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Ultra-Trace Determination of Phthalate Ester Metabolites in Seawater, Sediments, and Biota from an Urbanized Marine Inlet by LC/ESI-MS/MS

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This study presents results of an analytical method developed for the quantification of monoalkyl phthalate esters (MPEs) in seawater, sediments, and biota. The method uses accelerated solvent extraction, solid-phase extraction, and liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS). Results show the method is robust and can provide trace measurement of several MPE analytes at low parts per trillion levels in water and low parts per billion levels in sediments and biological tissues. Analyte recoveries varied between 70% and 110%. Method detection limits (MDLs) varied between 0.19 and 3.98 ng/L in seawater and between 0.024 and 0.99 ng/g in sediment and biota, which is approximately 10−50 times lower than previously reported MDLs using gas chromatography mass spectrometry. We applied the method to field collected samples of seawater, sediments, and tissues of mussels, crabs, and fish from False Creek, an urbanized marine inlet near Vancouver, Canada. The results indicate residues of several MPEs can be found in surface waters, sediments, and organism tissues of this marine system. Monoethyl phthalate (MEP), mono-*n*-butyl phthalate (M*n*BP), and mono-(2-ethylhexyl)-phthalate (MEHP) were frequently detected in all matrices. M*n*BP generally exhibited the highest concentrations among MPEs analyzed. Detectable concentrations of MPEs varied from 1 to 600 ng/L in seawater, 0.1 to 20 ng/g dry wt in sediments, and 0.1 to 600 ng/g wet wt in biota. Observed concentrations of low molecular weight MPEs in mussels were found to be significantly higher ($P < 0.05$) than those of corresponding parent DPEs (e.g., M*n*BP > DBP). Mono-*iso*-nonyl-phthalate (MoC9) and mono-*iso*-decyl phthalate (MoC10), which were routinely detected in water and sediments, were not detected in False Creek biota, indicating negligible uptake and/or in vivo bioformation of these high molecular weight MPEs. The ability to measure MPEs in complex environmental samples provided by this LC/ESI-MS/MS method expands the capability for future biomonitoring and risk assessment of phthalate plasticizers.

Introduction

Dialkyl phthalate esters (DPEs) are high production volume (HPV) chemicals used widely as commercial plasticizers and in various applications and products, including textiles, medical equipment, electronics, and personal care products (*1*). Discharge of phthalates into the environment can occur via industrial, municipal, and household waste streams (*2, 3*). Worldwide production of DPEs is estimated at approximately five million tons per year (*4*). DPEs have been detected in environmental samples (*5, 6*) as well as tissues and fluids of wildlife and humans (*7, 8*). Biomonitoring of DPEs is important because due to the fact elevated exposure to some of these compounds can cause reproductive and developmental impacts in animals (*9*−*11*).

Monoalkyl phthalate esters (MPEs) are the primary degradation and/or biotransformation products of DPEs. MPEs can be formed via abiotic and microbial degradation of DPEs in sediments, soils, and water (*12*−*14*). DPEs do not biomagnify in aquatic organisms and food webs (*7, 15*), likely due to metabolic transformation of those compounds to corresponding MPEs (*12*−*14, 16*−*19*). Pharmacokinetic analyses of DPEs in laboratory animals show that DPE metabolism involves (i) the hydrolysis of DPE to MPE and corresponding alcohol, (ii) the β-oxidation of the alcohol via a carboxylic intermediate to acetate and carbon dioxide, (iii) various oxidations (via microsomal mixed function oxidases and mitochondrial enzymes) of the alkyl chain of the MPE, and (iv) conjugation of the MPE with glucuronic acid by uridine 5′-diphospho-glucuronosyltransferase (UDPGT) (*16*−*19*), (Figure 1). Given the large production volumes of commercial phthalate ester plasticizers, monitoring of key degradation products and metabolites (i.e., MPEs) in environmental media and wildlife is important and will aid in the assessments of the impacts of phthalate ester use.

Several studies have reported MPE residues in human fluids, including saliva, urine, serum, and milk (*8, 20*−*31*). However, MPE concentration data in environmental media and wildlife are sparse in part because of the lack of analytical methods with adequate sensitivity and specificity for those matrices. Developing robust analytical techniques for ultratrace MPE analysis in complex environmental and biological matrices is an important first step toward monitoring the occurrence and distribution of these compounds in natural environments.

Previous studies have reported analytical methods for the identification and quantification of MPEs in aqueous solution (*23, 32, 33*), river water and suspended solids (*5*), marine sediments (*34*), and biological fluids (*20*−*25, 28, 31, 32, 35*). These methods have utilized a variety of instrumentation, including HPLC-UV (*29, 32*), GC-FID (*30*), GC-ECD (*33*), LC/ESI-MS/MS (*21*), LC/APCI-MS/MS (*25*), and GC/MS (*5, 20, 34*). Also, a variety of sample enrichment and purification approaches have been investigated, including solid phase extraction (*21, 22, 31*), solid phase microextraction (*23*), and column chromatography (*20*). To our knowledge, there are no published methods for extraction and analysis of MPEs in biological tissues. Generating accurate MPE concentration data for water, sediments, and aquatic organisms is critical for better understanding the long-term fate of these widely used commercial substances.

The objectives of the present study are to develop and evaluate analytical techniques for ultratrace analysis of MPEs

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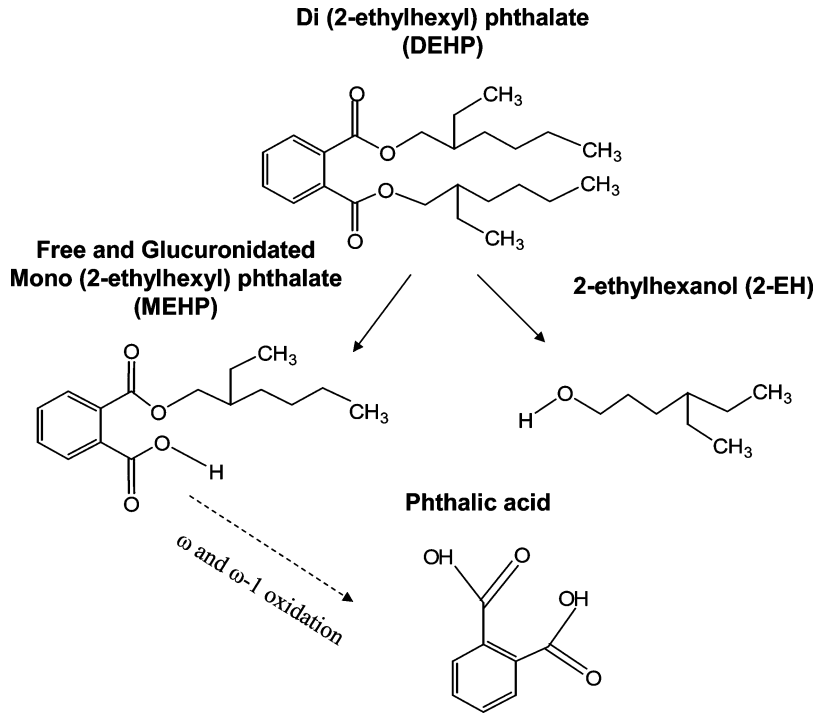

FIGURE 1. Schematic illustration of in vivo biotransformation of di-(2-ethyl hexyl) phthalate (DEHP).

and to apply these techniques to determine current MPE concentrations in water, sediment, and biota of an aquatic ecosystem. Specifically, we present novel methods for the extraction, cleanup, and liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) quantification of MPEs at ppt levels in seawater and ppb levels in sediments and aquatic biota. We compared the separation and quantification of MPEs using LC/ESI-MS/MS to those achieved using previously described GC/MS-based methods (*5*). Using the newly developed LC/ESI-MS/MS method, we measured concentrations of six single MPE isomers and four MPE isomeric mixtures in field collected samples of seawater, sediments, bivalves, crabs, and fish collected from an urbanized marine inlet in Vancouver, Canada. MPE concentrations were compared to measured concentrations of the corresponding parent DPEs.

Nomenclature. This study investigates six single MPE isomers [monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (M*n*BP), monobenzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP), and mono-*n*-octyl phthalate (M*n*OP)] and four MPE isomeric mixtures [mono-*iso*-hexyl phthalate (MoC6), mono-*iso*-heptyl phthalate (MoC7), mono-*iso*-nonyl phthalate (MoC9), and mono-*iso*-decyl phthalate (MoC10)], where the numbers represent the monoester alkyl chain length. Physical−chemical properties of MPEs, including molecular weights (MW, g/mol), aqueous solubility (C_{Sw}, mg/L), acid dissociation constants (pK_a), octanol−water partition coefficients (log K_{OW}), and distribution coefficients (log *D*) are shown in Table S1 of the Supporting Information.

Experimental Section

Materials. Standards of MMP, MEP, M*n*BP, MBzP, MEHP, M*n*OP, MoC7, MoC9, and MoC10 were obtained from Exxon Mobile Biomedical Laboratory (Annandale, NJ). MoC6 was purchased from Waco Chemicals USA, Inc. (Richmond, VA). Native monoiso-nonyl phthalate (MiNP) as well as three isotope-labeled internal surrogate standards (IS) (i.e., MEP-$^{13}C_4$, M*n*BP-$^{13}C_4$ and MEHP-$^{13}C_4$) and one isotope-labeled method performance or recovery standard (RS), (i.e., MiNP-$^{13}C_4$) were purchased from Cambridge Isotope Laboratories (Andover, MA). Individual stock (range, 0.1−100 ng/μL) and calibration solutions (range, 0.1−250 pg/μL) were prepared in acetonitrile and stored at 4 °C in the dark (Table S2 of the Supporting Information). All solvents were HPLC grade (EMD Chemicals, Darmstadt, Germany), and reagent water was high purity HPLC grade (Fisher, Fairlawn, NJ). Solid phase extraction cartridges, OasisHLB 500 mg/6 cm^3 (for water samples) and OasisMAX 150 mg/6 cm^3 (for sediments and tissue samples), were purchased from Waters Corp. (Milford, MA). Ottawa sand standard 20−30 mesh, 28% ammonium hydroxide, and formic acid were purchased from Fisher (Fairlawn, NJ), Anachemia (Ville St-Pierre, QC), and Fluka (Switzerland), respectively.

Samples. From May to September, 2004−2006, samples of seawater, marine sediments, invertebrates, and fish were collected from False Creek, an urbanized marine inlet, in Vancouver, British Columbia. This marine system (False Creek) is the same location as our previous field study of DPEs between 1999−2001 (*7, 36*) (Figure S1 of the Supporting Information). Individual water samples were collected from four unique locations in the inlet using 4 L glass bottles, which had been rinsed with water, acetone, and methanol, then baked overnight at 350 °C, and rinsed several times with methanol. Water samples were stored at 4 °C before analysis. Bottom sediments (top 10 cm layer) were collected using a solvent-rinsed petit ponar. Samples were placed on solvent-rinsed aluminum, while the top 0.5−1.0 cm layer was removed with a metal spoon and transferred into a glass vial. Blue mussels (*Mytilus edulis*) were collected along the intertidal region during low tide and stored in clean jars. Stainless steel crab and prawn traps with bait were used to collect samples of Dungeness crabs (*Cancer magister*) and white-spotted greenling (*Hexogrammos stelleri*). Collected sediments, mussels, crabs, and fish were kept at −20 °C in the dark before being analyzed. To compare MPE concentrations to corresponding parent compounds (DPEs), we also analyzed field samples for individual DPEs, including dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*iso*-butyl phthalate (DIBP), di-*n*-butyl phthalate (DBP), benzyl-butyl phthalate (BBP), di-(2-ethyl hexyl) phthalate (DEHP), di-*n*-octyl phthalate (DnOP), and di-*n*-nonyl phthalate (DnNP)

as well as the DPE isomeric mixtures (C6−C10), using GC/MS and LC/ESI-MS/MS methods (*36*).

Sample Extraction and Cleanup. Prior to LC/ESI-MS/MS analysis, MPEs were extracted from tissue and sediment samples using accelerated solvent extraction (ASE 200, Dionex Corporation, Sunnyvale, CA). Tissue samples were thawed, dissected, and homogenized (Sorval Omni-Mixer) prior to extraction. Sorval Omni-Mixer parts were cleaned between samples by washing with water, acetone, methanol, and dichloromethane. Sediment samples were homogenized in a similar fashion. Approximately 10 g of sediment, 10 g of fish and crab muscle tissue, or 2 g of mussel homogenate was weighed and spiked with a 25 μL solution containing isotope-labeled internal standards (300 ng each of MEP-$^{13}C_4$, M*n*BP-$^{13}C_4$, and MEHP-$^{13}C_4$) and blended with ∼10 g Ottawa sand, previously baked at 500 °C. Samples were further homogenized with mortar and pestle and transferred to 33 mL stainless steel ASE cells, with any void filled with Ottawa sand. I-Chem vials used for collecting extracts were dish washed and rinsed with acetone and methanol. Water was chosen as the extracting solvent for its compatibility with SPE cleanup and selectivity, minimizing coextractive interferences. Quantitative recoveries were achieved using the following ASE conditions: 70 °C, 700 psi, heating (5 min), static step (5 min), and a 60% flush through 3 cycles. Extracts were allowed to cool to room temperature before adding glacial acetic acid (10 drops) to adjust the pH to ∼2.5. Tissue and sediment extracts were further cleaned using 150 mg Oasis MAX cartridges, which were conditioned with 5 mL of acetonitrile, 5 mL of methanol, and 5 mL of water. Extracts were passed through the cartridge at 1 mL/min, followed by washes of 6 mL of 2% ammonium hydroxide, and 6 mL of methanol. MPEs were finally eluted with 6 mL of 2% acetic acid in methanol.

Seawater samples of 500 mL were acidified to pH ∼2.5 by adding concentrated formic acid. Water samples were first passed through 500 mg Oasis HLB cartridges and then conditioned with 5 mL methanol and 5 mL water. Cartridges were then washed with 3 mL of water, followed by 6 mL of a 35% methanol/water solution. MPEs were finally eluted with 5 mL of ethyl acetate, followed by 5 mL of acetonitrile. Samples were evaporated to 0.5 mL under a gentle stream of high purity nitrogen and transferred with methanol to 2 mL autosampler vials and evaporated to near dryness. Eluates were evaporated to dryness to remove any formic acid. All extracts were resuspended in 0.5 mL of methanol and spiked with 50 μL (600 ng) of recovery standard (MiNP-$^{13}C_4$) prior to LC/ESI-MS/MS.

Analysis of MPEs via LC/ESI-MS/MS. The HPLC system used was a Dionex P680 (Dionex, Sunnyvale, CA), and separations were performed on a 150 mm × 3 mm i.d. stainless steel analytical column packed with Synergi RP-MAX 4um C12 (Phenomenex, Torrance, CA). The column temperature was kept constant at 28 °C using a Dionex TCC-100 thermostatted column compartment. All compounds of interest were eluted from the column in a single chromatographic run using a 10 μL injection volume and gradient elution program operating at 300ul/min. Mobile phase A was 0.01% acetic acid in methanol, and mobile phase B was 0.01% acetic acid in HPLC grade water. The gradient program was 80% A and 20% B held for 5 min, increased to 90% A and 10% B over 2.5 min, held at 90% A for 12.5 min, and then returned to initial conditions (80% A, 20% B) over 1 min and held for 9 min. Mass analysis was carried out using a Sciex API 5000 mass spectrometer (MDS Sciex, Ontario, Canada) operating in negative electrospray ionization multiple reaction monitoring (MRM) mode. Two transitions for most analytes were monitored (Table S3 of the Supporting Information), except monobenzyl phthalate (MBzP), for which only one suitable transition was monitored, with a dwell time of 40 ms. Optimal source parameters were as follows: ionspray voltage −4500 V, curtain gas flow 20 arbitrary units (au), nebulizer gas flow 40 au, turbo-ion spray gas flow 40 au, and turbo-ion spray temperature 300 °C.

Quantitation and QA/QC. Quantification was based on 10-point calibration curves generated for each analyte (calibration standard range CS1−CS10 = 0.1−250 pg/μL) (Table S2 of the Supporting Information). Surrogate internal and recovery standards were added to all samples, and analyte concentrations were all IS recovery corrected. The criteria for quantitation were (a) the representative ion of the specific analyte was detected at the exact m/z at unit resolution during the entire chromatographic run, (b) the retention time of a specific analyte had to be within a 15 s to that obtained during analysis of the authentic calibration standards, and (c) the signal-to-noise ratio of the representative ion had to be ≥3. Procedural or method blanks (n = 2) and a sample duplicate (n = 1) were employed with every batch of 12 samples to monitor potential background contamination and reproducibility. Method detection limits (MDLs) for individual MPEs were calculated on the basis of 3 times the signal-to-noise in low-level spike samples.

To determine whether DPEs were being converted to MPEs during sample handling, extraction, and cleanup, we conducted a series of spike matrix experiments using native DPEs. We were particularly interested in assessing the potential conversion of DPEs to MPEs during ASE extraction and SPE cleanup, which exposes the samples to temperatures of 70 °C, pressures of 700 psi, and a pH range of 2.5 to 12.5. Specifically, samples of fish tissue (n = 3) and sediments (n = 3) were spiked with 25 μL of a DPE mixture prepared in methanol (containing 500−1000 ng each of DMP, DEP, DIBP, DBP, BBP, DEHP, DnOP, and DnNP) and carried through the extraction and cleanup procedure. Triplicate analyses of those unspiked matrices were then conducted in the same manner. Resulting MPE levels in spiked and unspiked samples were then measured by LC/ESI-MS/MS.

Spike Recovery Experiments and Method Validation Parameters. To evaluate the accuracy and precision of MPE separation and quantification using this LC/ESI-MS/MS method, we conducted several spike recovery experiments. In particular, 10 g of marine sediments from a remote location (i.e., clean matrix) was spiked with the 10 native MPEs in triplicate at concentrations of 100 and 300 ng/g. Similarly, triplicate samples of seawater were spiked with native MPEs at 200 ng/L. Three procedural blanks were included with each triplicate analysis. Average recoveries and relative standard deviations (RSD, %) were determined for triplicate analyses. In addition, average recoveries and RSDs were determined for isotope-labeled MPE surrogates ($^{13}C_{12}$ MEP, $^{13}C_{12}$M*n*BP, and $^{13}C_{12}$ MEHP) spiked into water (n = 9), sediments, (n = 10), mussels (n = 10), fish muscle (n = 9), and Ottawa Sea Sand (n = 8).

Comparative Analyses Using GC/MS. For comparison, we investigated the use of GC/MS for separation and quantification of MPEs. Specifically, we utilized a previously reported method by Suzuki et al. (*5*), which involved solid-phase extraction (SPE) and GC/MS determination of MPEs in water, to determine MPEs in False Creek water samples. This method requires derivatization (methylation) of MPEs with diazomethane prior to GC/MS. Separation and quantification performance using GC/MS was compared to results using the newly developed LC/ESI-MS/MS method. Details regarding SPE and GC/MS analysis of MPEs in water samples are available in ref *5*.

Data Analysis and Statistics. Chemical concentration data were reported as geometric means (GM), in units of ng/L in seawater, ng/g dry wt in sediments, and ng/g wet wt in biota. Asymmetric errors were calculated as 1 standard deviation (SD) and 95% confidence intervals (CI_{95}). Mean concentra-

TABLE 1. List of Mono-Alkyl Phthalate Esters (MPEs) and Corresponding LC/ESI-MS/MS Parameters[a]

analyte	LC/ESI-MS/MS parameters: ion transition (*m*/*z*) primary → secondary	LC/ESI-MS/MS parameters: RT (min)	method validation parameters: sediment (100 ng/g) % Rec (RSD) *n* = 3	method validation parameters: sediment (300 ng/g) % Rec (RSD) *n* = 3	method validation parameters: seawater (200 ng/L) % Rec (RSD) *n* = 3	MDL water (ng/L)	MDL sediment (ng/g)	MDL tissue (ng/g)
monomethyl phthalate (MMP)	179 →107, 77	5.38	42 (10)	34 (5)	63 (6)	1.6	0.08	0.39
monoethyl phthalate (MEP)	193→121, 77	5.88	84 (5)	70 (2)	77 (17)	3.9	0.20	0.99
monobutyl phthalate (M*n*BP)	221→77, 149	8.24	98 (1)	91 (3)	86 (12)	1.7	0.08	0.42
monobenzyl phthalate (MBzP)	225→147	8.34	99 (4)	90 (6)	122 (11)	0.22	0.01	0.06
mono-*iso*-hexyl phthalate (MoC6)	249→121, 77	11.1	106 (1)	104 (7)	119 (8)	1.3	0.07	0.33
mono-*iso*-heptyl phthalate (MoC7)	263→113, 121	12.2	109 (1)	104 (4)	93 (12)	0.67	0.03	0.17
mono-2-ethylhexyl phthalate (MEHP)	277→134, 119	13.2	104 (1)	104 (5)	116 (18)	0.19	0.01	0.05
mono-*n*-octyl phthalate (M*n*OP)	277→127, 119	14.3	102 (1)	101 (5)	93 (2)	0.50	0.03	0.14
mono-*iso*-nonyl phthalate (MoC9)	291→139, 121	14.1	103 (2)	99 (1)	100 (3)	0.48	0.02	0.12
mono-*iso*-decyl phthalate (MoC10)	305→155, 121	16.3	82 (8)	87 (7)	58 (7)	2.7	0.13	0.67

[a] Parameters include retention times (RT), mass transitions (*m*/*z*), and method validation parameters; % recoveries and method detection limits (MDLs) are for seawater, sediments, and tissues.

TABLE 2. Recoveries of Internal Standards and % RSD of Mono-Alkyl Phthalate Esters in Various Matrices

matrix	*n*	^{13}C-MEP % recovery (RSD)	^{13}C-M*n*BP % recovery (RSD)	^{13}C-MEHP % recovery (RSD)
seawater	9	50 (14)	72 (21)	71 (25)
sediment	10	83 (5)	86 (4)	99 (6)
mussels	10	73 (4)	99 (5)	118 (6)
greenling	9	50 (14)	72 (21)	71 (25)
sand	8	81 (8)	89 (6)	97 (13)

tions were only calculated if frequency of detection was ≥60%. Also, when calculating means, we substituted a value of 1/2 MDL for nondetectable concentrations. One-way analysis of variance (ANOVA) tests were used to assess concentration differences between different compounds (e.g., MPE versus DPE) and between different matrices and organisms (mussels versus fish).

Results and Discussion

LC/ESI-MS/MS Method Performance. Results of spike recovery experiments are summarized in Table 1. Recoveries of native MPEs in sediment varied from 70% to 109%, except for MMP, which exhibited recoveries between 34% and 42%. The relative standard deviation (RSD, %) for individual analytes was ≤10%, demonstrating good precision. Recoveries of native MPEs in spiked seawater varied from 58% to 122%, with RSDs < 18%.

Average surrogate recoveries were also generally high for all matrices (Table 2). Recoveries varied from 71% to 118%, except for ^{13}C-MEP, which exhibited lower recoveries (50% on average) in seawater and muscle tissue. Lower recoveries of ^{13}C-MEP were likely attributed to poor retention of the more polar monoester during loading of the solid phase extraction cartridge. Taking into account the wide variation in the matrices between samples, even within similar sample types, we found the method performed well, with RSDs varying from 4% to 25% for all internal standards.

MDLs ranged between 0.024 and 0.20 ng/g in sediment and fish muscle, 0.047 and 0.99 ng/g in mussels, and 0.19 ng/L and 3.98 ng/L in seawater (Table 1). MDLs of individual MPEs in seawater and sediments using this LC/ESI-MS/MS method are 10−50 times lower compared to those previously reported by GC/MS (*5*). For example, Suziki et al. (*5*) reported GC/MS-derived MDLs in river water of 10 ng/L for M*n*BP and MEHP and 30 ng/L for MMP.

DPE spiking experiments showed no apparent conversion of DPEs to MPEs during sample extraction and cleanup. Specifically, concentrations of MPEs observed in unspiked and spiked matrices exhibited no significant difference ($p < 0.05$). This indicates that endogenous levels of DPEs as well as potential DPE contamination that may occur during sample preparation and extraction will not impact the concentration measurement of MPEs during sample analysis. Suzuki et al. (*5*) also observed no hydrolysis of DPEs to MPEs during preparation of river water samples under acidic conditions. Calafat et al. (*31*) previously observed the degradation of DPEs to MPEs in milk samples (via hydrolysis by milk esterase). However, employment of formic or phosphoric acid effectively halts this activity (*31*). In the same study, the authors observed no significant conversion of MPEs to phthalic acid during preparation of milk samples (*31*).

Advantages of LC/ESI-MS/MS over GC/MS. Previous studies have utilized GC/MS to quantify MPEs in water and saliva (*5, 20*). MPEs measured by GC/MS requires derivatization (methylation or ethylation). One limitation of this approach is that MMP (in the case of methylation) and MEP (in the case of ethylation) are transformed into their corresponding DMP and DEP, respectively. Residues of DMP or DEP, already present in samples and from background contamination during derivatization, may artificially elevate levels of MMP or MEP. Using this approach, therefore, requires us to simultaneously determine the corresponding diesters (i.e., DMP or DEP) before and after derivatization.

Lin et al. (*36*) previously demonstrated that LC/ESI-MS was superior to GC/MS for the separation and quantification of isomeric mixtures of the phthalate diesters (C6−C10) in environmental and biological samples. Accurate determination of high molecular weight (MW) DPE isomeric mixtures in the environment is important because of their high production volumes (*37*). Determination of the corresponding high MW MPE isomeric mixtures is equally important. The LC/ESI-MS/MS method presented in this study enables the direct measurement of isomeric mixtures of monoesters in water, sediments, and biota. A comparison of GC/MS and LC/ESI-MS/MS chromatograms for the isomeric mixtures of MoC6, MoC7, MoC9, and MoC10 is shown in Figure S2 of the Supporting Information. Under GC/MS conditions with electron impact (EI) ionization, the fragment at *m*/*z* 163 is the common ion for most MPEs (once methylated). This is a major limitation in using GC/MS for the determination of MPE isomeric mixtures by GC/MS, primarily because of the occurrence of coeluting isomers with varying composition of alkyl substitution. For example, we observed a high degree of overlap between MoC9 and MoC10 isomers as well as C6 and C7 during GC/MS analysis of methylated MPEs (Figure S2 of the Supporting Information). LC/ESI-MS/MS allows

TABLE 3. Range (min−max) of Mono-Alkyl Phthalate Ester Concentrations in Seawater, Sediments, and Aquatic Biota from False Creek, Vancouver, Canada[a]

	sediment (ng/g dry wt) (n = 10)	seawater (ng/L) (n = 10)	blue mussels (ng/g wet wt) (n = 10)	Dungeness crab (ng/g wet wt) (n = 10)	white spotted greenling (ng/g wet wt) (n = 10)
			individual MPEs		
MMP	1.28−4.16 (100%)	0.42−20.1 (100%)	4.43−21.82 (100%)	ND (0%)	ND (0%)
MEP	0.45−3.63 (100%)	4.41−38.83 (100%)	5.63−25.54 (100%)	0.29−2.61 (100%)	ND (0/10)
M*n*BP	5.30−20.11 (100%)	50.9−107.8 (100%)	75.0−585 (100%)	8.66−38.2 (100%)	6.63−60.9 (90%)
MBzP	0.19−3.02 (100%)	ND−6.05 (90%)	ND−1.74 (90%)	ND (0/10)	ND (0/10)
MEHP	0.33−0.84 (100%)	45.49−57.2 (100%)	3.30−6.72 (100%)	0.39−1.13 (100%)	0.24−1.1 (90%)
M*n*OP	0.04−0.20 (100%)	ND−1.06 (10%)	0.34−0.43 (100%)	ND (0%)	ND (0%)
			isomeric mixtures		
MoC6	ND−0.53 (90%)	ND−0.52 (90%)	0.61−1.55 (100%)	ND (0%)	ND (0%)
MoC7	0.05−0.07 (100%)	2.71−6.61 (100%)	1.83−2.97 (100%)	0.017−0.31 (100%)	ND (0%)
MoC9	0.01−1.83 (100%)	ND−29.30 (60%)	ND (0%)	ND (0%)	ND (0%)
MoC10	ND−0.14 (90%)	2.23−8.25 (100%)	ND (0%)	ND (0%)	ND (0%)

[a] Values in parentheses represent the frequency of detection (% samples detected). ND = not detectable.

for the monitoring of specific ions $[M - H]^-$ and subsequent transitions for each of the MPEs. Thus, while LC/ESI-MS/MS cannot resolve individual isomers within a given isomeric mixture, it does allow for the complete separation and quantification of individual isomeric mixture homologues (i.e., MoC6, MoC7, MoC9, and MoC10) (Figure S2 of the Supporting Information).

Occurrence and Distribution of MPEs in an Urbanized Marine Inlet. Observed concentration ranges and frequency of detection (%) of MPEs in samples from False Creek, including seawater (n = 10), sediments (n = 10), mussels (n = 10), Dungeness crabs (n = 10), and white spotted greenling (n = 10), are summarized in Table 3. The LC/ESI-MS/MS method provided baseline resolution of all targeted analytes.

High MW MPEs (MoC9 and MoC10), which were routinely detected in water and sediments, were not detected in samples of mussels, crabs, or fish from False Creek (Table 3). Mean concentrations of the various MPEs in seawater ranged between 0.31 and 63 ng/L, levels which are several orders of magnitude below laboratory-derived effect concentrations (*38*). The dominant MPEs in seawater were M*n*BP and MEHP, which combined accounted for 80% of total MPEs. Suzuki et al. (*5*) reported similar findings of MPEs in river water from Japan. Mean concentrations of MPEs in sediments varied from 0.06 to 9.92 ng/g dry wt. Lower MW monoesters (MMP, MEP, and M*n*BP) comprised 90% of total MPEs in sediments. Previous analyses of DPEs in False Creek sediments (*36*) showed C8 (mainly DEHP) was the dominant DPE (comprising 70% of total DPE burden), along with higher MW diesters, C9 and C10 (25%). Low MW diesters (DMP, DEP, DiBP, DnBP, and BBP) accounted for the remaining 5%.

Mean concentrations of MPEs in mussels ranged from 0.61 ng/g wet wt for MoC6 to 183 ng/g wet wt for M*n*BP. M*n*BP was the dominant monoester in mussels, followed by MEP, MMP, and MEHP. Only MEP, M*n*BP, MEHP, and MoC7 were detected in crab tissue. In fish tissue (Greenling), M*n*BP and MEHP were the only MPEs detected (Table 3). Observed concentrations of M*n*BP and MEHP in crabs and white spotted greenling were significantly lower (P < 0.05) compared to those in mussels. For example, mean concentrations of M*n*BP in Dungeness crabs (mean = 18.2 ng/g, CI_{95} = 4.3−76.4) and white-spotted greenling (mean = 11.1 ng/g, CI_{95} = 2.1−60.8), were an order of magnitude lower than M*n*BP levels observed in blue mussels (mean = 183 ng/g, CI_{95} = 33.9−989).

A comparison of MPE and DPE concentrations observed in seawater, sediments, and blue mussels from False Creek is shown in Figure 2. MPE concentrations in sediments were substantially lower than DPE sediment concentrations. MEHP

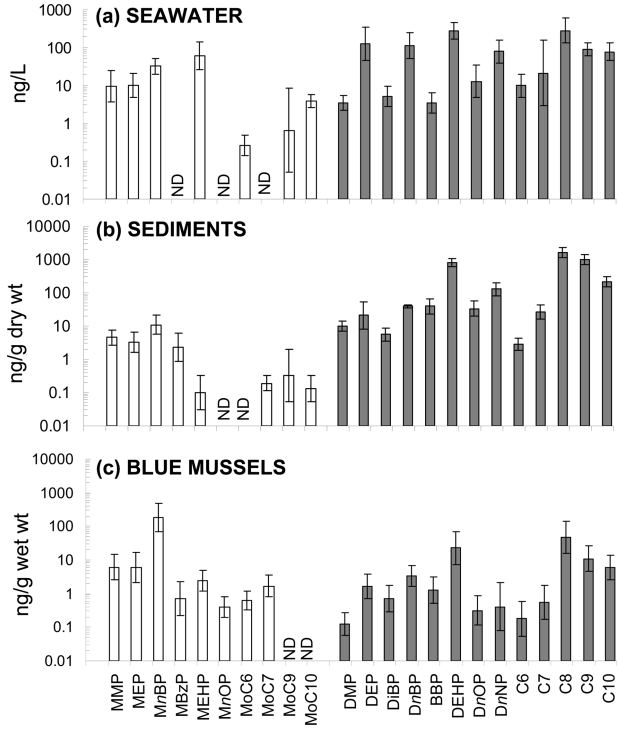

FIGURE 2. Measured concentrations of monoalkyl phthalate esters (white bars) and dialkyl phthalate esters (gray bars) in (a) seawater (ng/L), (b) sediment (ng/g dry wt), and (c) blue mussels (ng/g wet wt) from False Creek, Vancouver, BC. Plotted data are geometric means (bars), and errors represent the range of 1 SD.

was not detected in sediments (<0.01 ng/g), while mean DEHP concentrations exceeded 800 ng/g dry wt. In contrast, MPE concentrations in seawater and biota were generally comparable but somewhat lower than DPE concentrations in those matrices. Conversely, concentrations of low MW MPE (MEP, MMP, and M*n*BP) were significantly higher (P < 0.05) than corresponding DPE concentrations in mussels. The reason for an increased prevalence of low MW MPEs in mussels may be due to higher rates of degradation and/or biotransformation of corresponding low MW DPEs.

Concentrations of high MW DPEs (C9, C10) in biota were relatively low, despite a predominance of those compounds in sediments. Lin et al. (*36*) reported similar DPE profiles, which was attributed to the fact these very hydrophobic DPEs are likely sorbed to particulate and dissolved matter, thereby reducing their bioavailability. Similarly, high MW MPEs,

which are detected in water and sediments, are absent in biota, indicating negligible uptake or bioformation of these compounds.

Implications for Risk Assessment. MPEs are relatively water-soluble ionic compounds formed through the degradation and/or metabolism of DPEs. Because the pK_a of MPEs ranges between approximately 3.6 and 4.2 (Table S1 of the Supporting Information), MPEs are expected to be present as ionized molecules in a typical marine environment. Their estimated log D values, which range from −0.06 to 4 at pH 6, are lower than those of corresponding phthalate diesters, indicating smaller partition coefficients into organic carbon and lipids of biota. Also, free and conjugated forms of MPEs can also be further metabolized and eliminated by organisms (*16*−*19*). However, the results of the present study indicate residues of several MPEs can be found in surface waters, sediments, and organisms from an urban marine environment.

This study provides a simple analytical method for rapid quantitative determination of monoalkyl phthalate esters by LC/ESI-MS/MS in various environmental and biological matrices. The method is robust and can provide trace measurement of several MPE analytes at low parts per trillion levels in water and low parts per billion levels in sediments and biological tissues. Future investigations will focus on evaluating the sources, fate, and bioaccumulation behavior of individual DPEs and corresponding MPEs in this urban marine food web. This work aims to strengthen future biomonitoring and risk assessments of phthalate ester plasticizers.

Supporting Information Available

Physical−chemical properties of MPEs; calibration solution composition and concentrations and spiking amounts of internal surrogate and recovery standards; analyte retention times, ion transitions, and LC/ESI-MS/MS operating conditions and optimal source parameters (Tables S1−S3); map of study area; and chromatographs showing MoC6, MoC7, MoC9, and MoC10 isomeric mixtures by GC/MS and LC/ESI-MS/MS (Figures S1−S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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