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Monitoring of Phthalic Acid Monoesters in River Water by Solid-Phase Extraction and GC-MS Determination

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An analytical method for monitoring 10 phthalic acid monoesters in river water was investigated by solid-phase extraction, methylation with diazomethane, and GC-MS. Two cartridge-type solid phases packed with octadesyl-coated silica (C_{18}) and styrenedivinyl polymer (PS-2) and one disk-type solid phase made from octadesyl-coated styrene-divinylbenzene polymer (SDB-XD) were investigated in solid-phase extraction. PS-2 gave the highest recoveries of the three solid phases, and recoveries of more than 80% of the monoesters in filtered water samples were obtained at pH 2 to 3 with PS-2 at the spiked level of 0.1 μg L^{-1}, except for monomethyl-phthalate (MMP), in which more than 72% of the monoesters were recovered. For the monoesters in the suspended solids (SS), an acidic methanol extract of SS was added to purified water acidified to pH 2, and the monoesters were extracted with PS-2. The recoveries of the monoesters in SS were more than 80%, but the recoveries of MMP were more than 57%. The method detection limit (MDL) of each phthalic acid monoester in 500 mL of water sample and in 2 mg of dry weight of SS ranged from 0.010 to 0.030 μg L^{-1} and from 1 to 11 μg g^{-1}, respectively. Monitoring of phthalic acid monoesters in the Tama River in Tokyo was conducted every month from March 1999 to February 2000 using the present method. MMP, mono-*n*-butyl-phthalate (MBP), and mono-(2-ethylhexyl)-phthalate (MEHP) were detected at concentrations of 0.030−0.0340, 0.010−0.480, and 0.010−1.30 μg L^{-1}, respectively, in the filtered water samples but were not detected in SS. Dimethyl-phthalate (DMP), di-*n*-butyl-phthalate (DBP), and di-(2-ethylhexyl)-phthalate (DEHP) were detected in the river water at concentrations of 0.010−0.092, 0.008−0.540, and 0.013−3.60 μg L^{-1}, respectively. Diethyl-, di-*iso*-butyl-, and benzylbutyl-phthalates were also detected at concentrations of nanograms per liter, whereas the corresponding monoesters did not appear. The concentrations of MBP and MEHP in the river water were slightly lower than those of the corresponding diesters at the majority of sampling sites and sampling times.

Introduction

Phthalic acid diesters are well-known contaminants in environmental water, soil, and the atmosphere. They also have been detected in human serum and plasma due to their widespread use as plasticizers. The phthalates di-*n*-butyl-phthalate (DBP) and di-(2-ethylhexyl)-phthalate (DEHP) have been studied for metabolism and toxicity in mammals and environmental distribution, and general reports have been already published (*1*, *2*). DEHP was found to produce liver tumors in rodents (*3*, *4*) and to be a tumor promoter in the skin and liver in mice (*5*, *6*). In recent years, phthalic acid diesters have appeared as important pollutants in environmental samples as endocrine disrupting chemicals which influence the genitals because they have binding activity for the estrogen receptor (*7*, *8*) and cause proliferation of MCF-7 cells (*9*).

Phthalic acid monoesters such as mono-*n*-butyl-phthalate (MBP) and mono-(2-ethylhexyl)-phthalate (MEHP) are metabolites of phthalic acid diesters by various tissue lipases (*10*, *11*) and some esterases (*12*) in mammals. MBP has also been identified as a transient intermediate in culture solutions of microorganisms (*13*). MEHP has been found in intravenous solutions that were stored in medical grade poly(vinyl chloride) (PVC) bags (*14*). MBP and MEHP have also been detected in serum and plasma products packed into plastic containers (*15*) and in water from medical grade PVC tubing (*16*). Among the phthalic acid monoesters, MEHP has several biological effects. It caused peroxisome proliferation in rat liver (*17*), stimulated the growth of sertoli cells and genocytes in neonatal rats (*18*), had toxic effects on cultured kidney epithelial cells (*19*), and was found to damage DNA in human blood cells (*20*) at relatively low concentrations. These previous reports suggest that monitoring of phthalic acid monoesters, as well as phthalic acid diesters, in environmental and drinking water is necessary.

Analytical methods for phthalic acid monoesters in serum and plasma products have been previously published. In these methods, the monoesters were determined by reverse-phase HPLC-UV (*15*, *16*), GC-FID (*21*), and GC-ECD (*14*), and their detection limits were several milligrams per liter. These analytical methods might be inadequate for phthalic acid monoesters in environmental water samples because the parent compounds, phthalic acid diesters, in river water samples have been mostly detected at lower than microgram per liter levels (*1*, *2*). Only a few reports regarding analytical methods and monitoring data for phthalic acid monoesters in environmental water samples have been published.

In Japan, phthalate plasticizer was produced industrially at 416 840 tons [DEHP, 68.1%; DBP, 3.0%; di-methyl-phthalate (DMP), 0.4%; di-ethyl-phthalate, 0.2%; benzyl-*n*-butyl-phthalate, 0.5%; and the others, 27.8%] in 1999 (*22*). DEHP, DBP, and benzyl-*n*-butyl-phthalate have been detected in river water samples in Japan at microgram per liter levels (*23*). The objectives of this study were to develop an analytical method for determining the corresponding monoesters in river waters at nanogram per liter levels using soil-phase extraction and GC-MS and to examine the concentrations of the monoesters and corresponding phthalic acid diesters in the Tama River in Tokyo.

Experimental Section

Synthesis of Phthalic Acid Monoesters. The method of Snell (*16*) for the synthesis of MEHP was modified for the synthesis of the phthalic acid monoesters listed in Table 1. For the synthesis of monoesters with a substituted alkyl chain length of alcohol of more than 3, 10 mmol of phthalic anhydride

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TABLE 1. Purity of Phthalic Acid Monoesters and Retention Times, Monitor Ions, and Linearity of Calibration Curves of Their Methyl Derivatives on GC-MS

			free acid		methyl derivative			
						monitor ions[c] (*m/z*)		calibration
phthalic acid monoester	abbreviation	M. W.	purity [a] (%)	M. W.	tR[b] (min)	A	B	curve[d] (γ)
mono-methyl-phthalate	MMP	180	98.0	194	12:52	163	194	0.9999
mono-ethyl-phthalate	MEP	194	98.9	208	13:48	163	149, 176	0.9999
mono-iso-propyl-phthalate	MIPRP	208	94.7	222	14:15	163	149, 181	0.9998
mono-*n*-propyl-phthalate	MPRP	208	97.7	222	14:59	163	149, 181	0.9999
mono-iso-butyl-phthalate	MIBP	222	99.4	236	15:36	163	149, 181	0.9999
mono-*n*-butyl-phthalate-d_4	MBP-d_4	226	97.8	240	16:08	167	153, 185	0.9998
mono-*n*-butyl-phthalate	MBP	222	99.7	236	16:09	163	149, 181	0.9998
mono-*n*-pentyl-phthalate	MPEP	236	97.1	250	17:20	163	149, 181	0.9998
mono-cyclohexyl-phthalate	MCHP	248	97.0	262	19:31	163	149, 181	0.9999
mono-(2-ethylhexyl)-phthalate-d_4	MEHP-d_4	282	96.7	296	20:02	167	153, 185	0.9998
mono-(2-ethylhexyl)-phthalate	MEHP	278	98.8	292	20:03	163	149, 181	0.9998
mono-benzyl-phthalate	MBZP	256	97.4	270	21:07	163	91, 164	0.9998
mono-*n*-octyl-phthalate	MOP	278	97.2	292	21:11	163	149, 181	0.9998

[a] Estimated by reverse-phase HPLC at UV 265 nm. [b] Typical retention times. [c] A, ions for quantification; B, ions for confirmation. [d] Correlation coeffient when injecting a 1 μL of the methyl derivative mixtures from 0.01 to 0.2 mg L^{-1} into GC-MS.

(purity 99.5%, Wako Pure Chemical Industry, Osaka, Japan) and 15 mmol of *iso*-butyl-, *n*-butyl-, *n*-pentyl-, cyclohexyl-, benzyl-, 2-ethylhexyl-, or *n*-octyl-alcohol (purity more than 98%, Wako Pure Chemical Industry) were heated at 100−120 °C for 3 h. For the synthesis of MMP, ethyl-, *n*-propyl-, and *iso*-propyl-phthalates, phthalic anhydride (10 mmol) and 10 mL of methanol, ethanol, *n*-propanol, or 2-propanol, respectively (purity more than 98%, Wako Pure Chemical Industry), were refluxed at 100 °C for 6 h, and then the alcohols were removed under vacuum. The residual liquid of each monoester was added to 200 mL of 1% sodium hydrogen carbonate solution, and the phthalic acid diester and/or long-chain alcohol were removed by shaking twice with 50 mL of diethyl ether. Subsequently, phthalic acid monoesters were extracted by shaking twice with 50 mL of diethyl ether from the solution after acidifying to pH 4 with 5 N HCl. The ether solution was dehydrated by passing it through sodium sulfate anhydrous and evaporated using a rotary evaporator at 40 °C. For synthesis of the surrogate compounds, mono-*n*-butyl phthalate-d_4 (MBP-d_4) and mono-2-ethylhexyl phthalate-d_4 (MEHP-d_4), phthalic anhydrous with hydrogens substituted by deuterium at the 2, 3, 4, and 5 positions of the benzene ring (purity 98%, Cambridge Isotope Laboratories, Inc., MA) were used. The purity of each synthesized monoester obtained by reverse-phase HPLC (column, TSK-gel ODS 120T, 4.6 mm i.d. × 250 mm, Tosoh, Tokyo; column oven temperature, 40 °C; UV, 265 nm; solvent, acetonitrile−20 mM phosphate buffer (pH 4) (50:50, v/v); flow rate, 1.2 mL min^{-1}) is listed in Table 1. The phthalic acid diesters levels in the synthesized monoester samples were measured by injecting 1 μL of 10 mg L^{-1} monoester in acetone without methylation with diazomethane into GC-MS under the conditions described below and was estimated to be less than 1% of the corresponding monoesters.

Sampling Sites of River Waters. Water samples were collected every month from May 1999 to February 2000 at the six sites in the Tama River which flows through the Tokyo metropolitan area and empties into Tokyo Bay as illustrated in Figure 1. The Aki and Asa Rivers flow into the Tama River near sampling sites 5 and 6, respectively. The population around the six sampling sites is 3 841 000, and the populations around sampling sites 1 and 5, and site 6 are about 6 and 18%, respectively, of the total population in 1999. Effluent from sewage plants runs into the river at sampling sites 2, 3, 4, and 6. The water samples were taken using amber glass containers (2 L), precleaned with acetone, and brought back to the laboratory. In the case of the analysis of phthalic acid monoesters, all water samples were filtered within 8 h after

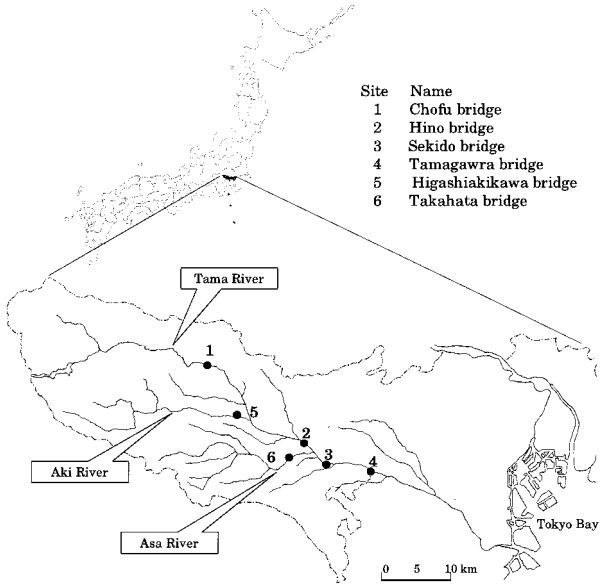

FIGURE 1. Sampling site locations of river water in the Tama River in Tokyo.

sampling through a glass filter AP-40 (pore size, 1 μm; internal diameter, 47 mm; Millipore, Bedford, MA), which was precleaned with acetone. The suspended solid (SS) trapped on the filters was kept in glass flasks precleaned with acetone.

Solid-Phase Extraction of Phthalic Acid Mono- and Diesters in River Water. Attention was needed to prevent contamination of MBP and MEHP during sample preparation. The solid-phase extraction cartridges, PS-2 and C_{18} (Waters, Milford, MA), were prewashed with dichloromethane (CH_2Cl_2), acetone, methanol, and purified water adjusted to pH 3 with 0.01 N HCl, 10 mL of each, before use. The disk cartridge, SDB-XD (3M, St. Paul, MN), was prewashed by refluxing with acetone in the Soxhlet-extraction apparatus for 3 h and storing the disk cartridge in acetone at room temperature. Commercially available organic solvents for phthalate analysis and the purified water prepared by PURIC-MX for HPLC grade (Organo, Bunkyo-ku, Tokyo) were used to decrease the blank levels. Sampling bottles and all glassware such as pipets and test tubes were washed with acetone and kept in a clean box after air-drying. It is also necessary to avoid the use of plastic products containing the phthalates.

For analysis of phthalic acid monoesters in the filtered water samples, 5 μL of 10 mg L^{-1} MBP-d_4 and MEHP-d_4 in acetone were spiked into 500 mL of the filtered water samples. Phthalic acid monoesters in the water samples were extracted by passing the water samples through the solid-phase extraction cartridge, PS-2 or C_{18} with Sep-pack concentrator (Waters), at a flow rate of 15 mL min^{-1} after acidifying the water samples to pH 2 with 1 N HCl. In the case of SDB-XD, the monoesters were extracted with the disk set to disk-holder (3M) at a flow rate of about 100 mL min^{-1}. The cartridges and the disk were dehydrated by passing air through them for 30 and 5 min, respectively, and then the monoesters were eluted with 5 mL and then 2 mL of CH_2Cl_2. The CH_2Cl_2 solution was dehydrated by passing the solutions through the small glass column packed with sodium sulfate anhydrous (Na_2SO_4), which was washed by CH_2Cl_2, and then concentrated to 0.5 mL under the stream of nitrogen (N_2). The blank sample of the filtered river water was prepared without water-sample loading to the solid-phase cartridge or the disk SDB-XD and was subjected to the same procedures as the filtered river samples. The monoesters in the filtered river water samples were analyzed in duplicate samples at one sampling site.

For the phthalic acid monoesters in SS trapped on the glass filter AP-40, the filter was put into a 100-mL flask, and 5 μL of 10 mg L^{-1} MBP-d_4 and MEHP-d_4 in acetone was spiked on the filter. After addition of 100 μL of 5 N HCl to the filter, the monoesters were extracted twice by sonication for 5 min with 5 mL of methanol. About 10 mL of the combined solution was added to 500 mL of the water made using PURIC-MX and acidified to pH 2 with HCl, and then the monoesters were analyzed in accordance with the methods for the filtered water samples. The blank sample of SS was prepared without water-sample loading to AP-40 and was subjected to the same procedures as SS. To measure the content of SS in river water, the same river water (500−1000 mL) was passed through the AP-40, and then the weights of AP-40 were measured after drying for 4 h at 105 °C. The analysis of the monoesters in SS and measurement of SS content were performed in duplicate samples at one sampling site.

Phthalic acid diesters in river water samples were extracted by using Empore Disk SDB-XD according to the EPA methods (*24*) without filtration. Attention was also needed to prevent contamination of DBP and DEHP during sample preparation. The procedures described below were performed at the clean bench with attached active carbon filter (Dalton, Los Angeles, CA). Each surrogate compound of phthalic acid diester listed in Table 4, with hydrogen substituted by deuterium at 2, 3, 4, and 5 positions of the benzene ring (purity more than 98%, Kanto Chemical Co., Chuo-ku, Tokyo), was spiked into the river water at a final concentration of 0.1 μg L^{-1}. An aliquot of water samples (250−500 mL) was passed through the disk at a flow rate of 50−100 mL min^{-1}. The cartridge was dehydrated by passing air through it for 5 min, and then the phthalates were eluted with 10 mL of CH_2Cl_2. The CH_2Cl_2 solution was dehydrated by passing the solutions through the small glass column packed with Na_2SO_4 and then concentrated to 1000-fold under a stream of N_2. The resulting solutions spiked with the internal standard fluoranthene-d_{10} (IS) at final concentrations of 0.1 μg L^{-1} were injected into the GC-MS. The blank sample was prepared without water-sample loading to the disk SDB-XD and was subjected to the same procedures as the river samples. The diesters in river water samples were analyzed in duplicate samples at one sampling site.

Methylation of Phthalic Acid Monoesters. The diazomethane was prepared by adding 1 g of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine to 5 N sodium hydride solution, and then diazomethane gas was trapped to 20 mL of ice-cold diethyl ether. The diazomethane solution was dehydrated using 0.1 g of potassium hydroxide granule. A total of 0.25 mL of diazomethane in diethyl ether solution was added to the concentrated extracts obtained from the filtered water and SS samples, and the mixture was allowed to stand for 30 min at room temperature. The solution was concentrated to 0.5 mL under a stream of N_2. The resulting solutions which were spiked with internal standard fluoranthene-d_{10} (IS) at a final concentration of 0.1 μg L^{-1} were injected into the GC-MS.

GC-MS Conditions. GC-MS conditions for phthalic acid mono- and diesters were as follows: GC model, HP-5890 series II (Hewlett-Packard, Palo Ato, CA); injector temperature, 220 °C; column head pressure, 80 kPa; carrier gas, helium; auto sampler, HP-7673 (Hewlett-Packard); sample size, 1 μL (split-less injection, purge on time for 1 min; glass wool was not stuffed in the split-less insert); analytical column, HP5-MS, 0.25 mm i.d. × 30 m, film thickness 0.25 μm (J&W Scientific, Folsom, CA). The GC oven temperature was programmed as follows: held at 50 °C for 1 min, increased from 50 to 200 °C at 10 °C min^{-1} and from 200 to 300 °C at 6 °C min^{-1}; MS model, Automass II mass spectrometer (JEOL, Akishima, Tokyo); ionization potential, 70 eV; ionization current, 300 μA; ion source temperature, 220 °C; temperature of transfer line between GC and MS, 250 °C. For the fragmentation study, methylated phthalic acid monoesters were measured by scanning at ranges from 50 to 500 m/z. For the measurement of phthalic acid mono- and diesters in water sample, single ion monitoring (SIM) using the fragment ions listed in Table 1 was performed. The base peak, m/z 163 or 167, was used for quantification of the monoesters. The concentrations of MBP and MEHP in the filtered water and SS samples were corrected with MBP-d_4 and MEHP-d_4, and the other monoesters were determined with IS. The ion used for quantification of the phthalic acid diesters was m/z 149, except for DMP, in which m/z 163 was used, and each molecular ion peak was used for confirmation. The concentrations of each diester in river water samples were corrected with a corresponding surrogate compound.

Results and Discussion

Analytical Conditions of Phthalic Acid Monoesters in Water Sample. The mass spectra of some phthalic acid monoesters methylated with diazomethane are presented in Figure 2. A weak molecular ion peak of less than 0.1% and an ion at m/z 149 were observed for each methylated monoester, except for DMP. The fragment ions at m/z 163 and 181 were common ions in the methylated monoesters that have alkyl chain lengths beyond 2 and may be due to the cleavage of long chain alkyl groups. The fragment ions at m/z 167 and 185, and the molecular ion at m/z 240 of methylated MBP-d_4, correspond to the fragment ions at m/z 163 and 181 and the molecular ion at m/z 236, respectively, of methylated MBP. In the case of methyl-benzyl phthalate, the intensity of the fragment ions at m/z 164 is relatively higher than the other compounds, and the fragment ion at m/z 181 is less than 0.1%. DMP and methylethyl phthalates appeared as ions at M-32, which may be due to cleavage of the methyl group, but did not appear at m/z 181 or appeared at less than 0.1%. Phthalic acid diesters generally appeared as the fragment ions of M-R, M-2R, M-COOR, and M-2COOR (*25*), and the fragment ion at m/z 149 is well-known as a characteristic ion for these compounds. Phthalic acid diesters, except for DMP, corresponding to the monoesters investigated in this study did not appear as fragment ions at m/z 163 and 181. From these results, the monitor ions of the methylated monoesters were defined as shown in Table 1.

Typical retention times and linearity of calibration curves of phthalic acid monoesters under the GC-MS conditions are listed in Table 1. The correlation coefficients for the peak area ratios of the methylated monoesters to IS compound vs concentrations from 0.01 to 0.2 mg L^{-1} of the monoesters

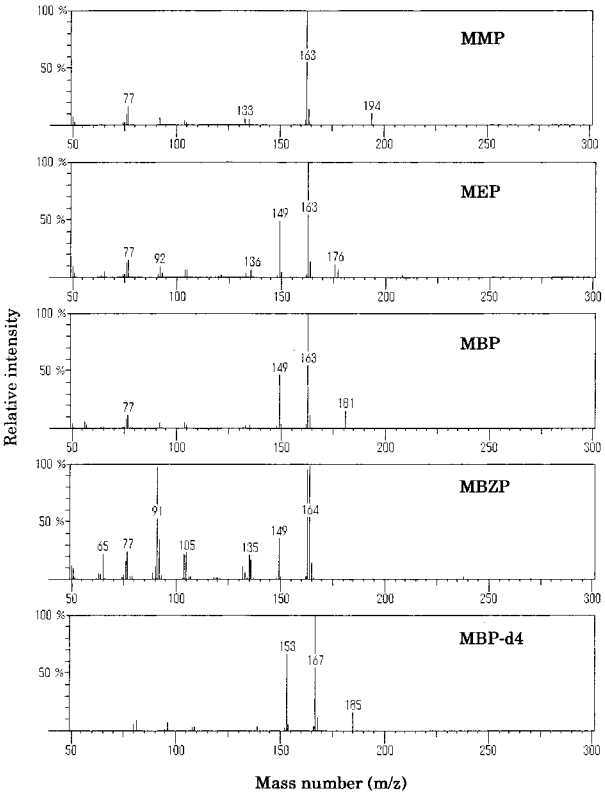

FIGURE 2. Mass spectra of phthalic acid monoesters methylated with diazomethane.

were 0.9998 to 0.9999. The relative standard deviations of the retention times and peak areas injected with 0.1 ng of the methyl derivatives of phthalic acid monoesters into the GC-MS were less than 0.5 s and 0.9% ($n = 5$), respectively, under these conditions.

Elution solvents for phthalic acid monoesters from the solid-phase cartridge PS-2 were examined. The monoesters were eluted at high efficiency (more than 74%) by CH_2Cl_2 and diethyl ether from PS-2 cartridge, and their recoveries were higher than those using benzene (Table 2). Effects of pH of the water sample on the recoveries of phthalic acid monoesters were evaluated by using purified water. Phthalic acid monoesters are weakly acidic compounds due to the presence of a carboxyl group; therefore, the pH of water samples affects the extraction efficacy on solid-phase extraction. The recoveries of phthalic acid monoesters ranged from 76 to 107% at pH 2 and 3 but decreased gradually at pH values greater than 3 (Table 2). The phthalic acid monoesters scarcely break through the PS-2 cartridge at sample volumes of 200 to 800 mL at the concentration of 0.1 μg L^{-1} (Table 2). Comparison of solid-phase type to recoveries of phthalic acid monoesters was conducted with river water samples (Table 3). The recoveries of MMP and MEP from the filtered river water were 72 and 88% for PS-2, 59 and 86% for SDB-XD, and 10 and 51% for C_{18}, respectively. The recoveries of the other monoesters were more than 81% for the three solid phases at the spiked level of 0.1 μg L^{-1}. These results suggested that a solid-phase extraction cartridge or disk using styrene-divinylbenzene polymer is suitable for efficient extraction of phthalic acid monoesters having short-chain alkyl groups as compared to a silica-based solid-phase extraction cartridge.

The recoveries of phthalic acid monoesters from SS were listed in Table 3. The phthalic acid monoesters spiked at 50 ng into SS trapped on AP-40 were extracted twice with methanol, acetone, or CH_2Cl_2 and sonicated after acidification with HCl. The acetone or methanol extract was added to the purified water and acidified to pH 2 with HCl, and then the monoesters were analyzed in accordance with the methods for the filtered water samples. The recoveries of the monoesters from SS were more than 77%, except for MMP, when acidic methanol or acetone was used (Table 3). The recoveries of MMP from SS using acidic acetone and methanol were 20 and 57%, respectively. The low recoveries of MMP from SS might be due to slightelution of the sample from the PS-2 cartridge while passing the acidic solution containing 2% methanol or acetone because recoveries of MMP from the filtered water sample were more than 72% with PS-2. In the case of CH_2Cl_2, the extract was dehydrated by Na_2SO_4 and concentrated under a stream of N_2 and then methylated with diazomethane. The recoveries of MEHP and MOP from SS with CH_2Cl_2 were more than 85%, whereas the recoveries of the other monoesters were less than 50% (Table 3).

The method detection limits (MDLs) associated with 500 mL of the filtered river water using PS-2 extraction were estimated as the product of the standard deviation of seven replicate samples at a 0.1 μg L^{-1} level and the two-tailed t-statistics for $n = 6$ degrees of freedom at the 95% confidence interval. The MDLs for the monoesters in SS were similarly investigated by spiking 50 ng of the monoesters into 2 mg of SS trapped on AP-40 using acidic methanol and PS-2 extraction. As shown in Table 4, the estimated MDLs of the monoesters in the filtered river water ranged from 0.010 to 0.014 μg L^{-1} except for MMP, which was estimated at 0.030 μg L^{-1}. The MDLs of the monoesters in SS were estimated from 1 to 5 ng, except for MMP, which was estimated at 11 ng.

Formation of phthalic acid monoesters during sample preparation due to hydrolysis of phthalic acid diesters under acidic conditions was examined. The diesters listed in Table 4 at the final concentrations of 1 μg L^{-1} were added to 0.001 and 0.01 N HCl solutions at room temperature, and then the monoesters corresponding to the diesters were analyzed using PS-2 extraction and GC-MS. The concentrations of phthalic acid monoesters were lower than the MDLs after standing for 18 h in the HCl solutions. These results suggest that formation of phthalic acid monoesters due to acidic hydrolysis does not occur during sample preparation.

The major problem during the analysis of phthalic acid diesters is the blank value. Controlling the blank values of DBP and DEHP at low levels is difficult because they are present in many apparatuses and equipment made of plastic in the laboratory or organic solvents. Similarly contamination by MBP and MEHP appeared during sample preparation. The blank values of these compounds could be decreased reproducibly at low levels by using solvents and apparatus free of the compounds and by performing sample preparation in the clean bench with attached active carbon filter. The blank values of DBP, DEHP, MBP, and MEHP in the resulting solutions were 0.011 ± 0.005, 0.007 ± 0.004, 0.010 ± 0.004, and 0.013 ± 0.003 mg L^{-1}, respectively ($n = 12$). The other phthalic acid mono- and diesters were not detected in the resulting solutions of sample blank.

From these results, the extraction conditions of phthalic acid monoesters in river water and SS were fixed as shown in Experimental Section.

Monitoring of Phthalic Acid Mono- and Diesters in the Tama River. The monitoring results of phthalic acid monoesters in the Tama River were listed in Table 4. MBP and MEHP were detected at high frequencies at all sampling sites, and their maximum concentrations were 0.480 and 1.30 μg L^{-1}, respectively. For the tributary rivers, the concentrations of MBP and MEHP in the Asa River (site 6) were higher than those in the Aki River (site 5) as shown in Table 4. These phthalic acid monoesters did not appear in the resulting solution from SS trapped on the glass filter AP-40. When the monoesters were spiked into the river water samples at the final concentrations of 0.1 μg L^{-1} before filtering with AP-40,

TABLE 2. Effects of Eluent, pH, and Applied Volume of Water Samples on Recovery of Phthalic Acid Monoesters

phthalic acid monoester	recovery (%)[a]											
	eluent[b]			pH[c]					sample volume (mL)[d]			
	CH_2Cl_2	diethyl ether	benzene	2	3	4	5	6	200	400	600	800
MMP	74	76	24	76	76	66	10	10	77	74	70	77
MEP	90	86	24	90	88	83	12	11	89	84	84	81
MIPRP	88	92	29	90	92	89	56	33	85	89	85	94
MPRP	93	94	28	94	92	88	57	42	90	97	95	99
MIBP	91	92	31	95	93	90	74	63	88	91	89	97
MBP-d_4	90	95	31	94	93	88	72	61	93	97	96	94
MBP	91	94	31	92	101	96	57	53	98	98	99	103
MPEP	91	93	35	100	95	93	65	63	97	100	102	102
MCHP	95	94	38	95	89	94	63	61	97	102	108	105
MEHP-d_4	96	94	38	100	98	107	77	74	100	100	99	99
MEHP	98	98	39	92	106	96	80	66	101	99	102	105
MBZP	95	96	38	94	89	90	64	61	99	97	90	100
MOP	91	88	37	99	101	105	84	79	89	87	86	93

[a] Solid-phase extraction cartridge, PS-2; values were mean of two experiments. [b] Sample, 500 mL of purified water acidified to pH 2 with HCl; spiked level, 0.1 μg L^{-1}; eluent, 5 mL. [c] Sample, 500 mL of purified water acidified with HCl; spiked level, 0.1 μg L^{-1}; eluent, 5 mL of CH_2Cl_2. [d] Sample, purified water acidified to pH 2 with HCl; spiked level, 0.1 μg L^{-1}; eluent, 5 mL of CH_2Cl_2.

TABLE 3. Recovery of Phthalic Acid Monoesters in Filtered River Water and Suspended Solid by Solid-Phase Extraction[a]

phthalic acid monoester	filtrated river water recovery (%)[b]				suspended solid on AP-40 recovery (%)[c]		
	PS-2		SDB-XD	C_{18}			
	0.1 μg L^{-1}	1.0 μg L^{-1}	0.1 μg L^{-1}	0.1 μg L^{-1}	acetone	methanol	CH_2Cl_2
MMP	72 ± 8	73 ± 3	59 ± 15	10 ± 10	20 ± 3	57 ± 8	11 ± 2
MEP	88 ± 4	80 ± 2	86 ± 4	51 ± 1	77 ± 3	84 ± 4	10 ± 2
MIPRP	88 ± 1	81 ± 2	88 ± 1	89 ± 1	91 ± 3	90 ± 2	14 ± 2
MPRP	94 ± 1	85 ± 1	92 ± 2	92 ± 1	90 ± 2	94 ± 2	13 ± 2
MIBP	97 ± 3	90 ± 1	91 ± 3	93 ± 2	102 ± 2	103 ± 2	20 ± 2
MBP-d_4	97 ± 4	90 ± 3	87 ± 5	87 ± 5	92 ± 3	95 ± 4	22 ± 3
MBP	90 ± 1	88 ± 2	89 ± 1	96 ± 1	97 ± 2	93 ± 2	24 ± 3
MPEP	104 ± 1	95 ± 2	102 ± 1	106 ± 2	103 ± 3	97 ± 2	40 ± 4
MCHP	90 ± 2	102 ± 3	102 ± 3	107 ± 2	105 ± 3	96 ± 7	50 ± 5
MEHP-d_4	103 ± 5	95 ± 4	91 ± 3	91 ± 3	92 ± 4	93 ± 5	85 ± 8
MEHP	91 ± 3	99 ± 2	96 ± 2	97 ± 3	88 ± 3	90 ± 2	87 ± 9
MBZP	96 ± 1	103 ± 4	105 ± 2	107 ± 1	94 ± 3	103 ± 5	39 ± 4
MOP	98 ± 2	106 ± 1	104 ± 4	107 ± 2	84 ± 3	88 ± 5	113 ± 17

[a] River water samples from site 2 in Figure 1 were filtered with the filter AP-40. [b] Values were mean ± SD (n = 5); sample volume, 500 mL; acidified to pH 2 with HCl; eluent, 5 mL of CH_2Cl_2; final volume 0.5 mL. [c] Values were mean ± SD (n = 5); spiked amount, 50 ng; suspended solid, 2 mg. Phthalic acid monoesters were extracted twice with 5 mL of methanol, acetone, or dichloromethane and sonicated for 5 min after addition of 100 μL of 5 N HCl. Acetone or methanol extract was added to 500 mL of purified water acidified to pH 2 with HCl, and phthalic acid monoesters were extracted with PS-2; final volume, 0.5 mL. Dichloromethane extract was dehydrated with Na_2SO_4 and concentrated under N_2 gas, and then methylated with diazomethane; final volume 0.5 mL.

more than 90% of the monoesters were recovered in the filtered water sample, except for MMP, in which more than 70% were recovered. This suggests that phthalic acid monoesters are scarcely adsorbed by SS in river water samples because the monoesters dissociate at pH 6 to 7 in the river water samples. MMP was also detected at some sampling sites at concentrations from 0.030 to 0.340 μg L^{-1}. In this analytical method, MMP could not be measured independently due to the methyl derivatization with diazomethane because more than 83% of DMP was recovered at the spiked level at 0.1 μg L^{-1} under the conditions for the monoesters with PS-2. Phthalic acid in the water acidified to pH 3 with HCl was not sufficiently recovered. Consequently, the concentrations of MMP were calculated by subtracting the values obtained from the diesters analysis from the values of the monoester.

The concentrations of DBP and DEHP in the Tama River ranged from 0.01 to 0.540 and 0.013−3.60 μg L^{-1}, respectively (Table 4). DMP, di-ethyl-, di-*iso*-butyl-, and benzyl-*n*-butylphthalates were also detected in water samples at concentrations from 0.01 to 0.310 μg L^{-1}, but the corresponding phthalic acid monoesters did not appear (Table 4). During the most recent monitoring for endocrine disrupting chemicals at sewage plants constructed near this sampling site, which was conducted by the Tokyo metropolitan government, DBP and DEHP in influent sewage were detected at concentrations from 1.3 to 9.5 μg L^{-1} and from 6.9 to 31 μg L^{-1}, respectively. On the other hand, their concentrations in effluent water from the plants decreased to lower than 0.2 μg L^{-1}. In this study, the concentrations of DBP and DEHP at sites 2, 3, 4, and 6 were higher than those of the sewage drainage. At site 4, which was located in the lowest part of the river in this study, the average flow rate of the river water in 1999 was 1 270 000 m^3 day^{-1}, and the sewage drainage comprises 40 to 50% of the river water. The area around the Asa River is low spread of the sewage system, and the relatively high concentrations of DBP, MBP, DEHP, and MEHP were observed at site 6. The concentrations of these compounds in river water at sites 1 and 5, which run into a relatively low population area, were lower than the other sampling sites. These results suggest that the contamination by phthalates in the Tama River might be influenced by direct inflow of sewage or surface water rather than drainage from sewage plants.

TABLE 4. Monitoring of Phthalic Acid Mono- and Diesters in the Filtered Water Samples from the Tama River from March 1999 to February 2000[a]

		site 1		site 2		site 3		site 4		site 5		site 6	
phthalate ester	MDL[b] (μg L^{-1})	dt	conc (μg L^{-1}) min−max	dt	conc (μg L^{-1}) min−max	dt	conc (μg L^{-1}) min−max	dt	conc (μg L^{-1}) min−max	dt	conc (μg L^{-1}) min−max	dt	conc (μg L^{-1}) min−max
Monoester													
MMP[c]	0.030 (11)	4	ND−0.070	5	ND−0.170	5	ND−0.190	4	ND−0.340	3	ND−0.110	6	ND−0.061
MEP	0.014 (3)	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
MIPRP	0.010 (1)	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
MPRP	0.010 (2)	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
MIBP	0.012 (1)	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
MBP	0.010 (2)	9	ND−0.050	10	ND−0.150	11	0.012−0.100	8	ND−0.480	10	ND−0.100	10	ND−0.170
MPEP	0.012 (2)	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
MCHP	0.010 (5)	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
MEHP	0.010 (1)	9	ND−0.120	11	0.021−0.580	11	0.023−0.460	11	0.020−1.30	10	ND−0.170	10	ND−0.870
MBZP	0.013 (4)	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
MOP	0.012 (4)	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
Diester													
methyl (DMP)	0.008	2	ND−0.010	2	ND−0.040	1	ND−0.052	3	ND−0.092	2	ND−0.010	3	ND−0.082
ethyl	0.004	0	ND	10	ND−0.071	10	ND−0.073	10	ND−0.310	3	ND−0.011	10	ND−0.120
iso-propyl	0.008	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
n-propyl	0.010	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
iso-butyl	0.002	0	ND	2	ND−0.022	1	ND−0.011	1	ND−0.033	0	ND	2	ND−0.023
n-butyl (DBP)	0.008	11	0.010−0.091	11	ND−0.230	10	ND−0.260	11	ND−0.540	10	ND−0.150	11	0.042−0.400
n-penthyl	0.002	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
cyclohexyl	0.002	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND
2-ethylhexyl (DEHP)	0.004	10	0.013−0.520	11	0.110−1.46	11	0.15−3.20	11	0.082−3.25	11	0.021−0.18	12	ND−3.60
benzyl-*n*-butyl	0.004	2	ND−0.010	8	ND−0.060	8	ND−0.020	9	ND−0.061	3	ND−0.012	11	ND−0.031
n-octhyl	0.006	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND

[a] ND, less than MDL; dt, detection times; sampling was conducted every month. [b] MDL (μg g^{-1}) for SS in parentheses. [c] Values were calculated by subtracting the values of the analysis for diesters from the values of the analysis for monoesters.

The previous reports suggest that the first biodegradation pathway for DBP and DEHP is the hydrolysis of the diesters to the corresponding monoesters and that the monoesters undergo enzymatic ring cleavage and then mineralize in river water and activated sludge (*26*), freshwater sediments (*27*), and freshwater hydrosoil (*28*). The estimated half-lifes of DBP and DEHP in estuarine and freshwaters under laboratory conditions ranged from 1 to 14 days (*29*, *30*) and from 5 to 35 days (*26*, *27*), respectively. The aerobic biodegradation of DBP, DEHP, di-*iso*-octyl phthalate, and di-*iso*-nonyl phthalate decreased at low temperatures (*27*). On the other hand, chemical hydrolysis of DEHP was not observed in practice; the half-life was estimated to be more than 100 years in water at pH 8 and 30 °C (*31*). In a study of humans exposed to DEHP, it was found that MEHP and some ω- and ω-1-hydroxylation products of MEHP were excreted in urine (*32*, *33*). As shown in Figure 3, the concentrations of MBP and MEHP in the Tama River water were slightly lower than those of DBP and DEHP, respectively, at most sampling sites and sampling times. The concentrations of MMP in the river water were higher than those of DMP. The concentrations of phthalic acid mono- and diesters at site 4 were low in the summer and high in the winter (Figure 3). These results suggest that the phthalic acid monoesters detected in the Tama River might be attributed to sewage containing the monoesters in urban areas or biodegradation of phthalic acid diesters by some microorganisms. Some monoesters might appear in the river that is contaminated by the diesters as well as in the Tama River.

In conclusion, these results prove that this analytical method for phthalic acid monoesters using soil-phase extraction and GC-MS was relatively simple and that it could be used to detect the analytes at nanogram per liter levels in river waters. The present monitoring indicated that MMP, MBP, and MEHP were detected in the Tama River in Tokyo at concentrations from nanogram to microgram per liter. The concentrations of MBP and MEHP were slightly lower than those of the corresponding phthalic acid diesters and higher in the winter than in the summer. Thus far, interest in phthalate esters in aquatic environments has been mainly focused on diesters. The phthalic acid monoesters such as MEHP have several modes of biological action in mammals. The study of phthalic acid monoesters in environmental water

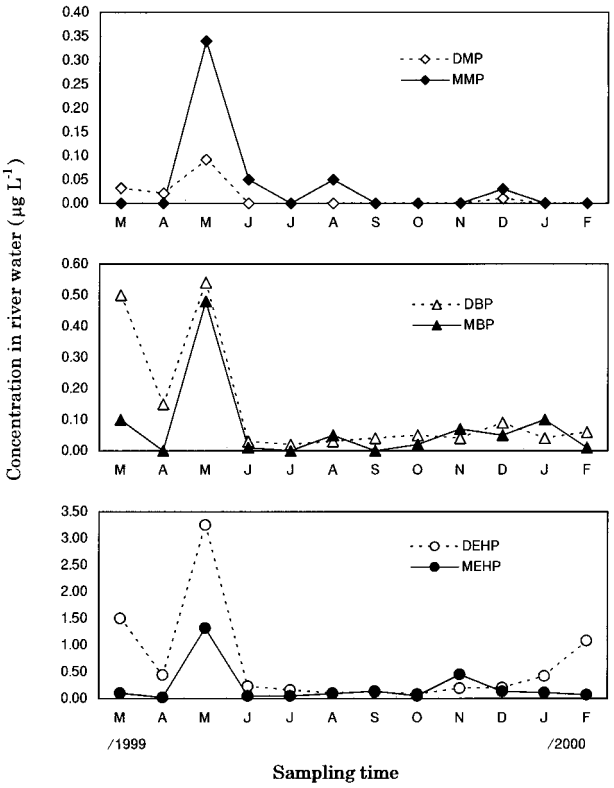

FIGURE 3. Seasonal changes of phthalic acid mono- and diesters in river waters at sampling site 4.

should be conducted in addition to the corresponding phthalic acid diesters.

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