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Contribution of brominated organic disinfection by-products to the mutagenicity of drinking water

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Abstract The activity inducing chromosomal aberrations of the mixture of brominated disinfection by-products (DBPs) was approximately three times higher than that of the chlorinated counterparts for the same hypohalous acid dose. With the combination of chromosomal aberration test and a new analytical technique to differentiate total organic chlorine (TOCl) and total organic bromine (TOBr), it was found that TOBr was correlated to the mutagenicity of chlorinated waters. It was also implied that for a bromide-to-TOC ratio of 0.1 (mg/mg C), brominated DBPs could account for at least 29% of the total toxicity of DBPs formed during chlorination. On the other hand, bromate ion, a major ozonation DBP, was not a major contributor to the activity inducing chromosomal aberrations of the water treated with an ozone/chlorine sequential process. Therefore, ozonation is one possible option to reduce the health risk caused by DBPs even in the presence of bromide.

Keywords Brominated disinfection by-products; chlorination; chromosomal aberration test; ozonation

Introduction

When a source water is chlorinated in drinking water treatment process, not only chlorinated disinfection by-products (DBPs) but also brominated DBPs are produced. Naturally occurring bromide ion (Br^-) in source waters is easily oxidized to hypobromous acid (HOBr) (Pinkernell *et al.*, 2001), and then HOBr reacts with natural organic matter (NOM) to form brominated compounds (Richardson *et al.*, 1999a). These brominated DBPs have been gathering more attention recently because studies have suggested that small brominated compounds such as bromoacetic acids are much more mutagenic to mammalian cells than their chloro counterparts (Plewa *et al.*, 2002). Also, Nobukawa and Sanukida (2001) demonstrated that genotoxicity of chlorinated water greatly increases with Br^- using Ames test and micronuclei formation assay.

However, while the above results imply the importance of controlling brominated DBPs during drinking water treatment processes, many questions on the contribution of brominated DBPs to the toxicity of finished water remain unanswered. One of these questions is the relative toxicity of the mixture of brominated DBPs to that of chlorinated DBPs. Past studies only consisted of comparative studies on simple DBPs or toxicity evaluation of a mixture that contained both chlorinated and brominated DBPs. It is of practical importance to quantitatively identify raw water characteristics and treatment conditions with which the contribution of brominated DBPs is of concern.

Ozonation is commonly used to decompose the precursors of chlorinated DBPs (Richardson *et al.*, 1999b). Studies have suggested that preozonation can destroy reaction sites in NOM for chlorinated DBP formation, and reduce the toxicity of finished water (Patterson *et al.*, 1995). However, little information is available on the effect of Br^- on the toxicity of water treated by an ozone/chlorine sequential process. Also, the relative toxicity of ozonation DBPs in the presence of Br^- (e.g., bromate ion (BrO_3^-)) to the entire toxicity of finished water is not fully understood.

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The main objective of this study is to answer the two unanswered questions mentioned above. This study consisted of three sets of experiments. First, chromosomal aberration test was performed to compare the mutagenicity of brominated DBPs with that of chlorinated DBPs. These two mixtures were separately prepared by the reactions between humic acid and corresponding hypohalous acids (i.e., HOBr and hypochlorous acid (HOCl)), so that the activity inducing chromosomal aberrations of brominated and chlorinated DBPs could be evaluated separately. In the second part of this study, total organic bromine (TOBr) and total organic chlorine (TOCl) of the mixtures of DBPs formed during chlorination in the presence of Br^- were measured by the combination of a combustion furnace and an ion chromatograph, and were compared to the results of chromosomal aberration test. With this new analytical technique, it was possible to determine the contribution of TOBr and TOCl to TOX. Lastly, the activity inducing chromosomal aberrations of the DBPs formed during the ozone/chlorine sequential treatment both with and without Br^- was evaluated. Of particular concern was the effect of pH during ozonation on the toxicity and the contribution of BrO_3^-, an ozonation DBP, to the total toxicity of finished water.

Experimental

Sample preparation

Humic acid solution. Concentrated humic acid solutions (TOC = 750–1,000 mg/L) were used as model NOM solutions to perform the chromosomal aberration test without concentrating samples after reactions. The concentrated humic acid solutions were prepared by dissolving approximately 3 g of humic acid (Aldrich) in 1 L of 0.01 N NaOH solution for 24 hr with vigorous mixing, readjusting pH to 7.0, and filtering through a glass fiber filter (GS25, Advantec).

HOBr solution. To minimize the effect of liquid bromine ($Br_{2(aq)}$), a bromide-free HOBr solution for direct bromination was prepared by oxidizing Br^- (0.23 M KBr solution) with a NaOCl solution (Wako). The concentration of the stock solution was measured by direct UV absorbance at 266 nm (Beckwith *et al.*, 1996). A commercial NaOBr solution could not be used for this purpose because it contains approximately equal amount of Br^- and thus $Br_{2(aq)}$ was not negligible at a high dose around pH7.0 (Snoeyink and Jenkins, 1980). Similar consideration was not necessary for HOCl solution because the equilibrium constant for hydration is much lower than that for $Br_{2(aq)}$.

Chlorination and bromination. Chlorination and bromination were performed by adding NaOCl solution (Wako) or the HOBr stock solution to the mixture of the concentrated humic acid solution, a phosphate buffer solution (final concentration = 50–175 mM), and a KBr (Wako) solution (if necessary). The samples were stored in dark place at 20°C for 24 hours before chromosomal aberration test and chemical analysis unless otherwise noted.

Ozone/chlorine sequential treatment. Concentrated humic acid solutions (pH 6.0, 7.0 or 8.0) were ozonated in an impinger (inner volume = 30 mL) in semi-batch mode. Ozone was generated from ultrapure oxygen using a Mitsubishi OS-1N-A ozone generator. The flow rate of ozone was 1.5 mg/min. After ozonation, the pH of the solution was readjusted to 7.0 with NaOH or H_2SO_4, and the solution was chlorinated following the procedure descried above. The readjustment of pH was necessary to evaluate the effect of pH during ozonation.

Chromosomal aberration test

Chromosomal aberration test using Chinese hamster lung cells (CHL/IU, Dainihon Pharmaceutical) was performed to evaluate the initiating activity in the carcinogenesis process of humic acid solutions treated by chlorination, bromination, and an ozone/chlo-

rine sequential treatment. The details of the test procedure is described elsewhere (Itoh *et al.*, 1996). The number of abnormal cells was counted by visual observation under a microscope. For each sample, 100 metaphases were analyzed. The ratio of the sample volume to the media volume was fixed to 1/6. The incubation time after contacting with sample solutions was 24 hr. To minimize the decomposition of DBPs, no quenching reagent was added to the samples since chlorine and bromine concentrations were less than the concentration range where cytotoxicity on CHL cells is significant.

Chemical analysis

TOX analysis. TOX (TOBr+TOCl) was analyzed by following the Standard Methods (1995). A Mitsubishi Chemical TOX-10Σ TOX analyzer was used for the analysis.

Differentiation of TOBr and TOCl. The detail of the procedure is described elsewhere (Echigo *et al.*, 2000). But, briefly, the off gas from a TOX furnace that contains HCl and HBr corresponding to TOCl and TOBr was trapped into distilled water, and these Cl^- and Br^- were separately quantified by an ion chromatograph. A TOX analyzer (TOX-10Σ, Mitsubishi Chemical) was used as a furnace. A Shimdzu VP-10 system was used for ion chromatographic analysis.

Bromate analysis. Bromate ion concentration was determined by ion chromatography with a post-column derivatizing method (Wagner *et al.*, 1999). Samples were diluted prior to the analysis.

Results and discussion

Activity inducing chromosomal aberrations of the brominated and chlorinated DBPs

The activity inducing chromosomal aberrations of the mixture of brominated DBPs was approximately three times higher than that of chlorinated DBPs at the same doses of hypohalous acids (Figure 1). For example, at 750 mg Cl/L, the number of abnormal chromosomes was 60 per 100 cells for the mixture of brominated DBPs, while the number for chlorinated DBPs were 23 per 100 cells. Two explanations are possible for this result. One is the difference of the toxicity between brominated DBPs and chlorinated DBPs on molar basis (i.e. TOX basis). The other one is the difference of the amount of halogenated compounds produced by the two hypohalous acids. To test these hypotheses, the number of

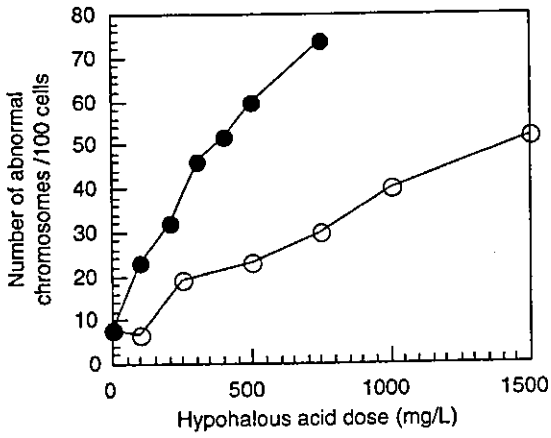

Figure 1 Comparison of the activity inducing chromosomal aberrations of brominated DBPs from the reaction between HOBr and humic acid with that of chlorinated DBPs from the reaction between HOCl and humic acid. Conditions: humic acid concentration, 1,000 mg C/L; reaction time, 24 hours; temperature, 20°C. The ratios of the hypohalous acid doses to TOC were similar to a chlorine-to-DOC ratio in actual drinking water treatment practice

chromosomal aberrations per unit TOX (mmol) was calculated (Table 1). From this result, it can be clearly seen that the activity inducing chromosomal aberrations of the mixture of brominated DBPs per unit TOX is roughly two to six times higher than that of chlorinated DBPs, while the TOX formation per unit hypohalous acid addition was almost identical for HOBr and HOCl. Thus, it can be said that the mixture of the brominated DBPs is more toxic than that of chlorinated DBPs.

With this difference on the magnitude of the activity inducing chromosomal aberrations between brominated DBPs and chlorinated DBPs shown in Table 1, rough estimation of the contribution of brominated DBPs to the total mutagenicity is possible for a model raw water. For example, Echigo *et al.* (2000) reported that the TOBr-to-TOX ratio of a model chlorinated water (Br^- = 100 μg/L; chlorine dose = 4.0 mg Cl/L; DOC = 3.0 mg/L) was approximately 7%. Hence, assuming the effect of all the DBPs are additive and the chemical structures of both NOMs are similar, it is estimated that brominated DBPs account for roughly 12–30% of the activity inducing chromosomal aberrations of the model drinking water.

Activity inducing chromosomal aberrations of chlorination DBPs in the presence of Br^-

Effect of Br^- concentration on the activity inducing chromosomal aberrations of chlorinated water. The implication from the comparison of the toxicity of brominated DBPs and chlorinated DBPs led us to investigate more realistic situations. That is, chlorination in the presence of Br^-. Figure 2 illustrates that with increasing Br^- concentration, the number of abnormal chromosomes increases. This tendency was in agreement with the results of Ames assay and micronuclei formation test (Nobukawa and Sanukida, 2001). Also, this increase of the number of abnormal chromosomes corresponded to the formation of TOBr (Figure 3).

For the initial Br^- concentration of 100 mg/L (Br^-/TOC = 0.1 mg/mg), the number of abnormal chromosomes was 1.4 times higher than that without Br^-. Hence, at least 29% (= 0.4/1.4) of the activity inducing chromosomal aberrations could be attributed to brominated DBPs under this condition. This percentage fell in the range estimated from the relative activity inducing chromosomal aberrations of brominated DBPs and chlorinated DBPs given in the previous section and the TOBr and TOCl data (17–39%).

Assuming that TOC of a typical source water is 2 mg/L, the corresponding Br^- concentration to the Br^-/TOC ratio = 0.1 is 200 μg/L. Average Br^- concentration in the United States is 100 μg/L (Westerhoff *et al.*, 1998), and 200 μg/L is relatively high but not uncommon. Serious attention should be paid to brominated DBPs for the raw waters that contains Br^- above this level. Also, a linear interpolation suggested a 7–20% increase of chromosomal aberrations for the Br^-/TOC ratio of 0.05 (100 μg/L for TOC = 2 mg/L) compared to the number of abnormal cells without Br^-.

Table 1 Activity inducing chromosomal aberrations of the reaction products of the reaction between HOBr and humic acids and those between HOCl and humic acid on TOX basis. See the caption of Figure 1 for the reaction conditions

Halogenating reagent	Dose (mg Cl/L)	TOX (mg Cl/L)	TOX/dose (mg/mg)	Number of abnormal chromosomes per TOX (count/100 cells mmol)
HOBr	100	14.4	0.14	56.7
	200	21.3	0.11	53.3
	500	60.8	0.12	35.0
HOCl	250	35.0	0.14	19.3
	750	97.1	0.13	11.0
	1,500	185.7	0.12	9.9

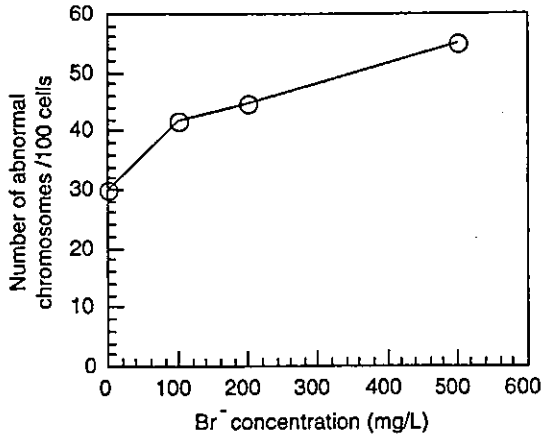

Figure 2 Effect of initial Br^- concentration on the activity inducing chromosomal aberrations of chlorinated humic acid solution. Conditions: humic acid concentration, 1,000 mg C/L; reaction time, 24 hours; temperature, 20°C; chlorine dose, 1,500 mg/L

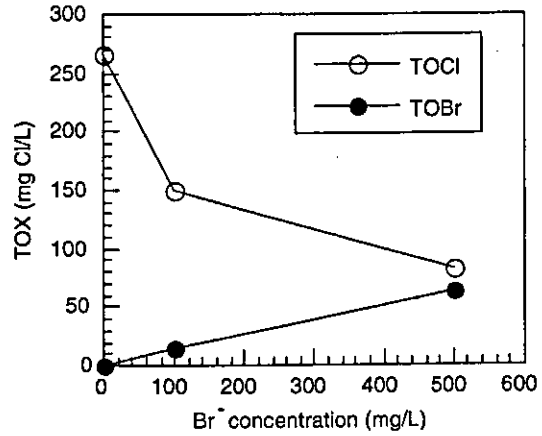

Figure 3 Effect of initial Br^- concentration on the distribution of TOBr and TOCl during chlorination of a concentrated humic acid solution. See the caption of Figure 2 for reaction conditions

The effect of reaction time on the activity inducing chromosomal aberrations was also investigated (Figure 4). The number of abnormal chromosomes was highest after 24 hours, and then decreased regardless the initial concentration of Br^-. After 96 hours, the numbers of abnormal chromosomes were almost identical with and without Br^-. This result appears to be in agreement with a general observation that hydrolysis of brominated compounds is faster than chlorinated counterparts (Larson and Weber, 1994).

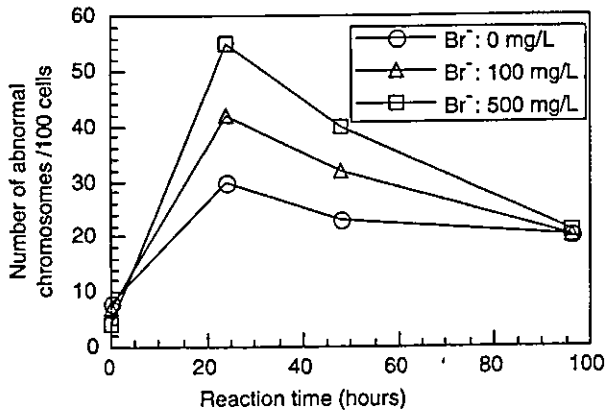

Figure 4 Effect of reaction time on the activity inducing chromosomal aberrations of chlorinated humic acid solution. Conditions: humic acid, 1,000 mg C /L; temperature, 20°C; chlorine dose, 1,500 mg/L

Competition between HOBr and HOCl to humic acid. In Figure 5, the ratio of Br^- to HOCl and the ratio of TOBr to TOX are compared. The latter was higher than the former, though certain reaction time is required for the formation of HOBr before reacting with NOM. If the reactivities of HOBr and HOCl to humic acid are similar, the Br-to-HOCl ratio should be less than the TOBr-to-TOX ratio (i.e., below the dashed line in the figure). Thus, bromination of humic acid by HOBr has to be kinetically preferential to chlorination. Indeed, it is known that the reactions between phenolic compounds and HOBr are much faster than those with HOCl (Echigo and Minear, 2001; Gallard *et al.*, 2003). Also, it was indicated that bromination of humic acid is faster than chlorination (Qualls and Johnson, 1983; Echigo, 2002).

Effect of preozonation on the activity inducing chromosomal aberrations of chlorinated humic acid

The effect of preozonation on the chlorinated humic acid with and without Br^- is shown in Figure 6. Regardless the presence of Br^- and pH (6–8), the number of abnormal chromosomes decreased with ozonation. Therefore, the ozone/chlorine sequential treatment is an effective way to improve the safety of finished water with and without Br^- compared to chlorination.

The effect of pH during ozonation (note: chlorination was conducted at pH7.0) was not as evident as the pH effect during chlorination (Kowbel *et al.*, 1984). But the number of abnormal chromosomes was slightly higher at higher pH, especially with Br^-. The first possible explanation would be the contribution of BrO_3^- since in general higher BrO_3^- formation is observed for higher pH (Pinkernell *et al.*, 2001). However, comparing BrO_3^- concentration range (0.0–1.1 mg/L) in this experiment with a reported dose-response curve

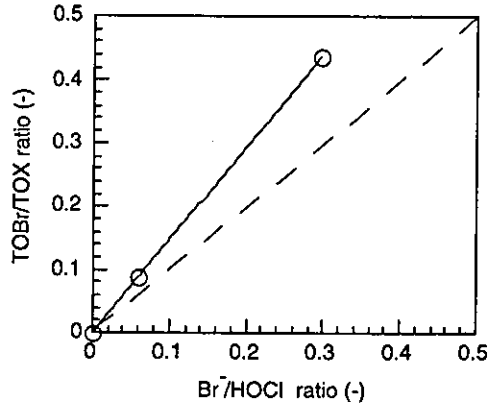

Figure 5 Comparison of the Br^--to-HOCl ratio with the TOBr-to-TOX during chlorination of a concentrated humic acid solution. See the caption of Figure 4 for the reaction conditions. Dashed line is the hypothetical line for Br^-/HOCl = TOBr/TOX

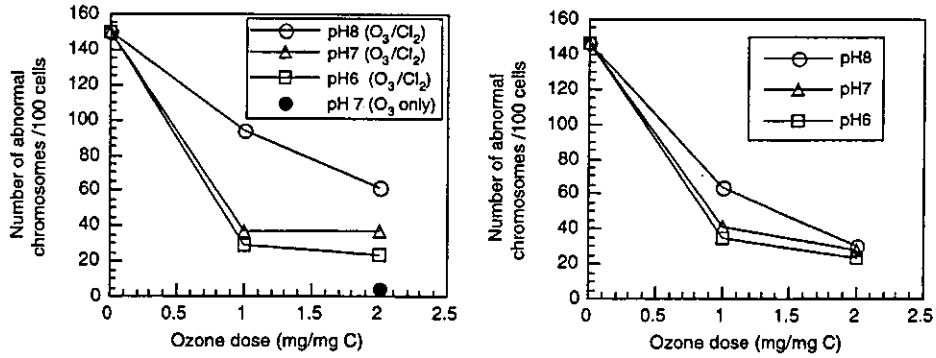

Figure 6 Effect of ozonation on the activity inducing chromosomal aberrations of chlorinated humic acid solution with (left, Br^-/TOC = 0.05 mg/mg) and without (right) Br^-. Conditions: humic acid concentration, 750 mg DOC/L; reaction (chlorination) time, 24 hours; temperature, 20°C; chlorine dose, 1,500 mg/L

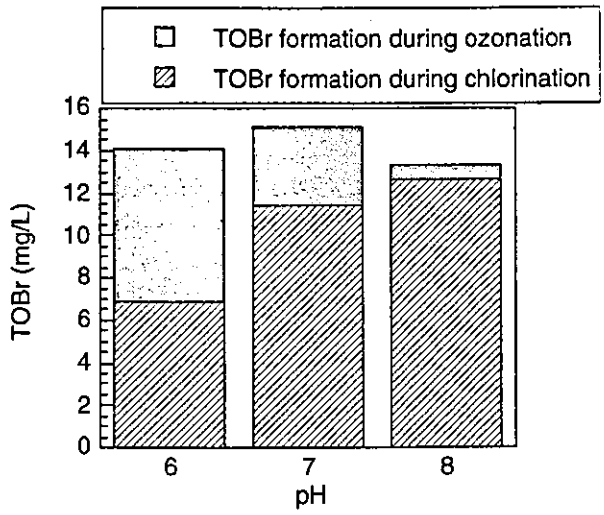

Figure 7 Effect of pH on the formation of TOBr during the ozone/chlorine sequential treatment. Ozone dose was 2 mg/mg C. See the caption of Figure 6 for other reaction conditions

(Life-science Information Center, 1998), it was implied that BrO_3^- could not be a major contributor to the activity inducing chromosomal aberrations of this solution. Also, the contribution of reaction products during ozonation (i.e., without chlorine addition) was small (see the solid circle in Figure 6 (left)). Thus, main contributors to the activity inducing chromosomal aberrations of the water treated with the ozone/chlorine sequential treatment are the DBPs formed during the chlorination stage.

TOX formation decreased by approximately 20% with preozonation (data not shown in figures). Also, while no clear pH dependence was observed for TOX and TOBr formed in the ozone/chlorine sequential treatment, the TOBr formed during the chlorination stage in the ozone/chlorine sequential treatment did depend on pH and higher TOBr observed for high pH (Figure 7). This may be related to the difference of the activity inducing chromosomal aberrations for different pH during ozonation.

Conclusions

This study demonstrated that brominated organic DBPs can be major contributors to the activity inducing chromosomal aberrations of the finished water. Other major findings from this study are listed below.

- The activity inducing chromosomal aberrations of the reaction products of the reaction between humic acid and HOBr was two to six times higher than those between humic acid and HOCl on TOX basis.
- For a bromide-to-TOC ratio of 0.1 (mg/mg), brominated DBPs accounted for at least 29% of the activity inducing chromosomal aberrations of DBPs formed during chlorination.
- With increasing initial Br^- concentration, both TOBr and the number of abnormal chromosomes increased. That is, TOBr formation corresponded to the toxicity of the chlorinated water.
- With the differentiation technique between TOBr and TOCl, it was implied that bromination of humic acid is dominant over chlorination in the initial phase of chlorination process.
- Bromate ion, a major ozonation DBP, was not a major contributor to the activity inducing chromosomal aberrations of the water treated with the ozone/chlorine sequential treatment.
- Ozonation is a possible option to reduce the health risk caused by chlorination DBPs even in the presence of Br^-.

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Determination and quantitation of sulfonylurea and urea herbicides in water samples using liquid chromatography with electrospray ionization mass spectrometric detection

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Abstract

A multianalyte method has been developed for the confirmation and quantitation of five sulfonylureas, bensulfuron-methyl, imazosulfuron, pyrazosulfuron-ethyl, flazasulfuron and halosulfuron-methyl, and for three ureas, siduron, dymron (daimuron) and diuron (DCMU) in water. Samples were extracted from water by off-line solid-phase extraction (SPE) with a polystyrene polymer cartridge (PS2), an ODS C_{18}-bonded silica cartridge (C_{18}) and an *N*-vinylpyrrolidone polymer cartridge (Oasis). Analyte determination and quantitation were performed by liquid chromatography with mass spectrometry (LC–MS). Extraction efficiency experiments demonstrated the ability of this method to extract sulfonylureas and ureas from water samples. Confirmatory analysis was carried out by LC-electrospray mass spectrometry (LC–ESI–MS) instrumentation equipped with a single-quadrupole mass filter. MS data acquisition was performed by a single or two-ion selected ion monitoring (SIM) program. It is required for confirmation that LC–MS retention times of the analytes are within 1% of the retention times of the standards, and that the molecular ion or characteristic fragment ion is present for each analyte. Fragment ions from distinctive structures must be obtained to identify and characterize specific herbicide molecules. These were obtained by controlled decomposition of sulfonylurea and urea adduct ions after suitably adjusting the electrical field in the desolvation chamber. The eight herbicides were also measured in fortified pure water (water purified by a milli-Q system), tap water and river water. Average recoveries of the eight analytes from water samples were in the range of 70–120% with relative standard deviations (R.S.D.s) of <20%. The limit of quantitation (LOQ) for each of the eight herbicides was between 10 and 100 ng l^{-1}.

Keywords: Sulfonylurea herbicides; Urea herbicides; LC–ESI–MS; SPE; Water samples

1. Introduction

Herbicides are used in the management of rice paddies, golf courses, and other types of fields. They are carried into the surrounding environment by aquifers, and are widely distributed in the environment. There is concern that agricultural chemicals may have an adverse influence on ecosystems, and that their use can result in contamination of tap water. Sulfonylurea herbicides were first introduced in 1982 by DuPont Agricultural Products. Sulfonylureas are labile and weakly acidic compounds. Compared to other older herbicides, sulfonylureas and ureas are used in much lower concentrations and are degraded in soil more rapidly. Therefore, very low concentrations (parts-per-billion) of these herbicides are to be expected in the water supply. We carried out an analysis of these compounds in water using the analytical methods of liquid chromatography with ultraviolet detector (LC–UV) [1–7], capillary electrophoresis with ultraviolet detector (CE–UV) [8–11], LC with mass spectrometry (MS) [12–19], immunoassay [20,21], bioassay [22,23] and radio immunoassay [3]. Sulfonylureas and ureas are generally not directly amenable to separation by gas chromatography (GC) because of their extremely low volatility and thermal instability. GC has been used in conjunction with diazomethane derivatization [24–26], pentafluorobenzyl bromide derivatization [27], and hydrolysis followed by analysis of the aryl sulfonamides [28]. Sulfonylureas and ureas can be separated using reversed-phase high-performance liquid chromatography (HPLC), but they are not readily identified by standard HPLC detectors. These substances generally do not have

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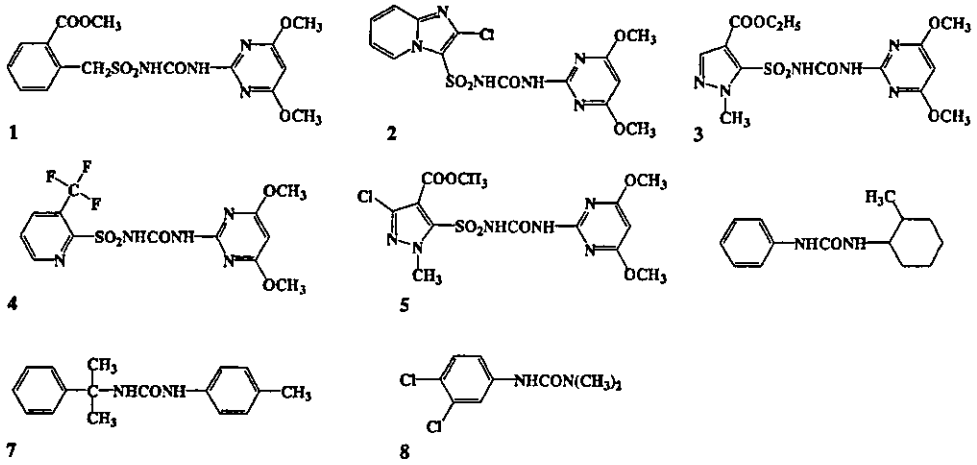

Fig. 1. Structures and common names of the eight herbicides. 1: Bensulfuron-methyl; 2: imazosulfuron; 3: pyrazosulfuron-ethyl; 4: flazasulfuron; 5: halosulfuron-methyl; 6: siduron; 7: dymron; 8: diuron (DCMU).

extremely strong UV or visible light absorption that would allow selective and high-sensitivity detection. For confirmatory and quantitative trace analysis of compounds having thermal instability or extremely low volatility, such as the compounds of interest in this study, the use of LC–MS is required. LC–MS offers chromatographic separation without thermal decomposition, high sensitivity, mass selectivity and structural information. There is increasing demand for accurate detection and characterization of herbicides, in order to evaluate their levels in the environment. Therefore, the establishment of an accurate multicomponent analysis method is of practical importance.

The herbicides investigated here are in widespread use in Japan, but there have been few reports describing measuring methods or estimating quantities of these herbicides found at different locations. Therefore, it is necessary to quickly develop suitable analytical methods, which must have high selectivity and high sensitivity, for the identification and measurement of compounds containing sulfonylureas and ureas. We have developed a new LC–MS methodology for quantitation of these herbicides in various kinds of water, such as tap water, and raw water, etc.

There have also been few reports concerning recovery rates obtained by evaluating the differences in an SPE cartridge. We report here the results of analysis of water samples, including pure filtered water, tap water and river water by an SPE using PS2, C_{18} and Oasis, and using LC–ESI–MS in the positive ionization mode.

2. Experimental

2.1. Chemicals

Common names and structures of the five sulfonylureas and three ureas evaluated here are shown in Fig. 1. Analytical grade standard bensulfuron-methyl (99.7%), imazosulfuron (99.7%), pyrazosulfuron-ethyl (99.9%), halosulfuron-methyl (100%), siduron (98.9%), dymron (100.0%) and diuron (100.0%) were purchased from Wako Osaka, Japan. Analytical grade standard flazasulfuron (99.9%) was purchased from Hayashi, Osaka, Japan.

Acetonitrile and methanol used as HPLC grade solvents were purchased from Wako Osaka, Japan. Acetic acid was purchased from Wako. Deionized water used for sample preparation and LC mobile phase was prepared by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. LC–ESI–MS analysis

LC was carried out using an Agilent 1100 series (Agilent, Waldbornn, Germany) instrument equipped with a Rheo-

Table 1
SIM Conditions for monitoring five sulfonylureas and three ureas in water, along with retention times

Compound	M_r	Channel mass		Retention time (min)
	MW	*m/z*	Composition	
Bensulfuron-methyl	410.4	411	M + H	12.396
		412	M + 2H	
Imazosulfuron	412.8	415	M + 3H	12.621
Pyrazosulfuron-ethyl	414.4	415	M + H	14.846
		437	M + Na	
Flazasulfuron	407.3	408	M + H	12.611
		409	M + 2H	
Halosulfuron-methyl	434.8	435	M + H	15.494
		457	M + Na	
Siduron	232.3	233	M + H	13.978
		255	M + Na	14.406
Dymron	268.4	269.1	M + H	16.171
		291.1	M + Na	
Diuron (DCMU)	233.1	233	M + H	10.924
		255	M + Na	

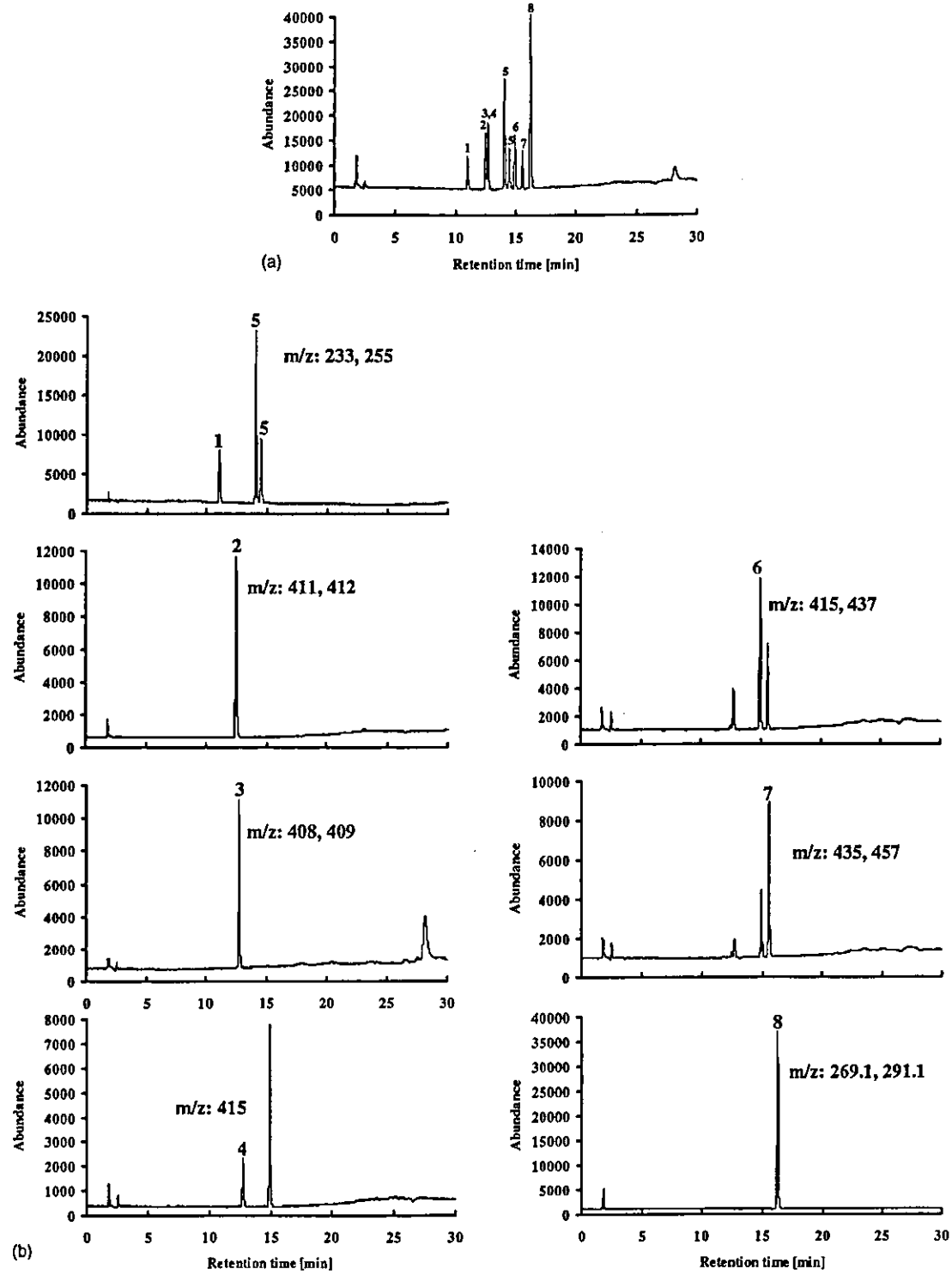

Fig. 2. (a) Chromatogram of a standard solution by LC–ESI–MS using selected ion monitoring (SIM). (b) Signals for each ion. The concentration of each compound was 0.02 mg l^{-1} in acetonitrile:water = 1:9 (v/v). Peaks—1: diuron (DCMU); 2: bensulfuron-methyl; 3: flazasulfuron; 4: imazosulfuron; 5: siduron; 6: pyrazosulfuron-ethyl; 7: halosulfuron-methyl; 8: dymron.

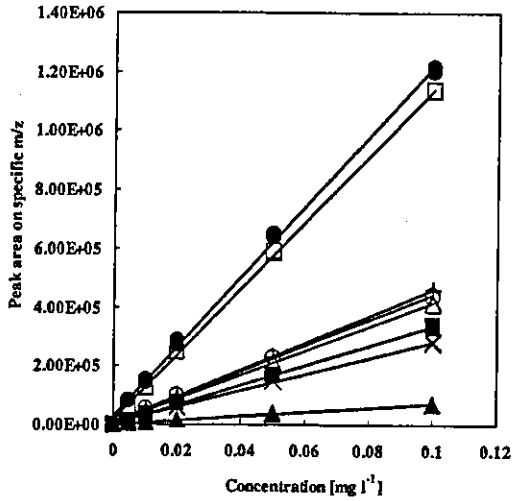

Fig. 3. Calibration curves of eight herbicides analyzed by LC–ESI–MS, with detailed signals shown for each ion. (○) Bensulfuron-methyl; (▲) imazosulfuron; (△) pyrazosulfuron-ethyl; (+) flazasulfuron; (■) halosulfuron-methyl; (□) siduron; (●) dymron; (×) diuron (DCMU).

dyne Model 7750 injector. The analytical column was a Zorbax Eclipse XDB-C_{18} (Agilent) 250 mm × 4.6 mm, 5 μm particle size. The mobile phases were 0.15% (v/v) acetic acid (A) and 100% acetonitrile (B). The herbicides were separated with the following gradient program: maintaining 60% A for 5 min; followed by a linear gradient from 60% A at $t = 5$ min to 20% A at $t = 20$ min; maintaining 20% A for 10 min and returning linearly to 60% A in 7 min. These LC conditions were described by Rodriguez and Orescan [18]. The column temperature was 40 °C, the flow-rate was 1.0 ml min^{-1} and the injection volume was 25 μl.

The MS system was an Agilent 1100 series (Agilent) quadrupole equipped with an electrospray ionization (ESI) source. The instrument was operated in the positive ionization mode. The operating conditions for ESI were nebulizer gas (nitrogen) 414 KPa; drying gas (nitrogen) flow 10.0 l min^{-1}; capillary voltage 4000 V and gas temperature 350 °C. The fragmentor voltage was kept at 120 V.

Table 2
Linear regression data for the eight herbicides investigated by LC–ESI–MS [calibration range 0, 0.005–0.1 mg l^{-1} (six data points)]

Compound	$y = bx + c$	r^2	LOD[a] (mg l^{-1})
Bensulfuron-methyl	$y = 4 \times E10^6\ x + 7688.7$	0.9992	0.005
Imazosulfuron	$y = 701937\ x + 1441.4$	0.9982	0.005
Pyrazosulfuron-ethyl	$y = 4 \times E10^6\ x + 4243.2$	0.9997	0.005
Flazasulfuron	$y = 5 \times E10^6\ x + 4884.8$	0.9996	0.005
Halosulfuron-methyl	$y = 3 \times E10^6\ x + 1447.6$	0.9997	0.005
Siduron	$y = 1 \times E10^7\ x + 11506$	0.9996	0.005
Dymron	$y = 1 \times E10^7\ x + 27683$	0.9984	0.005
Diuron (DCMU)	$y = 3 \times E10^6\ x + 2438$	0.9995	0.005

[a] LODs were evaluated by using a signal/noise (3) ratio of more than 3.

2.3. Preparation of standard solutions

Individual stock solutions (100 mg l^{-1}) of each analytical standard were prepared in acetonitrile. Next, the analytical standard mixtures were prepared by diluting each herbicide stock solution with acetonitrile to a final concentration of 12.5 mg l^{-1}. These stock solutions were stored at 4 °C. The 12.5 mg l^{-1} standard stock solution was appropriately diluted to prepare of five working standards; 0.005, 0.01, 0.02,

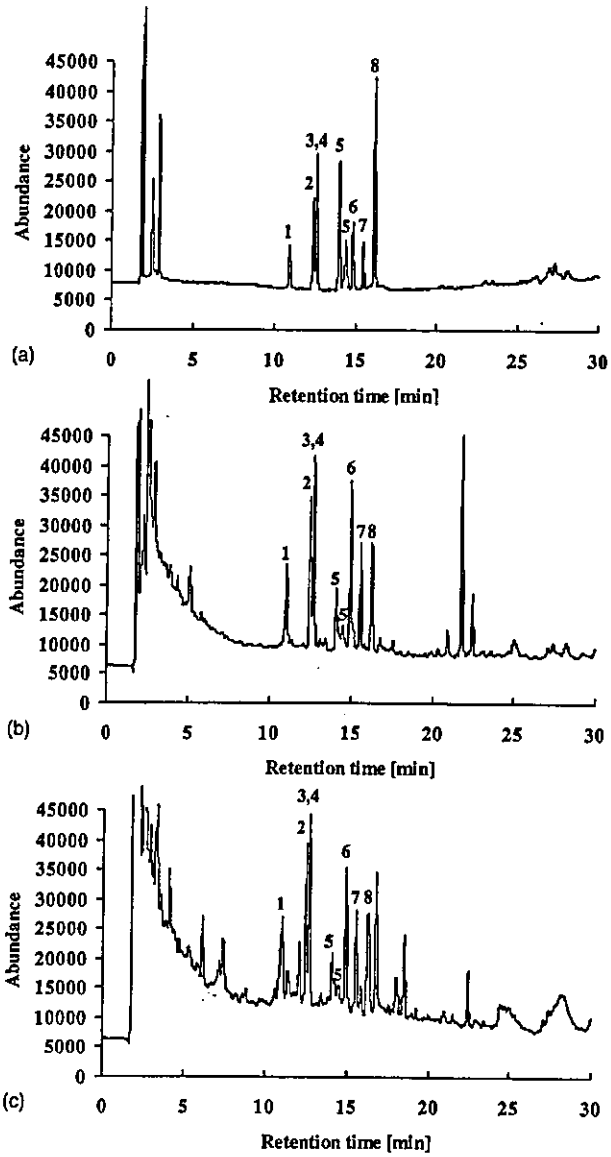

Fig. 4. Chromatogram of water sample fortified with the eight herbicides, using the LC–ESI–MS selected ion monitoring (SIM). (a) 40 ng l^{-1} in fortified pure water, (b) 100 ng l^{-1} fortified tap water (except for siduron and dymron, which were fortified to 20 ng l^{-1}) and (c) 100 ng l^{-1} fortified river water (except for siduron and dymron, which were fortified to 20 ng l^{-1}) Eight herbicides were concentrated by Oasis. Peaks—1: diuron (DCMU); 2: bensulfuron-methyl; 3: flazasulfuron; 4: imazosulfuron; 5: siduron; 6: pyrazosulfuron-ethyl; 7: halosulfuron-methyl; 8: dymron.

Table 3
Average recoveries, relative standard deviations (precision) and limits of quantitation (LOQs) on extracting five sulfonylureas and three ureas from pure water

Compound		20 ng l^{-1} [a]			40 ng l^{-1} [a]		
		Recovery (%)[b]	R.S.D. (%)	LOQ (ng l^{-1})	Recovery (%)[b]	R.S.D. (%)	LOQ (ng l^{-1})
Bensulfuron-methyl	PS2	93.8	1.5	2.8	106.8	0.8	3.3
	C_{18}	99.0	0.6	1.2	104.9	1.6	6.5
	Oasis	110.6	2.6	5.7	98.4	1.4	5.5
Imazosulfuron	PS2	81.4	0.4	0.6	105.0	3.0	12.5
	C_{18}	95.8	5.0	9.6	108.8	2.3	10.1
	Oasis	95.4	0.3	0.6	93.0	2.9	10.8
Pyrazosulfuron-ethyl	PS2	45.2	4.0	n.d.	72.8	1.3	3.7
	C_{18}	92.5	1.6	2.9	98.5	0.4	1.7
	Oasis	108.5	0.3	0.6	91.9	1.7	6.2
Flazasulfuron	PS2	31.9	1.8	n.d.	65.3	1.0	n.d.
	C_{18}	65.2	1.2	n.d.	72.3	0.2	0.7
	Oasis	90.2	1.6	2.9	89.1	0.4	1.5
Halosulfuron-methyl	PS2	70.9	2.8	3.9	108.6	3.5	15.3
	C_{18}	95.7	5.7	11.0	103.1	1.2	4.8
	Oasis	104.7	2.4	5.1	96.2	3.2	12.3
Siduron	PS2	102.8	1.4	2.8	113.5	0.8	3.7
	C_{18}	96.7	0.6	1.1	105.6	0.4	1.8
	Oasis	125.5	2.2	n.d.	111.7	1.1	4.7
Dymron	PS2	105.1	1.8	3.8	119.7	1.7	8.3
	C_{18}	101.0	2.1	4.3	115.8	1.0	4.8
	Oasis	109.8	3.7	8.1	107.3	0.5	2.3
Diuron (DCMU)	PS2	88.1	2.1	3.8	94.9	0.5	2.0
	C_{18}	88.6	2.9	5.1	94.8	0.3	1.1
	Oasis	87.7	2.7	4.7	91.0	0.9	3.2

n.d.: not determined.
[a] Fortified with pure water.
[b] Mean values from three determination.

0.05, and 0.10 mg l^{-1}. These were then used to analyze the fortified samples. The final solvent composition of the working standard solutions was 10:90 (acetonitrile:water).

2.4. Calibration procedure

The external standard method of calibration was used for this analysis. At least five standard solutions containing all eight compounds were analyzed, and calibration plots of the peak area as a function of the concentrations of analytes injected were linear over the range of 0, 0.005–0.10 mg l^{-1} in an automated sequence. Calibration curves were analyzed by LC–ESI–MS in selected ion mode (SIM mode), followed by detection of the signal from one or two of the more abundant daughter ions. These daughter ions were identified in scan mode during the acquisition of the mass spectrum of the selected ion. The injection was performed three times for each sample to test reproducibility.

2.5. Water samples

In this report, three types of water were analyzed: pure water, tap water and river water. The "pure" water was Milli-Q water. The tap water samples were collected from the tap in the laboratory. L(+)-ascorbic acid sodium salt (Wako) was added to tap water to 0.005% (w/v), and this eliminated the chlorine, which can react with and degrade some of the compounds of interest. River water samples were collected from the Tama river near Tokyo. The river water was filtered before use.

2.6. Analytical procedure

For recovery studies, three types of water samples (0.5 l of pure water, tap water and river water) were fortified with 1 ml of the composite standard solution. Afterwards, the fortified water samples were concentrated with the solid phase extraction (SPE) method as described previously [29]. The SPE was performed with PS2 cartridges that were prepacked with polystyrene polymer resin (Sep-Pak Plus PS2 Cartridges; PS2), C_{18} cartridges prepacked with ODS C_{18}-bonded silica resin (Sep-Pak Plus C_{18} Cartridges; C_{18}), and Oasis cartridges prepacked with *N*-vinyl-pyrrolidone polymer resin (Oasis HLB Plus Extraction Cartridges), all from Waters (Milford, MA, USA). The SPE mode was used for the pure water, tap water and river water samples to preconcentrate

a mixture of five sulfonylureas and three ureas. 0.5 l of each sample was extracted on the SPE cartridges packed with PS2, C_{18} and Oasis. Solid-phase cartridges were equilibrated with 5 ml acetonitrile (except OASIS which was conditioned with 5 ml methanol) then with 5 ml deionized water (Milli-Q water). Extraction of water samples was carried out at a 10 ml min^{-1} flow rate. After passing the sample through the cartridges, the cartridges were washed with 10 ml deionized water at a 5 ml min^{-1} flow rate. Air was then pulled through the cartridge for 40 min. The analytes were eluted from the cartridges with 3 ml acetonitrile (except Oasis, where 3 ml methanol was used) at a rate of 1–2 drops s^{-1}. After evaporating the samples to near dryness under a gentle nitrogen stream, the compounds were transferred into a final volume of 1.0 ml of a mixture of acetonitrile–water (ratio10:90).

3. Results and discussion

3.1. LC–ESI–MS analysis

The LC–ESI–MS system was used for quantitative analysis of water samples. The use of high-flow pneumatically assisted ESI run in positive mode is a soft ionization technique used for LC–MS applications. It is a superior interface for analysis of sulfonylurea and urea herbicides. In the present study, all of the herbicides chosen for investigation could be picked up by this detector. Selection of one or two ions for investigation was scheduled according to the following protocols, as detailed in Table 1. A typical chromatogram for the 0.02 mg l^{-1} standard solution containing the eight target analytes is shown in Fig. 2a. Although five of the eight herbicides were well separated, bensulfuron-methyl, imazosulfuron and flazasulfuron were not well separated in this chromatogram. Five sulfonylurea and three urea herbicides were detected by LC–ESI–MS in the selected ion monitoring (SIM). This was followed by extraction of the signal from one or two of the most abundant daughter ions. These were acquired in full scan mode during the acquisition of the mass spectrum of the selected ion. The ions used for SIM for each compound are summarized in Table 1. Each ion gave a strong ion signal with positive mode ESI, but imazosulfuron gave only a weak ion signal. Because bensulfuron-methyl will also be detected by *m/z* 413, which indicates the strong ion of imazosulfuron, the peaks of these compounds overlap, and it is difficult to quantitate each of

Table 4
Average recoveries, relative standard deviations (precision) and limits of quantitation (LOQs) on extracting five sulfonylureas and three ureas from tap and river water

Compound		Tap water (0.5 l)[a]			River water (0.5 l)[a]		
		Recovery (%)[b]	R.S.D. (%)	LOQ (ng l^{-1})	Recovery (%)[b]	R.S.D. (%)	LOQ (ng l^{-1})
Bensulfuron-methyl	PS2	39.2	1.0	n.d.	95.9	0.8	7.8
	C_{18}	72.2	1.1	7.8	64.3	0.6	n.d.
	Oasis	90.8	1.0	9.2	93.2	0.7	6.7
Imazosulfuron	PS2	61.4	3.1	n.d.	98.8	1.6	15.7
	C_{18}	73.8	5.3	38.3	75.6	0.9	6.6
	Oasis	88.6	0.5	4.5	82.7	3.4	27.7
Pyrazosulfuron-ethyl	PS2	47.8	5.0	n.d.	45.3	1.6	n.d.
	C_{18}	20.8	2.1	n.d.	9.1	2.1	n.d.
	Oasis	112.9	1.6	18.1	86.4	0.6	5.0
Flazasulfuron	PS2	33.3	1.5	n.d.	51.5	0.5	n.d.
	C_{18}	28.8	0.3	n.d.	29.2	0.7	n.d.
	Oasis	100.2	0.9	8.9	98.6	0.4	3.8
Halosulfuron-methyl	PS2	55.9	9.2	n.d.	63.2	11.4	n.d.
	C_{18}	27.5	1.6	n.d.	16.1	3.0	n.d.
	Oasis	81.4	2.5	20.4	81.8	3.0	24.5
Siduron	PS2	98.5	2.7	5.2	104.5	3.2	6.7
	C_{18}	95.9	1.5	2.9	95.0	1.6	3.1
	Oasis	108.1	3.7	8.0	110.2	0.6	1.4
Dymron	PS2	116.4	2.7	6.3	94.5	0.6	1.2
	C_{18}	112.2	2.8	6.4	89.3	3.6	6.5
	Oasis	108.0	1.6	3.6	80.9	1.2	2.0
Diuron (DCMU)	PS2	123.6	2.2	n.d.	124.8	2.2	n.d.
	C_{18}	131.1	1.1	n.d.	133.5	3.3	n.d.
	Oasis	115.6	0.8	9.3	107.2	6.1	65.7

n.d.: not determined.
[a] Fortified with 100 ng l^{-1}, except for siduron and dymron fortified with 20 ng l^{-1}.
[b] Mean values from three determination.

the compounds. Therefore, a peak found at *m/z* 415 was chosen for detection, even though the area under this peak was smaller, because it was possible to get better quantitative determinations without complicating peak overlaps. The compound siduron gave two peaks, because it had two isomeric forms. The area under the two peaks was calculated and summed to give the total amount of siduron in the samples. Fig. 2b shows an extracted ion chromatogram for a standard solution.

The concentration of acetic acid in mobile phase will change during the gradient elution. However, the separation behavior is dependent on the affinity on the surface of the solid phase according to the concentration of the organic solvent in mobile phase. Therefore, the change of the concentration of acetic acid might not become a problem for the analysis of the herbicides shown in this paper. The quantitation and reproducibility have been obtained under this method.

3.2. Calibration curves and limit of detection (LOD)

Calibration curves were established for the eight substances, each of which was analyzed at five different concentrations. Standard solutions containing 0, 0.005, 0.01, 0.02, 0.05, and 0.10 mg l^{-1} were analyzed by LC–ESI–MS in the SIM mode. Examples of calibration curves for the eight analytes are shown in Fig. 3. Linear regression data are shown in Table 2. The calibration curves were linear in the concentration range from 0 to 0.10 mg l^{-1} and the correlation coefficients were higher than 0.998 for all of the herbicides studied. This indicated that the methodology developed in this work performed well in quantitating these compounds.

The LODs were defined as three-times of standard deviation (S.D.) on the basis of three independent determinations. The LODs can be obtained using this methodology due to the high selectivity and sensitivity of the LC–MS system. The LODs using this method are 0.005 mg l^{-1} in standard solution for all herbicides.

3.3. Limit of quantitation (LOQ)

This method was validated using pure water, tap water, and river water. The standard mixture solution was added to pure water, tap water or river water, respectively, and the recovery by three kinds of solid phases, i.e. PS2, C_{18}, and Oasis, was examined using each 0.5 l sample. Water samples were fortified with five selected sulfonylurea and three selected urea herbicides. Water samples with 20 and 100 ng l^{-1} of each compound were prepared. Pure water samples were fortified with both 20 and 40 ng l^{-1} of each compound, while tap water and river water were fortified with 100 ng l^{-1} of each compound, with the exception of siduron or dymron, which were added to a final concentration of 20 ng l^{-1}. All samples were concentrated to 500-fold. Typical chromatograms of fortified pure water, tap water and river water samples are shown in Fig. 4. Average recoveries and relative standard deviations (R.S.D.s) are summarized in Tables 3 and 4.

LOQ was the lowest fortification level evaluated. The methods were used for determining with acceptable recoveries and precisions (70–120% and R.S.D. $\leq$ 20%, respectively). The LOQ is defined as the concentration that is 10-times the value S.D. on the basis of three independent determinations. The results of herbicide measurements are shown in Table 3 and Fig. 5 (fortified pure water) and Table 4 and Fig. 6 (fortified tap water and river water). The recoveries were between 70 and 120% and the R.S.D.s were less than 20%. It shows that n.d. does not satisfy these two conditions. In this investigation, the LOQ for the fortified waters was 10 ng l^{-1} for the seven compounds (except halosulfuron-methyl was 20 ng l^{-1}) in the pure water, using any of the three kinds of cartridge. Similarly, the LOQ in the case of tap water was 10 ng l^{-1} (except imazosulfuron, pyrazosulfuron-ethyl and halosulfuron-methyl, which had the LOQ of 100 ng l^{-1}), for river water the LOQ was 10 ng l^{-1} (except imazosulfuron, halosulfuron-methyl and diuron was 100 ng l^{-1}).

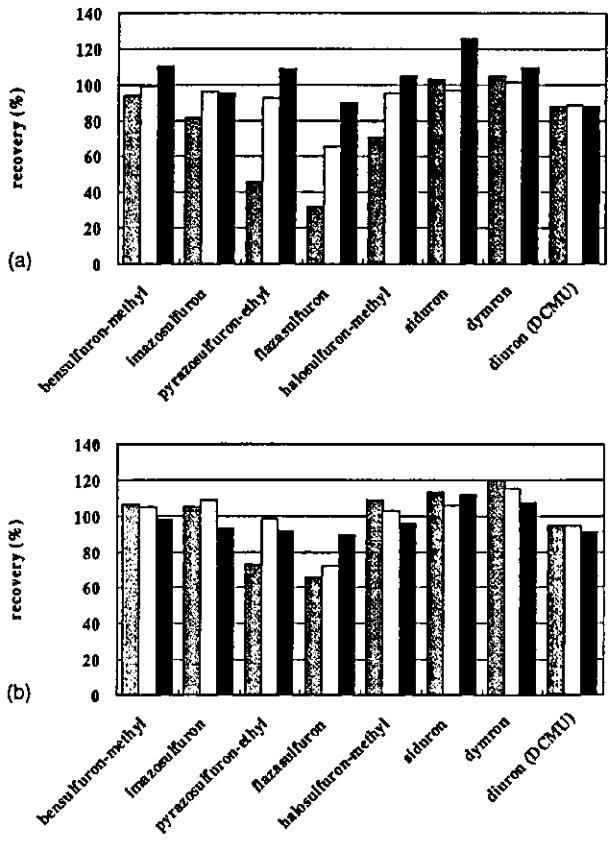

Fig. 5. Levels of herbicide recovery of from pure water, using PS2, C_{18} and Oasis cartridges. (a) Fortified with 20 ng l^{-1}; (b) fortified with 40 ng l^{-1}. (▨): PS2; (□): C_{18}; (■): Oasis.

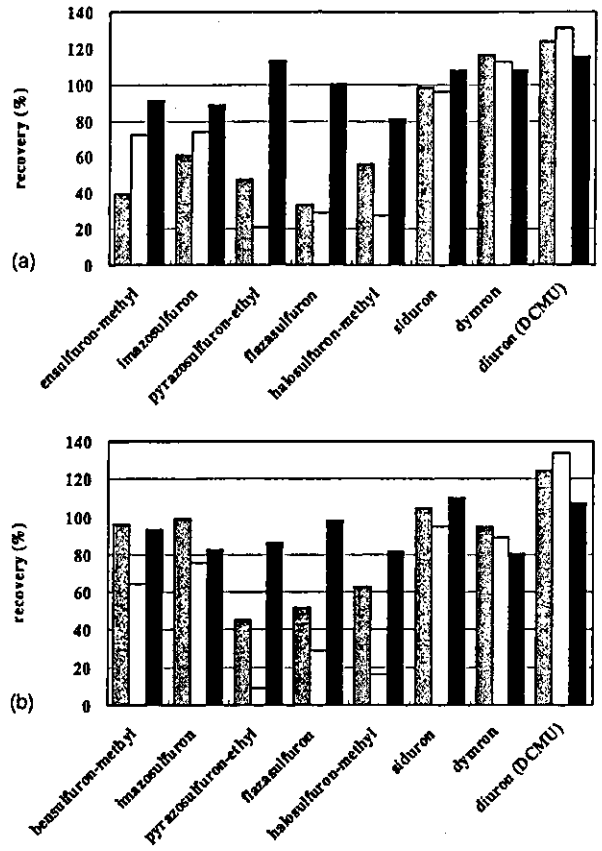

Fig. 6. Levels of herbicide recovery from water samples using PS2, C_{18} and Oasis cartridges. (a) Tap water; (b) river water. (▨): PS2; (□): C_{18}; (■): Oasis.

4. Conclusions

We have demonstrated that SPE–LC–ESI–MS is a sensitive and selective technique for the determination and quantitation of herbicides in environmental water samples. Very low detection limits can be reached due to the enhanced selectivity and high sensitivity obtained with this methodology. Furthermore, this method has clearly demonstrated good recoveries (70–120%), good precision (0.2% ≤ R.S.D. ≤ 5.7%), and good sensitivity by SPE using the three different types of cartridge.

Acknowledgements

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「事例報告」

太田川流域の浄水場における農薬類検出実態と原水中濃度の予測

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要旨：広島市太田川流域に位置する浄水場の原水及び浄水について2002年４月～11月に57種類の農薬類の検出状況を調べた。調査期間には原水、浄水ともに延べ20物質程度の農薬類が検出されたが、検出率、検出濃度の平均的なものはそれぞれ20%程度、10ng/L オーダーであった。なお、浄水で検出された農薬類について相対健康リスク（検出濃度／目標値）の総和を算出すると0.01オーダーと新水道水質基準の目標値１より２桁程度低かった。また、原水における検出濃度データを用いて、既往の簡易な統計予測モデルの適用について検討した。各農薬類の予測濃度と原水中最大濃度の差の中央値は0.1オーダー程度であり、精度良く予測できることを確認した。

キーワード：農薬、実態調査、相対健康リスク、統計的予測モデル
分類項目：農薬（120311）、河川水の調査研究（120603）、給配水系における調査研究（120605）、健康リスク評価（120106）

1.　はじめに

農薬は、樹木、農林産物などを害する動植物またはウィルスの防除に使用される殺菌剤、殺虫剤や農作物の生長促進剤、発芽抑制剤など、農業生産の安定化、農作業の能率化を図るために用いられる[1]。農薬取締法において2002年９月30日現在、有効登録されている農薬製品は5059件ある。また、国内の農薬総出荷数量はここ数年、横ばい状態であるが、30万ｔ以上使用されている。

近年、これらの農薬類による環境汚染が注目され、水環境実態[2~4]、水田からの流出動態[5, 6]、土壌中における挙動[7]など様々な研究がなされてきた。しかし、これらは法規制されている個々の農薬類を対象としたものが多く、データは蓄積されているが、地域毎に監視すべき農薬類を予測するには十分ではない。使用される農薬は時代とともに変わり、その変化に対応できる水道水質管理を考える場合、水源に検出される可能性のある農薬類を精度良く予測する手法を確立することは重要である。そこで、本研究では広島市太田川流域の浄水場について2002年４月～11月に57種類の農薬類の検出状況を調べ、その検出濃度データを用いて、既往の簡易な統計予測モデルの適用について検討した。

2.　調査方法

2.1　調査流域及び地点

本研究で調査した広島市太田川流域及び測定地点を図-1に示す。太田川は広島県西部を貫流する中国地方有数の河川で、中国山地の冠山に発し、大小72河川の支流及び島根県江の川水系土師ダム貯水池の分水を集め、広島湾に注ぐ。太田川と土師ダム貯水池上流域を合わせた水源流域面積は約1,800km^{2}であり、水源流域市町村の耕地面積は約65km^{2}である。

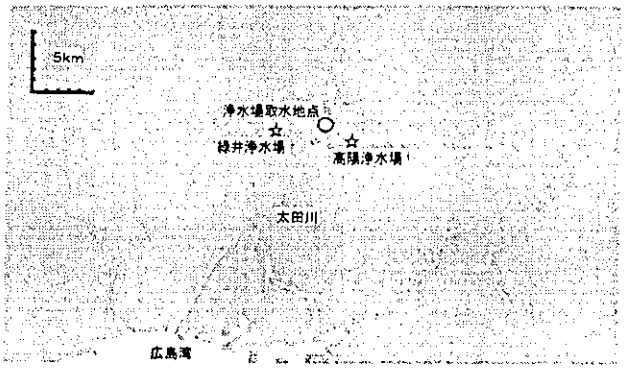

図-1　広島太田川流域

今回の測定場所は、広島市水道局高陽浄水場、緑井浄水場で、取水口は広島湾から約15km 上流部に位置する（図-1)。太田川表流水を取水し、ともに硫酸アルミニウム（太田川表流水が高濁度の場合はポリ塩化アルミニウム）を用いる凝集沈澱・急速沪過方式で浄化している。両浄水場の違いは沈澱池で、高陽浄水場は傾斜板を用いる横流式沈澱地を、緑井浄水場は複合形高速凝集沈澱地を採用している。

これらの浄水場の原水と浄水について2002年４月～９月に月３回、10～11月は月１回の頻度で農薬類の測定を行った。原水とは、取水場の沈砂池においてごみ、砂など浮遊物を自然沈澱させた後、ポンプで浄水場に送水される浄化前の水で、浄水とは、浄水場内の浄水池出口あるいは配水池出口の水である。浄水の塩素接触時間はおよそ10時間である。

2.2　農薬類の測定方法及び検出下限値

本研究で測定対象とした農薬類は2002年公布前の水道水質基準における基準項目、監視項目（オキソン体も含む)、ゴルフ場使用農薬や環境省の公共用水域等の水質評価指針項目など、合計57農薬である。前処理及び測定は、固相抽出-ガスクロマトグラフ法（SPE-GC)、固相抽出-ガスクロマトグラフ質量分析法（SPE-GCMS)、固相抽出誘導体化-ガスクロマトグラフ質量分析法(SPEder-GCMS)、ヘッドスペース-ガスクロマトグラフ質量分析法（HS-GCMS)、固相抽出-高速液体クロマトグラフ法（SPE-HPLC)、高速液体クロマトグラフ-ポストカラム法（HPLC-PC）で、上水試験方法に準じて実施した[5]。なお、HS-GCMS 法以外の方法の検水は、ガラス繊維沪紙（Millipore AP40）で沪過した試料を分析に供した。HS-GCMS 法では揮発性物質の測定であるので、沪過を行わない試料を分析に供した。

農薬類の検出下限値であるが（表-1)、HS-GCMS 法及び SPE-HPLC 法で測定した農薬類は目標とする検出下限値を定めて、装置の調整を行い、測定した。一方、SPE-GCMS 法、SPEder-GCMS 法及び SPE-GC 法は、検出下限値を、測定毎に標準液のピークとバックグラウンドの SN 比（３とした。）から定めた。

3.　調査結果及び考察

3.1　原水における農薬類

本研究で測定対象とした農薬類の検出数、検出濃度等をまとめたものを表-1に示す。高陽浄水場の原水（以下、高陽原水)、緑井浄水場の原水（以下、緑井原水）ではそれぞれ、延べ24物質、22物質が検出された。検出下限値が測定毎及び物質間でも異なるので、比較するには注意が必要であるが、参考までに述べると原水における検出数が６以上の物質は、高陽原水ではシマジン（CAT)、チオベンカルブ、フェノブカルブ（BPMC)、ベンタゾン、フルトラニル、ピリブチカルブ、プレチラクロール、カルプロパミドで、緑井原水ではこれらに加えてジクロルボス（DDVP）であった。

次に2002年４月～９月における高陽浄水場原水の農薬類検出濃度と太田川流域の農薬類予想散布量の推移を図-2に示す。予想散布量は太田川流域市町村における平成12年度農薬類含有薬剤販売量及び農協等が発行する稲作暦の散布予定時期から推測した。例えば農薬 a が薬剤 A に60%、薬剤 B に40%含まれており、薬剤 A が５月上旬に60kg、薬剤 B が５月上旬から５月中旬に40kg 散布される場合、農薬 a の予想散布量を、５月上旬では60×0.6+40×0.4÷２＝44kg、５月中旬では40×0.4÷２＝８kg のように算出した。各農薬類の濃度変動と散布時期を照らし合わせると散布直後から濃度が増加し、散布終了後に濃度が低下する傾向が認められた。なお、緑井浄水場の場合も、ほぼ同様な傾向が観察された。

次に各農薬の予想最大散布量と原水中最大濃度の比の対数値とオクタノール/水分配係数

表-1　本研究で測定した農薬類の測定方法及び検出状況等

濃度値の単位[ng/L]

	農薬（浄水道水質基準番号、農薬名、目標値(ng/Lで表示)）		測定関連		測定数	高陽浄水場								緑井浄水場							
			測定方法*1	検出下限値の中央値*2		原水				浄水				原水				浄水			
						検出数	最大値	中央値	最小値	検出数	最大値	中央値	最小値	検出数	最大値	中央値	最小値	検出数	最大値	中央値	最小値
1	チウラム	20000	SPE-HPLC	150	19	0				0				0				0			
2	シマジン（CAT）	3000	SPE-GCMS	2.0	19	9	30	3.0	1.2	3	7.0	4.4	1.5	7	20	2.8	1.2	2	7.0	4.1	1.1
3	チオベンカルブ	20000	SPE-GCMS	3.5	19	8	31	7.7	5.5	1	1.4	1.4	1.4	8	25	16	1.1	0			
4	1,3-ジクロロプロペン（D-D）	2000	HS-GCMS	60	19	0				0				0				0			
5	イソキサチオン	8000	SPE-GCMS	43	19	0				0				0				0			
	イソキサチオンオキソン		SPE-GCMS	125	19	0				0				0				0			
6	ダイアジノン	5000	SPE-GCMS	2.1	19	4	4.5	2.6	2.2	1	1.0	1.0	1.0	2	2.3	2.3	2.2	0			
	ダイアジノンオキソン		SPE-GCMS	7.0	19	0				0				0				0			
7	フェニトロチオン（MEP）	3000	SPE-GCMS	8.9	19	5	39	14	9.2	0				5	25	15	1.2	0			
	フェニトロチオンオキソン		SPE-GCMS	30	19	2	33	20	7.1	2	60	46	31	0				2	15	15	14
8	イソプロチオラン（IPT）	40000	SPE-GCMS	2.2	19	0				1	2.6	2.6	2.6	1	10	10	10	0			
9	クロロタロニル（TPN）	50000	SPE-GCMS	0.79	19	0				0				0				0			
10	プロピザミド	50000	SPE-GCMS	2.2	19	0				0				0				0			
11	ジクロルボス（DDVP）	8000	SPE-GCMS	0.90	19	4	7.7	4.9	0.60	6	5.7	3.0	0.40	6	6.7	1.5	0.30	3	2.6	1.9	1.4
12	フェノブカルブ（BPMC）	30000	SPE-GCMS	0.28	19	14	52	17	0.20	15	49	4.4	0.40	14	30	7.4	0.40	12	25	7.2	0.60
13	クロルニトロフェン（CNP）	100	SPE-GCMS	5.5	19	0				0				0				0			
15	イプロベンホス（IBP）	8000	SPE-GCMS	4.1	19	3	43	17	2.9	3	7.5	5.7	4.5	4	28	13	4.1	2	8.7	8.7	8.6
16	EPN	6000	SPE-GCMS	7.0	19	0				0				0				0			
	EPN オキソン		SPE-GCMS	24	19	0				0				0				0			
17	ペンタゾン	200000	SPEder-GCMS	1.4	19	19	300	80	8.4	19	220	36	6.4	19	270	98	8.1	9	160	21	7.7
18	カルボフラン（カルボスルファン代謝物）	5000	HPLC-PC	150	19	0				0				0				0			
19	2,4-ジクロロフェノキシ酢酸（2,4-D）	30000	SPEder-GCMS	2.4	19	0				2	17	10	3.5	0				1	14	14	14
20	トリクロピル	6000	SPEder-GCMS	1.9	19	3	80	27	22	2	32	24	15	2	41	34	27	1	32	32	32
21	アセフェート	80000	SPE-HPLC	300	18	0				0				0				0			
22	イソフェンホス	1000	SPE-GCMS	2.5	18	0				0				0				0			
23	クロルピリホス	30000	SPE-GCMS	2.7	18	0				0				0				0			
24	トリクロルホン（DEP）	30000	SPE-GC	18	18	0				0				0				0			
25	ピリダフェンチオン	2000	SPE-GCMS	11	18	0				0				0				0			
26	イプロジオン	300000	SPE-HPLC	120	18	0				0				0				0			