 5). A one-way ANOVA was applied to determine the differences between the five treatment processes and the source water, using the MR values of TA98 at the dose of 4 L/plate (Table 6).

Estrogenic Activity

Estrogenic activities induced by individual samples are shown in Figure 2. No significant estrogenic activity was observed in the blank, the deionized water control or in effluent from P1. The EC25-E2 of source water (36 mL) was higher than for any other sample. The decreasing sequence of estrogenic activity was as follows: source water (36 mL) > effluent P3 (45 mL) > effluent P5 (73 mL) > effluent P2 (131 mL) > effluent P4 (135 mL) > effluent P1 (none observed).

Table 3. Mutation Ratio of Organic Compounds in Source Water and Effluents from Process P1-P5 Assayed by *S. typhmurium* TA98 with or without S9 Metabolic Activation Mixture

Group	Doses (L/plate)	Solvent Control	Posivtive Control	MR					
				SW[d]	P1	P2	P3	P4	P5
+S9	4	0.96±0.04[a]	96.0±5.57[b]	3.20±0.40	2.50±0.32	1.51±0.01	1.57±0.04	2.71±0.34	1.31±0.04
	2			2.30±0.21	1.81±0.19	1.33±0.11	1.31±0.11	2.07±0.07	1.19±0.09
	1			1.41±0.17	1.28±0.24	1.03±0.07	1.08±0.11	1.57±0.10	1.01±0.03
	0.5			1.11±0.09	1.01±0.03	0.97±0.08	0.95±0.06	1.19±0.10	0.92±0.06
-S9	4	1.08±0.13[a]	9.10±0.44[c]	3.46±0.34	2.78±0.20	1.49±0.14	1.75±0.08	3.02±0.07	1.35±0.05
	2			2.56±0.11	1.99±0.11	1.55±0.15	1.52±0.08	2.23±0.15	1.38±0.07
	1			1.53±0.09	1.23±0.11	0.94±0.05	1.13±0.06	1.94±0.07	1.05±0.14
	0.5			1.31±0.06	1.03±0.05	1.05±0.06	1.01±0.14	1.41±0.04	1.15±0.02

Note. [a]DMSO; [b]2-Aminofluorene; [c]dexon; MR>2 with a dose-response effect is considered to be positive; [d]source water (SW).

Table 4. Mutation Ratio of Organic Compounds in Source Water and Effluents from Process P1-P5 Assayed by *S. typhmurium* TA100 with or without S9 Metabolic Activation Mixture

Group	Doses (L/plate)	Solvent Control	Posivtive Control[b]	MR					
				SW[d]	P1	P2	P3	P4	P5
+S9	2	0.99±0.01[a]	19.1±1.01[b]	0.99±0.09	0.99±0.18	0.97±0.11	0.95±0.03	1.06±0.12	1.00±0.17
	1			0.95±0.05	0.96±0.12	0.96±0.11	0.99±0.12	0.97±0.11	0.95±0.03
	0.5			1.02±0.05	0.95±0.03	0.98±0.02	1.00±0.09	0.95±0.02	1.01±0.05
-S9	2	1.03±0.06[a]	9.30±0.46[c]	1.01±0.08	0.94±0.05	0.99±0.09	0.90±0.10	1.12±0.21	1.06±0.09
	1			0.97±0.04	0.97±0.02	1.00±0.05	0.95±0.09	0.99±0.16	0.93±0.03
	0.5			0.98±0.11	0.92±0.07	1.00±0.03	0.90±0.06	0.94±0.05	0.95±0.05

Note. MR>2 with a dose-response effect is considered to be positive. [a]DMSO; [b]2-Aminofluorene; [c]sodium azide; [d]source water (SW).

Table 5. The Difference Between Adding S9 Metabolic Activation Mixture or Not

Paired Differences					*t*	*df*	*P*
Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
			Lower	Upper			
0.08	0.12	0.02	0.04	0.12	4.29	41	0.00*

Note. *significance at the 0.05 level (2-tailed).

Table 6. The Difference between Different Treatment Processes

(I)	(J)	Mean Difference (I-J)	Std. Error	*P*	95% Confidence Interval	
					Lower Bound	Upper Bound
sw[a]	P1	0.69*	0.13	0.00	0.42	0.96
	P2	1.83*	0.13	0.00	1.56	2.10
	P3	1.67*	0.13	0.00	1.40	1.94
	P4	0.46*	0.13	0.00	0.19	0.73
	P5	2.00*	0.13	0.00	1.73	2.27
P1	sw	-0.69*	0.13	0.00	-0.96	-0.42
	P2	1.14*	0.13	0.00	0.87	1.41
	P3	0.98*	0.13	0.00	0.71	1.24
	P4	-0.23*	0.13	0.09	-0.50	0.04
	P5	1.31*	0.13	0.00	1.04	1.58
P2	sw	-1.83*	0.13	0.00	-2.10	-1.56
	P1	-1.14*	0.13	0.00	-1.41	-0.87
	P3	-0.17*	0.13	0.02	-0.43	0.10
	P4	-1.37*	0.13	0.00	-1.64	-1.10
	P5	0.17*	0.13	0.02	-0.10	0.44
P3	sw	-1.67*	0.13	0.00	-1.94	-1.40
	P1	-0.98*	0.13	0.00	-1.24	-0.71
	P2	0.17*	0.13	0.02	-0.10	0.43
	P4	-1.21*	0.13	0.00	-1.47	-0.94
	P5	0.34*	0.13	0.02	0.07	0.61
P4	sw	-0.46*	0.13	0.00	-0.73	-0.19
	P1	0.23*	0.13	0.09	-0.04	0.50
	P2	1.37*	0.13	0.00	1.10	1.64
	P3	1.21*	0.13	0.00	0.94	1.47
	P5	1.54*	0.13	0.00	1.27	1.81
P5	sw	-2.00*	0.13	0.00	-2.27	-1.73
	P1	-1.31*	0.13	0.00	-1.58	-1.04
	P2	-0.17*	0.13	0.02	-0.44	0.10
	P3	-0.34*	0.13	0.02	-0.61	-0.07
	P4	-1.54*	0.13	0.00	-1.81	-1.27

Note. [a]sw=source water. *the mean difference is significant at the 0.05 level.

GC-MS Analysis

Qualitative identification of organic compounds of each water sample is detailed in Table 7. Under GC-MS conditions and organic compound concentrations used in this study, 15 kinds of organic compounds were identified in the source water. Fewer chemicals were present in effluents P1-P5 than in the source water. Two chemicals, 1,2-Benzene dicarboxylic acid and Di-n-butyl phthalate, were found in all water samples. In contrast, some substances such as γ-Sitosterol and Tetracosane were found in treated water but not the source water. Figure 2 is a representative chromatogram of the source water, showing Dimethyl phthalate with a retention time of 9.42 min as one of the main components.

DISCUSSION

In general, there are two approaches to evaluate the biological effects of aquatic organics. One is to apply chemical analyses for direct detection of kinds and amounts of organic compounds which have previously been identified as strong biological effect substances, such as polychlorinated biphenyl (PCB), dioxins, 3-chloro-4-(dicholoromethyl)-5-hydroxy-2 (5H)-furanone (MX) or di-(2-ethylhexyl) phthalate (DEHP). However, the concentration of harmful compounds in water is usually too low to detect by standard methods for water quality established by WHO or other countries. The other is to evaluate the total biotoxicity of organic compound mixtures with short-term bioassays. In this study, we used the Ames test and recombined yeast estrogen screen to evaluate the mutagenic and estrogenic effects of source waters and effluents from different water treatment processes. The data show that both mutagenic and estrogenic effects of the source water were reduced in treated waters, which suggested that the water treatment processes were effective for the removal of some biotoxic substances.

Mutagenicity and Estrogenicity of Water Samples from Different Processes

The mutagenicity levels of the water samples were comparable to those in a prior study that applied different bioanalytical assessment methods[23]. A conclusion that can be drawn from the Ames tests is that the organic compounds in the water mainly caused frameshift mutations, as evidenced by positive results in the TA98 strain but negative results with strain TA100. After adding the S9 fraction, the mutagenic activity was significantly reduced (t=4.292, P<0.01), indicating such organic

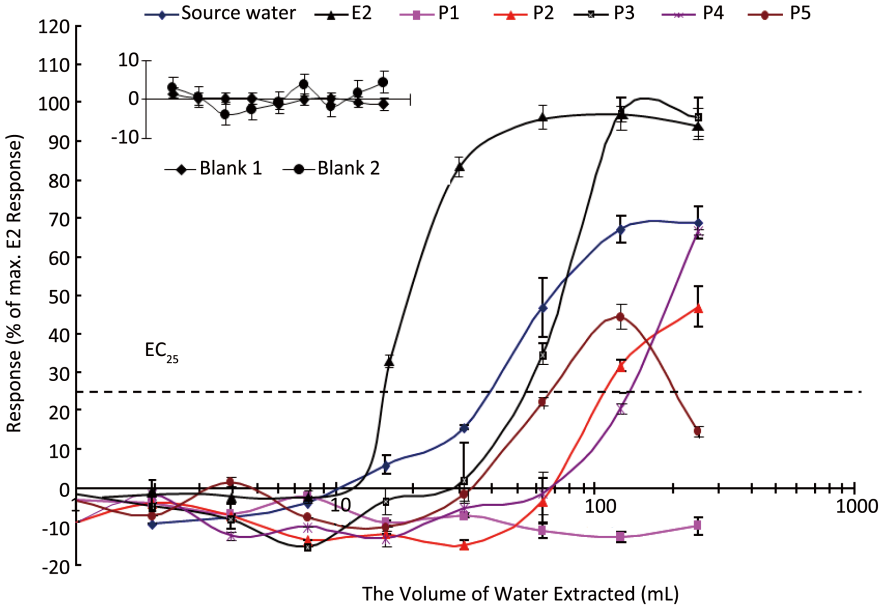

Figure 2. Estrogenic activity of organic compounds extracted from water samples and blanks tested by the yeast estrogen screen (YES). Arithmetic means and standard deviations (n=3) of absorbance data (A540 nm) are shown.

Table 7. Qualitative Results of the Organic Compounds in Source Water and Effluents from Process P1-P5 Identified by GC-MS

Compounds	GC Retention Time (min)	Present or Not in Samples					
		SW	1	2	3	4	5
Cyclohexanol	4.77	+	+	+	+		
Dimethyl phthalate	9.42	**+**	**+**	**+**	**+**	**+**	**+**
Nonadecane	9.80	+					+
Eicosane	10.15	+			+		+
Phenol	10.36	+	+				
Heneicosane	10.47	+	+	+	+		+
Hexadecanoic acid	10.49	+	+				
1-Hexadecene	10.52	+					
1,2-Benzene dicarboxylic acid	10.72	+	+	+	+	+	+
Tetradecane	10.77	+					
Docosane	10.78		+	+	+	+	+
1-Eicosanol	10.82	+			+		
Didodecyl phthalate	10.90	+					
γ-Sitosterol	10.95		+				+
Dibutyl phthalate	11.09	**+**	**+**	**+**	**+**	**+**	**+**
1-Octadecene	11.14	+	+	+	+	+	
Linoeic acid ethyl ester	11.17	+			+		
9,12-Octadecadienoic acid	11.22	+					
Tetracosane	11.37		+	+	+	+	
Cyclotetradecane	11.38	+					
ALLETHRIN	11.49	+			+		
Docosane	11.65	+	+	+		+	
Hexatriacontane	11.65					+	
1-Nonadecene	11.70	+					
Acetamide	11.73	+			+		
Fluazifop-P-butyl	11.82	+		+	+	+	+
Stigmasta-5,24(28)-dien-3-ol	11.89						+
Tricosane	11.92			+			
Cholest-5-en-30-ol	11.94		+				
2-Mercaptobenzothiazole	12.00	+			+		
Eicosane	12.20			+			
9-Octadecenamide	12.21	+					
Undecanone	12.24	+			+		
Octadecane	12.51			+	+		
Propiconazole	12.74	+		+		+	
2-Naphthalenamine	12.74			+	+		+
Propiconazole	12.77	+			+		+
Methanone	13.08	+					
Thiocyanic acid	13.08				+		
1H-indene	13.53	+					
9,9.11,12-Tetrahydro-2,3-dimethox	13.62			+	+		
9,9-Cyclolanostan-3-ol	13.98		+				
Acetamide	14.19	+					
Benzenamine	15.32					+	
1-chloro-4-deuteronapthalene	15.53					+	
2,7-Dimethyl-2[(E,E)-4',8',12'-tri	16.67	+					
Thiazolo[5,4-d]pyrimidine	18.04	+			+		
Stigmasterol	18.45	+			+		

compounds may be partly broken down or transformed into a less toxic substances. However, it cannot be ruled out that there may have been substances in the water responsible for indirectly causing frameshift mutations[24].

A one-way ANOVA was applied to determine the differences between the five treatment processes (Table 6). From the data, the five processes differed significantly from each other with respect to mutagenicity (all the *P* values are below 0.05). From Table 3, the mutagenicity of P5 (the only process that lacked a chlorination step), was the lowest of the processes, at all water extract doses. For P1-P4 effluents, mutagenicity may have been caused by the DBPs that formed as a consequence of chlorination, which is consistent with prior studies[23,25].

The estrogenic activity of source water was strongest, relative to P1-P5 effluents, as the source water had the lowest EC25-E2 (36 mL). After applying the different treatment processes, the estrogenicities decreased to different extents, indicating the potentially estrogenic compounds were removed by the treatment processes. However, some processes may have induced the formation of new compounds that have estrogenic potential. This may have been the case for P3. These results agreed with the study by Pereira et al.[26].

The Effects of Different Processes

The mutagenic effects for some samples did not correlate strictly with estrogenic effects; water with low mutagenicity could present high estrogenic effects. For example, the MR of effluent P1 was higher than the MR for effluents P2-P5, but no significant estrogenic effect was observed in P1. It is thus likely that most mutagenic and estrogenic compounds belong to different organic categories that cannot be removed effectively by the same treatment process. In this study, the water treatment processes we used did not produce ideal drinking water, which should have low biological effects (both low mutagenicity and estrogenic activity). However, Process 2 can be considered as a recommendable process (MR of 4 L extract <2, EC25-E2 =131 mL).

As mentioned before, the MR of effluent P5 (1.35, -S9) was lower than effluents from the treatment processes that included chlorine disinfection, which implied that use of chlorine can increase mutation risk. The MR (2.78, -S9) of effluent P1 was higher than effluent P2, P3, P5, which suggested that activated carbon was effective for removing mutagenic substances.

As an alternative disinfectant, the popularity of ozone, which is now extensively used in many countries, relies on strong oxidation with a low yield of byproducts. In our experience, ozone may play an important role in removing most organic compounds, although it still generates new byproducts[27-29]. Prior reports showed that the increase in mutagenicity following ozonation was still generally weaker and lower than with chlorine, suggesting that ozonation is a reasonable alternative to chlorination for minimizing mutagenicity of drinking water[30-31]. Pre-treatment with ozone had a profound influence on the subsequent treatment steps, particularly the coagulation step, which is an adjunct to pre-ozonation. However, the effects of pre-ozonation on removal of organics are still controversial according to some studies[27,32]. Based on the benefit of low-dosage ozone for removing organic matter, ozone was included as a pre-processing step in our study.

GAC/BAC filtration has been used in advanced water treatment for many years to control taste, odor and color of drinking water, and effectively remove mutagens. However, with prolonged use its absorbability and selectivity decline. Woo Hang Kim[33] found that BAC lost its absorbability after having been used for 20 months, because it was saturated with natural organic matter (NOM). In contrast, micropollutants with similar absorbability to that of phenol and bromophenol can still be removed by BAC even after a long period of operation and saturation by NOM. GAC appeared more effective for removing neutral rather than acidic mutagenic compounds[34-35]. Furthermore, in order to maintain adsorption and filtration efficacy, carbon columns may be back-flushed to loosen granules. This practice may release adsorbed organic compounds into the water used to back-flush. Although the initial back-flushing water was drained, the drain time or discharge capacity is hard to determine. In this study, the mutagenic effect of effluent P4 (MR=3.02) was higher than that of effluent P2 (MR=1.49), which implied a disadvantage of back-flushing.

Phthalates are widely used as plasticizers for polyvinyl chloride (PVC) resins and cellulose film coating. There are more than 60 kinds of phthalates produced and consumed for diverse purposes. Phthalates are considered to be EEs and may have toxic effects on reproductive development. Animal studies have proven that phthalates can cause

repetitive abortions and male sterility[36-39]. Pollution with phthalates is universal in water environments[40-43]. In this study, Dimethyl phthalate (DMP, RT=9.42) was presumed to be a main organic component in the source water from the Beijiang River. Besides, the results of GC-MS (Table 7) indicate that all effluents contain dimethyl phthalate and di-n-butyl phthalate, which could not be removed from the source water with any of the five processes used in this study.

The Biological Effects and the Contents

The combination of applying biological tests and instrumental analysis was an effective method for assessing water samples. In this study we applied the Ames tests to assess mutagenicity and the YES assay to evaluate estrogenicity of five combined water treatment processes, and GC-MS as an assistant method to detect specific compounds in the water samples. Under the GC-MS conditions described, the kinds and amounts of organic compounds identified in treated water were lower than that in source water (Table 7), which implied the positive effect of P1-P5. However, some substances such as γ-Sitosterol and Tetracosane were found in effluent (P1, P5) but not in source water, suggesting that water treatment processes add new compounds.

As mentioned above, P5 effluents showed the lowest mutagenicity. P5 didn't have a chlorination step whereas P1-P4 did, indicating that DBPs may have been formed during chlorination. However, the compounds shown in Table 7 were not typically associated with mutagenicity, and there were inconsistencies between the biological tests and GC-MS analysis. The latter may have been affected by various factors such as complicated pretreatment steps, detection limits and the stability of equipment. In contrast, the biological tests were effective for assessing the combined effects of mixed organic compounds, while GC-MS can hardly evaluate this[22].

With regard to estrogenicity, many kinds of phenols and phthalates were detected (Table 7). These compounds were potential environmental estrogens, with most of them detected in the source water and effectively removed by the treatment processes. The trend was consistent with the estrogenic activities presented by the YES assay.

CONCLUSIONS

The mutagenic and the estrogenic effect of source water could be decreased by all water treatment processes tested here, but to different extents. To decrease mutagenicity, processes P2 (preoxidation-GAC-NaClO), P3 (peroxidation-ozonation-BAC-NaClO), and P5 (peroxidation-GAC) would be better choices. To decrease estrogenic effects, process P1 would be the best. Generally, the effluent from P2 (ozone-GAC-NaClO) would be the ideal one as it appeared to have low biological effects overall. Some substances such as dimethyl phthalate, di-n-butyl phthalate, and 1, 2-Benzene dicarboxylic acid were very difficult to remove with any of the water treatment processes.

Detecting the compounds qualitatively and quantitatively and developing effective methods to remove these organic compounds will require further studies.

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