Comparison of extracts and toxicities of organic compounds in drinking water concentrated by single and composite XAD resins

Xue Zhou, Lunhui Xiang, Fenghong Wu, Xiaoling Peng, Hong Xie, Jiachun Wang, Kedi Yang, Wenqing Lu and Zhigang Wu

ABSTRACT

We compared extracts and toxicities of organic compounds (OCs) in drinking water concentrated by composite XAD-2/8 resin (mixed with an equal volume of XAD-2 and XAD-8 resins) with those extracted by single XAD-2 (non-polar) and XAD-8 (polar) resins. Drinking water was processed from raw water of the Han River and the Yangtze River in Wuhan section, China. The extraction efficiency of all resins was controlled at 30%. The types of extracted OCs were detected by gas chromatography–mass spectrometry, and the cytotoxicity and genotoxicity were assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and comet assays, respectively, in human hepatoma HepG2 cells. Our results showed that XAD-2/8 extracted a larger variety of OCs, compared with XAD-8 and XAD-2. The cytotoxicity and genotoxicity of extracted OCs were in the order of XAD-8> XAD-2/8> XAD-2 at almost all tested concentrations after 24 h treatment ($P < 0.05$). Our findings suggest that single XAD resin selectively extracts either polar or non-polar OCs, which would lead to over- or under-estimation of the toxicity of drinking water. Nevertheless, composite resin extracts both polar and non-polar OCs, and could be utilized as a useful extraction technique to evaluate the level and toxicity of OCs in drinking water.

Key words | cytotoxicity, drinking water, genotoxicity, HepG2 cells, organic compounds, XAD resin

Xue Zhou
Lunhui Xiang
Fenghong Wu
Xiaoling Peng
Hong Xie
Jiachun Wang
Kedi Yang
Wenqing Lu
Zhigang Wu (corresponding author)
State Key Laboratory of Environment Health (Incubation);
Key Laboratory of Environment and Health, Ministry of Education;
Key Laboratory of Environment and Health (Wuhan), Ministry of Environmental Protection;
Department of Occupational and Environmental Health,
School of Public Health,
Tongji Medical College,
Huazhong University of Science and Technology,
Wuhan 430030,
China
E-mail: *greatwuzg@sohu.com*

ABBREVIATIONS

BTEX	benzene, toluene, ethylbenzene, and xylenes
DDT	dichlorodiphenyltrichloroethane
DMEM	Dulbecco's minimal essential medium
DMSO	dimethyl sulfoxide
GC-MS	gas chromatography–mass spectrometry
HCHs	hexachlorocyclohexanes
LMPA	low melting point agarose
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
NMPA	normal melting point agarose
OCs	organic compounds
OCPs	organochlorine pesticides
PAEs	phthalate ethers
PAHs	polyaromatic hydrocarbons
PBDEs	polybrominated diphenyl ethers
PCBs	polychlorinated biphenyls
POPs	persistent organic pollutants

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INTRODUCTION

Due to industrial, agricultural, and domestic activities, natural surface waters are contaminated with a wide variety of organic compounds (OCs). Some of the OCs cannot be efficiently removed by conventional drinking water-purifying facilities, and have been detected in finished drinking water (Stackelberg *et al.* 2004, 2007). The presence of these OCs in drinking water has raised a growing public health

concern: the potential mutagenic, genotoxic, or carcinogenic effects to human bodies associated with long-term exposure to the OCs, even if their levels in drinking water are relatively low (Loper 1980; Buschini *et al.* 2008; Cantor 2010). Additionally, disinfection by-products, which are unintentionally created when disinfectants react with naturally occurring organic matter in source water, can induce adverse effects, such as bladder cancer, early-term miscarriage, birth defects, and respiratory diseases (Cantor 2010; Richardson & Postigo 2012). Therefore, it is of crucial importance to monitor the levels of OCs in drinking water and evaluate their mutagenicity, genotoxicity, and carcinogenicity.

Since OCs in drinking water are generally present at low to mid ng/L levels (Stackelberg *et al.* 2004; Benotti *et al.* 2009), which are usually under the detection limit of analytical instruments, they need to be concentrated before chemical analysis and toxicological test. XAD resins are non-ionic, macroporous polymers with a significant large surface area, and are effective in concentrating various trace OCs (Hewitt & Marvin 2005). According to the nature of the surface, XAD resins can be divided into polar and non-polar categories. XAD resins have been used to effectively extract OCs dissolved in water (Maurice *et al.* 2002), and the organics adsorbability depends on the category of the resin. For instance, XAD-2, a non-polar macroporous resin, has been shown to extract hexachlorocyclohexanes (HCHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs), and polyaromatic hydrocarbons (PAHs) in surface freshwater (Carroll *et al.* 2008; Wang *et al.* 2011). XAD-8, a polar macroporous resin, has been reported to extract phenol, esters, alkanes, aromatics, chlorine pesticides, fulvic acid, and humic acid in drinking water (Huang *et al.* 2004). Accordingly, XAD resins have been extensively used in screening water for the presence of mutagenic or genotoxic OCs (Guzzella *et al.* 2002; Takigami *et al.* 2002; Lemos *et al.* 2009; Alam *et al.* 2010; Kanno *et al.* 2010; Lee *et al.* 2010). In most of these studies, only single XAD resin was employed to extract OCs from water. Since single XAD resin selectively adsorbs either polar or non-polar OCs, it is inappropriate to assess the organic pollution level and their toxicity using one XAD resin to extract OCs. A resin that is composed of both polar and non-polar XAD resins may be utilized as an extraction technique to fully evaluate the level and the toxicity of OCs in water.

The Yangtze River, over 6,300 km long, is the longest river in China. The Han River is a tributary of the Yangtze River and merges into the Yangtze River at Wuhan, the capital of Hubei province in central China. However, severe contamination with organic pollutants, such as persistent organic pollutants (POPs) including OCPs, PAHs, PCBs, and phthalate ethers (PAEs) has been found in the Yangtze River (Liu *et al.* 2000; He *et al.* 2011). The Han River was also found to be contaminated with OCs, such as OCPs (Zhou *et al.* 2011). The Yangtze River and the Han River are the main drinking water sources of Wuhan. Consequently, contamination of drinking water processed from the two rivers with OCs becomes a serious public health problem.

The primary objective of this study was to explore the potential use of composite resin XAD-2/8 (mixed with an equal volume of XAD-2 and XAD-8 resins) to assess the level and the toxicity of OCs dissolved in drinking water in comparison with single XAD-2 and XAD-8 resin. For this purpose, gas chromatography-mass spectrometry (GC-MS) analysis was applied to analyze the types of extracted OCs, and the cytotoxicity and genotoxicity of extracted OCs were assessed by MTT and comet assays, respectively.

MATERIALS AND METHODS

Chemicals and materials

Amberlite™ XAD-2 and XAD-8 resins were purchased from Sigma (St Louis, MO, USA). Normal melting point agarose (NMPA), low melting point agarose (LMPA), and dimethyl sulfoxide (DMSO) were obtained from Amresco (Solon, OH, USA). Dulbecco's minimal essential medium (DMEM) and fetal calf serum were obtained from Gibco (Grand Island, NY, USA).

Water sampling

Drinking water (150 L) was collected from water treatment plants of the Han River and the Yangtze River in Wuhan, China in July 2008.

Extraction of OCs

Before serving as sorbents, XAD resins were pre-cleaned thoroughly with distilled water, followed by washing with diethyl ether, methyl cyanide, and methanol in Soxhlet, each for 8 h. The resins were then kept in methanol before use.

Extracting OCs from drinking water by XAD resins was according to the method described previously with slight modifications (Wilcox & Williamson 1986). Briefly, 30 L of drinking water was passed through glass chromatography columns filled with 100 mL XAD resins at a flow rate of 10 mL/min. Since UV-254 absorbance can be employed as an indicator of the concentrations of OCs in water, drinking water was measured by UV spectrophotometry at 254 nm before and after flowing through the columns. The extraction efficiency of the resins was calculated according to the following formula: extraction efficiency = (BOD-AOD)/BOD × 100%, where BOD and AOD represent the optical densities of drinking water before and after flowing through the columns, respectively. To make the amount of OCs extracted by different resins comparable, the extraction efficiency of all resins was controlled at 30% by flowing the water samples through the columns repeatedly. The XAD extracts were then eluted with methyl alcohol, acetone, ether, and methylene chloride in sequence, each with 100 mL. The eluate was concentrated to 10 mL by a rotary evaporator (Model Laborota 4000; Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) and condensed into a ceroleinlike state in a vacuum dry oven (Model DZF-6051; Shanghai Jinhong Instrument Co., Ltd, Shanghai, China) at 37 °C. The XAD extracts were stored at 4 °C before testing.

GC-MS analysis

The XAD extracts were dissolved in N-hexane (HPLC grade) and centrifuged at 2,000 rpm for 10 min. The supernatant was analyzed by a 6890N-5975B GC-MS system (Agilent, Santa Clara, CA, USA). The GC-MS parameters are summarized in Table 1.

Table 1 | GC-MS parameters

Parameter	Scale
Column	HP-5 MS, 30 m × 250 m × 0.25 μm
Injected volume	1 μL
Oven temperature program	
Initial temperature (°C)	40
Initial hold time (min)	5
Rate (°C/min)	4
Final temperature (°C)	280
Final hold time (min)	5
Carrier gas	Helium
Column flow rate (mL/min)	1
Interface temperature (°C)	280
Mass range (amu)	50–600
Ionization mode	EI
Electron energy (eV)	70
Scanner mode	Full-scan

Cell culture

Human hepatoma cell line HepG2 was kindly provided by Dr Firouz Darroudi (Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden, The Netherlands). The cells were cultured in DMEM at 37 °C in an incubator with a humidified atmosphere containing 5% CO_2. DMEM was supplemented with 10% fetal calf serum and 100 U/mL penicillin/streptomycin.

MTT assay

The cytotoxicity of XAD extracts was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, HepG2 cells were seeded in 96-well plates at 1×10^4 cells/well. Twenty-four hours after plating, the cells were treated with XAD extracts at concentrations corresponding to 10, 30, 50, and 100 mL drinking water per mL of culture medium for 24 h. The control cells were cultured in the presence of DMSO (final concentration <0.1%) and blank wells contained culture medium with no cells. Twenty microliters of MTT solution (5 mg/mL) was added to each well containing 100 μL culture medium at the end of treatment. After 4 h incubation at 37 °C, formazan crystals were dissolved with 100 μL DMSO and the absorbance was read at 490 nm using a microplate reader (Model 680; Bio-Rad, Hercules, CA, USA). The experiment was performed in six replicate wells. The percentage of the

viability of the cells was calculated according to the following formula: cell viability (%) = $(A490_{treated}\text{-}A490_{blank})/(A490_{control}\text{-}A490_{blank}) \times 100\%$. Results were expressed as percentage of control.

Comet assay

The comet assay was carried out according to the method described previously with slight modifications (Tice *et al.* 2000). Briefly, HepG2 cells were treated with XAD extracts at concentrations corresponding to 1, 10, and 30 mL drinking water per mL of culture medium for 24 h. Control cells were cultured in the presence of DMSO (final concentration <0.1%). Microscope slides were coated with 1% NMPA and 0.5% LMPA. Cells (3×10^4) were suspended in 80 μL 0.7% LMPA and rapidly layered onto the pre-coated slide. The slides were allowed to solidify, then immersed in freshly prepared lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, and 10% DMSO, pH 10) and incubated at 4 °C for 1.5 h. From that point on, all the steps were performed in the dark to prevent additional DNA damage. The slides were then placed randomly in a horizontal gel electrophoresis tank, which was filled with freshly prepared alkaline buffer (1 mM EDTA and 300 mM NaOH, pH >13) at 4 °C for 20 min. Thereafter, electrophoresis (0.7 V/cm, 300 mA) was conducted at 4 °C for 20 min. After electrophoresis, the slides were soaked in a neutralizing buffer (0.4 M Tris, pH 7.5) at 4 °C for 10 min. The slides were immersed in 100% methanol for 5 min and air-dried. Finally, the slides were stained with ethidium bromide (20 μg/mL). The comets were scored using an Olympus fluorescent microscope equipped with a 515–560 nm excitation filter, a 590 nm barrier filter, and a charge-coupled device camera. For each treatment group, 100 randomly selected cells (50 cells per replicate slide) were analyzed by CASP software.

Statistical analysis

Statistical analysis was performed using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). The data, presented as means ± SD, were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. The results were considered statistically significant at $P < 0.05$.

RESULTS

GC-MS analysis of XAD extracts

OCs in drinking water supplied from different sources (the Han River and the Yangtze River) were extracted by XAD-2, XAD-8, and XAD-2/8 resins. XAD extracts were analyzed by GC-MS, which is considered to be one of the most promising techniques for water analysis regarding its high sensitivity to OCs. As shown in Table 2, XAD extracts contained alkanes, alkenes, halogenated hydrocarbons, fatty acids, alcohols, aldehydes, ketones, ethers, phenols, lipids, phthalates, BTEX (benzene, toluene, ethylbenzene, and xylenes), amines, and heterocyclics. Our results showed that the types of OCs extracted by XAD-8 resin (72 in the Han River and 50 in the Yangtze River) were more than those extracted by XAD-2 resin (43 in the Han River and 39 in the Yangtze River). Meanwhile, composite XAD-2/8 resin could extract more types of OCs (110 in the Han

Table 2 | Classifications of XAD extracts of drinking water supplied from the Han River and the Yangtze River

	Han River			Yangtze River		
Classifications of organic compounds	**XAD-2**	**XAD-8**	**XAD-2/8**	**XAD-2**	**XAD-8**	**XAD-2/8**
Alkanes	21	34	41	12	17	20
Alkenes	4	11	10	5	0	3
Halogenated hydrocarbons	2	4	4	2	3	3
Fatty acids	0	1	3	2	2	3
Alcohols	0	2	11	0	1	2
Aldehydes	0	0	1	0	1	1
Ketones	0	1	7	0	3	6
Ethers	1	1	2	0	0	0
Phenols	2	2	2	3	2	5
Lipids	5	6	14	4	11	14
Phthalates	5	4	8	4	2	11
BTEX[a]	0	0	0	2	4	3
Amines	1	4	4	4	1	3
Heterocyclics	2	2	3	1	3	5
Total	**43**	**72**	**110**	**39**	**50**	**79**

[a]BTEX (benzene, toluene, ethylbenzene, and xylenes). The results of XAD-2/8 extracts were previously published (Xiang *et al.* 2010a).

River and 79 in the Yangtze River) than XAD-8 and XAD-2 resins, almost equal to the sum of them.

Cytotoxicity of XAD extracts

The cytotoxicity of XAD extracts from drinking water processed from raw water of the Han River (Figure 1(a)) and the Yangtze River (Figure 1(b)) in HepG2 cells was evaluated by MTT assay. The extraction efficiency of the XAD resins was controlled at 30%. Our results showed that XAD extracts induced cytotoxicity in a dose-dependent manner in HepG2 cells. XAD-8 extracts were more toxic than XAD-2 extracts at all treated concentrations for drinking water supplied from both rivers ($P < 0.05$). Moreover, the cytotoxicity of XAD-8 extracts was greater than that of XAD-2/8 extracts at concentrations corresponding to 30, 50, and 100 mL water sample per mL of culture medium for the Han River samples, and at concentrations corresponding to 50 and 100 mL water sample per mL of culture medium for the Yangtze River samples ($P < 0.05$). Compared with XAD-2 extracts, XAD-2/8 extracts were more toxic at the highest concentration for the Han River samples, and at all treated concentrations for the Yangtze River samples ($P < 0.05$). In summary, the order of increase in cytotoxicity in HepG2 cells was: XAD-8 extracts > XAD-2/8 extracts > XAD-2 extracts.

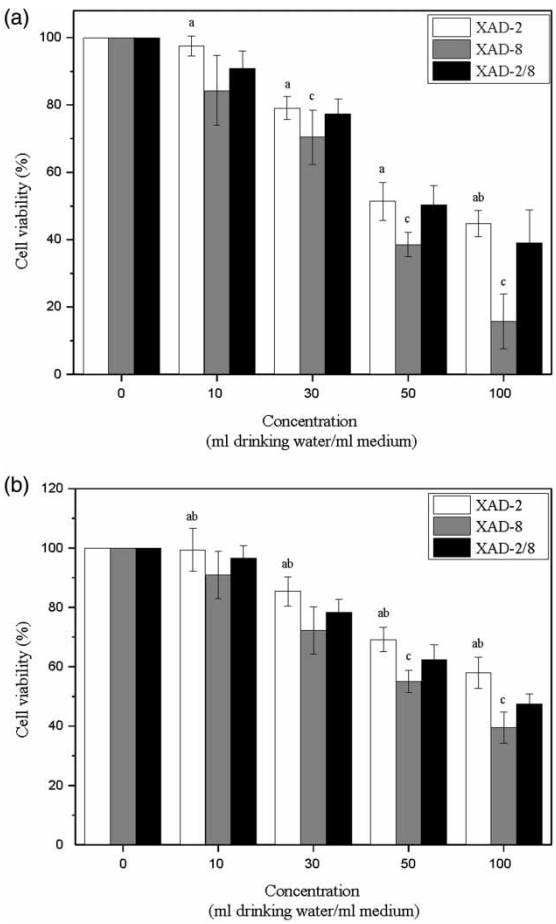

Figure 1 | Cytotoxicity of HepG2 cells treated with XAD-2, XAD-8, and XAD-2/8 extracts from drinking water processed from raw water of the Han River (a) and the Yangtze River (b). The cells were treated with XAD extracts at concentrations corresponding to 10, 30, 50, and 100 mL drinking water per mL of culture medium for 24 h. Results are expressed as the mean (±SD) of sextuples. [a]XAD-2 compared with XAD-8 at the same concentrations, $P < 0.05$; [b]XAD-2 compared with XAD-2/8 at the same concentrations, $P < 0.05$; [c]XAD-8 compared with XAD-2/8 at the same concentrations, $P < 0.05$.

Genotoxicity of XAD extracts

The genotoxicity of XAD extracts from drinking water processed from raw water of the Han River (Figure 2(a)) and the Yangtze River (Figure 2(b)) in HepG2 cells was examined by comet assay, which was widely used to detect genotoxic activity of water (Lu *et al.* 2004; Buschini *et al.* 2008). The extent of DNA damage was evaluated by Olive tail moment. The extraction efficiency of the XAD resins was controlled at 30%. Compared with control, DNA damage was significantly induced by XAD extracts in a dose-dependent manner in HepG2 cells. The DNA damage induced by XAD-8 extracts was more significant than that induced by XAD-2 extracts at all treated concentrations for drinking water supplied from the Han River and the Yangtze River ($P < 0.05$). Moreover, XAD-8 extracts induced more DNA damage than XAD-2/8 extracts at concentrations corresponding to 10 and 30 mL water sample per mL of culture medium for drinking water supplied from both rivers ($P < 0.05$). Furthermore, XAD-2/8 extracts induced more DNA damage than XAD-2 extracts at all treated concentrations for the Han River samples and at concentrations corresponding to 1 and 30 mL water sample per mL of culture medium for the Yangtze River samples ($P < 0.05$). In summary, the order of increase in DNA-damaging potency was: XAD-8 extracts > XAD-2/8 extracts > XAD-2 extracts, which is similar to that of the genotoxicity results.

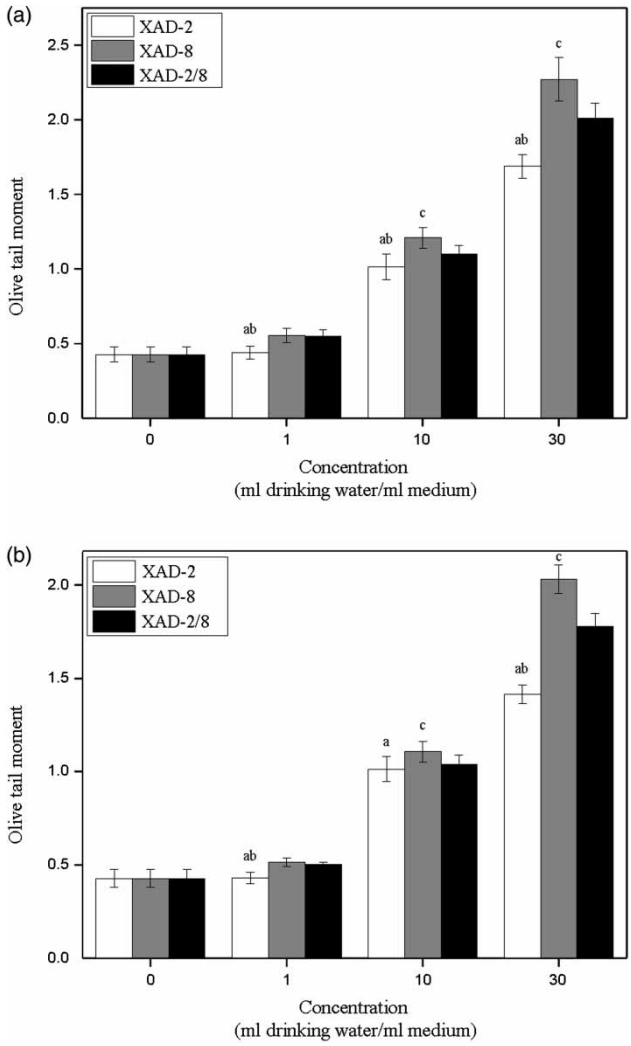

Figure 2 | Olive tail moment of HepG2 cells treated with XAD-2, XAD-8, and XAD-2/8 extracts from drinking water processed from raw water of the Han River (a) and the Yangtze River (b). The cells were treated with XAD extracts at concentrations corresponding to 1, 10, and 30 mL drinking water per mL of culture medium for 24 h. Results are expressed as the mean (±SD) of 100 randomly selected cells (50 cells per replicate slide). [a]XAD-2 compared with XAD-8 at the same concentrations, $P < 0.05$; [b]XAD-2 compared with XAD-2/8 at the same concentrations, $P < 0.05$; [c]XAD-8 compared with XAD-2/8 at the same concentrations, $P < 0.05$. The results of XAD-2/8 extracts were previously published (Xiang *et al.* 2010b).

DISCUSSION

In the present study, we compared the types of OCs in drinking water extracted by composite XAD-2/8 resin with those extracted by single XAD-2 and XAD-8 resins. Our results showed that single XAD resin only selectively and incompletely adsorbed OCs from drinking water. Composite XAD-2/8 resin was able to extract more types of OCs than single XAD-2 and XAD-8 resins, almost equal to the sum of the other two resins, suggesting that XAD-2/8 resin extracts a broader range of OCs from drinking water than single XAD-2 and XAD-8 resin.

Due to adsorption limitations, XAD resins are not able to completely extract all OCs from water, and different XAD resins have different extraction efficiencies. To the best of our knowledge, the extraction efficiencies of XAD resins are rarely reported, which make it impossible to compare the toxicity of XAD extracts among these studies. Our results showed that the static saturated extraction efficiencies of XAD-2, XAD-8, and XAD-2/8 resins were 67.8, 54.1, and 62.8%, respectively (data not shown). In our study, the extraction efficiencies of all XAD resins were all controlled at 30%. Therefore, the cytotoxic and genotoxic results of different XAD extracts were comparable.

Several studies have been conducted to investigate the genotoxicity of OCs from drinking water processed from raw water of the Yangtze River. For example, Lu *et al.* (2004) found that drinking water extracts could induce a significant increase in DNA migration and micronuclei frequencies in human hepatoma HepG2 cells. DNA damage was also detected in human peripheral blood lymphocytes treated with organic concentrates from drinking water originating from the Yangtze River (Li *et al.* 2006). However, a single resin (XAD-7 or XAD-2) was applied to extract OCs in these researches, which has been proved to selectively adsorb OCs from source water in our study. Thus, the results cannot reflect the actual genotoxic activity of drinking water.

The equal mixture of XAD-4 (a non-polar resin) and XAD-8 have been used to concentrate OCs from surface water to investigate the genotoxic activity of the water samples (Aleem & Malik 2003, 2005; Alam *et al.* 2010). However, the effectiveness of the combination of XAD-4/8 resin in concentrating genotoxic OCs has not been compared with that of XAD-4 or XAD-8 resin. In the present study, we used composite XAD-2/8 resin to extract OCs to test the cytotoxic and genotoxic activities of drinking water. The cytotoxicity and genotoxicity of XAD-2/8 extracts were compared with those of XAD-2 and XAD-8 extracts in HepG2 cells. HepG2 cell line, which expresses many xenobiotic metabolizing enzymes, has been proved

to be a suitable model for genotoxicity testing (Knasmuller *et al.* 2004). The cytotoxic and genotoxic effects were caused by the combined effect of all extracted OCs from drinking water. Both the cytotoxicity and genotoxicity of XAD-2, XAD-8, and XAD-2/8 extracts were in the following order: XAD-8 > XAD-2/8 > XAD-2. These results indicated that the polar extracts were more cytotoxic and genotoxic than the non-polar extracts. It is conceivable that any bias of adsorption to either polar or non-polar OCs extracted by a single resin would lead to over- or under-estimation of the toxicity of OCs in water. Therefore, it is not appropriate to use OCs extracted by a single XAD resin to assess the toxicity of OCs dissolved in water. Alternatively, composite XAD-2/8 resin can be used because it could more efficiently extract both polar and non-polar OCs in water.

CONCLUSION

Single XAD-8 or XAD-2 resin extracts less variety of OCs compared with composite XAD-2/8 resin, and selectively extracts either polar or non-polar OCs, which would lead to over- or under-assessment of the toxicity of drinking water. Therefore, composite XAD resins could be utilized as a useful extraction technique to evaluate the toxicity of OCs in water. When the toxicity of XAD extracts from different water sources are compared, the extraction efficiency should be kept the same. Otherwise, the toxicity would be incomparable due to different amounts of OCs adsorbed by different resins. Cytotoxicity and genotoxicity were detected in HepG2 cells treated with OCs extracted from drinking water using the Yangtze River and the Han River as source water. Therefore, special attention should be paid to the safety of the drinking water.

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