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Determination of Phthalate Ester Congeners and Mixtures by LC/ESI-MS in Sediments and Biota of an Urbanized Marine Inlet

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Phthalate esters (PEs) are a group of widely used commercial chemicals consisting of many different congeners. Concentrations of di(2-ethylhexyl) phthalate ester in the parts per million range have been observed in sediments from locations in North America and Europe. However, sediment and biota concentrations of other widely used PEs (i.e., dibutyl phthalate, diisononyl phthalate, and diisodecyl phthalate) are rare and often in doubt because of analytical difficulties. One of the problems is that commercial formulations predominantly consist of PEs with a specific molecular weight but include many isomers within each molecular weight class. Currently there are no analytical methods or required standards to fully separate PEs into the different molecular weight classes corresponding to the formulations from which they originate. Hence, ambient total and mixture-specific PE concentrations do not exist. This study presents a new method based on reversed-phase liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) for the quantitative determination of individual PEs, including six congeners on the U.S. EPA Priority pollutant list and several commercial PE isomeric mixtures, in complex environmental matrixes. The method is applied to determine the composition of PE concentrations in sediments and fish in an urbanized marine ecosystem. PE fingerprints in sediments show a predominance of high molecular weight PEs and match per capita consumption levels of PEs. Fingerprints in fish tissue show a predominance of low molecular weight PEs and do not match per capita consumption levels. The findings indicate that the higher molecular weight PEs are less biologically available than the lower molecular weight ones.

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Introduction

Dialkyl phthalate esters (PEs, Figure 1) are widely used as nonreactive plasticizers in vinyl plastics and in a broad range of industrial and consumer products. They range in molecular weight from 194.2 for dimethyl phthalate to 530.8 for ditridecyl phthalate (*1*). Most of the commercial PEs with alkyl chain lengths equal to or less than 8 carbon atoms are single isomers. PEs with alkyl chain lengths greater than 8 carbon atoms are typically complex mixtures of isomers. The broad range in the alkyl moiety of the compounds results in a broad range of physical, chemical, toxicological, and environmental properties (*1, 2*).

Because of the extensive use, high rate of global production of approximately 4.0 million t/yr, and categorization as U.S. EPA Priority pollutants (*3*), the environmental fate of PEs have been extensively studied in the laboratory and the field (*4*−*11*). Concentrations of certain phthalate esters, in particular DEHP, have been observed in the parts per million range in sediments in North America and Europe. Information about sediment and biota concentrations of other PEs such as DnBP, diisononyl phthalate (DiNP), and diisodecyl phthalate (DIDP), which are some of most widely used plasticizers in the plastics industry, are relatively rare and often in doubt because of difficulties associated with their measurement. This has precluded an analysis of the distribution, persistence, food-chain bioaccumulation, and potential ecological impacts of these global pollutants at a time when several PEs have been listed as priority pollutants in the United States, Canada, and several European countries and management options are being considered.

Several techniques including GC, LC, IR, NMR, and TLC have been used for the analysis of phthalates (*12*−*24*). The most common techniques for PE determination in environmental samples are gas chromatography with detection through flame ionization (*14*), electron capture (*16*), and mass spectrometry (*18*). Despite the availability of techniques, the analysis of PEs in environmental samples is subject to a number of unresolved analytical challenges. First, sample contamination during collection and especially during sample preparation is an overriding issue in the analysis of PE but in particular when using GC-based methods (*16, 25*). These require extensive cleanup of sample extracts involving large amounts of reagents and solvents, which can introduce PEs into the sample matrix. Although low femptogram detection of individual PE isomers can be achieved with high-resolution GC/MS instrumentation, this becomes inapplicable as the overall method detection limit is in most cases substantially higher due to sample contamination during the extensive cleanup procedures required for GC/MS-based methods. Second, incomplete separation of the large number of isomers present in commercial mixtures, which is particularly prevalent for GC methods, precludes an accurate determination of specific PE congeners, isomers, or commercial mixtures. Under GC/MS conditions with electron impact (EI) ionization PEs fragment extensively, and the molecular ion is not detected. The base peak in the EI mass spectra of most PEs is the characteristic phthalic anhydride fragment with $m/z = 149$. This becomes a limitation in identifying and quantifying isomers with varying composition of alkyl substitution that coelute but do not produce distinct molecular or fragment ions to confirm their structure. Other

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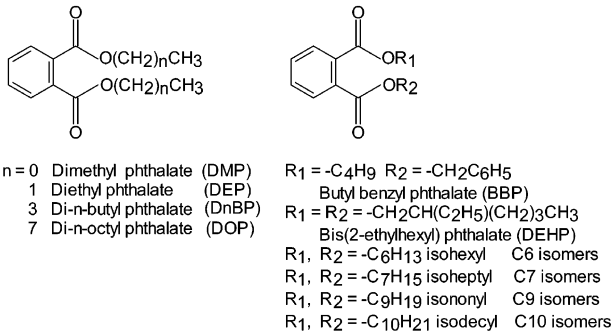

FIGURE 1. Structural formula of dialkyl phthalate esters.

GC-based methods with nonselective detectors such as ECD and FID similarly have limitations of nonspecificity. Grouping peaks in chromatograms within certain retention time "windows" has been used to address this problem, but this method is arbitrary and subject to error as the identity of the peaks is unclear and certain peaks may be incorrectly included or excluded. Third, analytical standards for many of the individual congeners in industrial PE mixtures are unavailable.

This paper presents the development, validation, and application of a novel analytical methodology based on liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) for the quantitative determination of PEs in complex environmental matrixes. The method relies on the formation of PE adducts $[M + Na]^+$ in LC/ESI-MS to separate and quantify PE mixtures even though the actual resolution of the LC chromatography is low. The lack of extensive cleanup steps to separate PEs from interfering compounds reduces the introduction of PEs into the sample during the process of sample preparation and analysis, hence reducing the detection limit of PEs. The paper explores the separation and quantitation of PEs using LC/ESI-MS and compares the new method to GC/MS, which has been the most commonly used method for PE determinations to date. Six individual PEs listed by the U.S. EPA as priority pollutants and five PE isomeric mixtures used in commercial products were measured at ultra-trace levels in the sediments and biota of an urbanized marine inlet to assess the sources and distribution profiles of these compounds in that ecosystem. Most of the prevalent lower molecular weight PEs as well as the higher molecular weight PE isomeric mixtures were detected in both sediments and biota. Detection of the higher molecular weight PE isomeric mixtures in environmental samples, at trace levels, has not been reported before. These compounds constitute a major percentage of the PEs used in various applications, and thus, their importance in understanding the fate of these compounds in the environment is significant, as described in this study.

Nomenclature

Single PE congeners are referred to by their systematic name (see Experimental Section). PE isomers are referred to as C6−C10, where the numbers correspond to the number of carbon atoms on each of the two alkyl chains.

Experimental Section

Materials. Standards of the individual phthalates: dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), and di(2-ethylhexyl) phthalate (DEHP) were purchased from Aldrich (Milwaukee, WI), and di-*n*-octyl phthalate (DnOP) was from American Biorganics (Niagara Falls, NY). Dinonyl phthalate (DnNP), diallyl phthalate (DAP), di-*n*-propyl phthalate (DnPP), bis-(2-methoxyethyl) phthalate (BMoEP), bis(4-methyl-2-pentyl) phthalate (BMPP), bis(2-ethoxyethyl) phthalate (BEoEP), di-*n*-pentyl phthalate (DnPeP), di-*n*-hexyl phthalate (DnHP), hexyl 2-ethylhexyl phthalate (HEHP), bis(2-*n*-butoxyethyl) phthalate (BBoEP), and dicyclohexyl phthalate (DCHP) were from AccuStandard (New Haven, CT); they are part of their EPA Method 8061 standard mixture. Standards of phthalate isomeric mixtures $(C_6H_4(COOR)_2$: R = C_6−C_{10}) [Jayflex DHP (mixture of C_6 isomers), Jayflex77 (mixture of C_7 isomers), and diisodecyl phthalate (mixture of C_{10} isomers)] were obtained from Exxon Chemical (New Milford, CT). Diisooctyl phthalate (mixture of C_8 isomers) was purchased from Aldrich, and diisononyl phthalate (mixture of C_9 isomers) was obtained from Aritech Chemical (Pittsburgh, PA). The isotope-labeled compounds DMP-d_4, DnBP-d_4, and DnOP-d_4 used as method internal standards (IS) and DEP-d_4 and BBP-d_4 used as method performance standards (PS) were purchased from Cambridge Isotope Laboratories (Andover, MA). Individual standard stock solutions were prepared at various concentrations in toluene as the spiking solutions were prepared in acetone. The calibration solutions were diluted from the stock solutions with methanol. All solutions were kept at 4 °C in the dark. Solvents were HPLC grade (EM Science, Darmstadt, Germany), and reagent water was high-purity HPLC grade (Burdick and Jackson, MI). Alumina (Neutral) was purchased from ICN Biomedicals (ICN Biomedicals, Meckenheim, Germany), and the Florisil (60−100 mesh) was purchased from Fischer Scientific (Pittsburgh, PA). Sodium acetate and anhydrous sodium sulfate (granular) were purchased from Aldrich.

Preparation of Glassware, Reagents, and Sampling Equipment. At the onset of our work, we realized that "distilled in glass" solvents contained considerable amounts of PEs and they were found to be the dominant source of background contamination. By distilling the solvents, we were able to reduce the level of background contamination 2−3-fold. As such, all the solvents used were doubly distilled. Later in our work we examined HPLC grade solvents for background PE contamination, and we realized that hexane was the only solvent that had considerable amounts of PEs. From that point onward we exclusively used HPLC grade solvents, and it was only necessary to double-distill the hexane. Glassware was detergent washed; rinsed with water, then acetone, doubly distilled hexane, and dichloromethane, respectively; baked at 400 °C for at least 10 h; and stored in clean aluminum foil. Prior to use, all glassware and sampling equipment (including the petit ponar) were rinsed with acetone, doubly distilled hexane, and dichloromethane, respectively. Mortar and pestle were cleaned using the same procedure as that for glassware but were baked at 150 °C for 10 h. Alumina and sodium sulfate were baked at 200 and 450 °C, respectively, for at least 24 h; cooled; and stored in a desiccator. Other materials such as Teflon stoppers, GC septa, and caps of sample vials (which decompose at elevated temperatures) were washed extensively with 1:1 dichloromethane/hexane (DCM/Hex).

Sample Collection. Marine surficial sediments and fish were collected at False Creek, a residential/industrial location in Vancouver's Inner Harbor. Four independent sediment samples were collected from each of four sampling stations, for a total of 16 samples. Surficial sediments were collected using a solvent-rinsed and oven-baked 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. Vials were kept at − 20 °C in the dark before being analyzed. Three striped seaperch (*Embiotoea lateralis*) were collected from each of three sampling stations in the harbor, for a total of 9 samples. The fish were collected with seine nets, euthanized, then immediately wrapped in solvent-rinsed aluminum foil, and kept at − 20 °C until analysis.

Sample Extraction and Cleanup. Fish muscle tissue was thawed, dissected, and homogenized prior to extraction. Skin

and bones were removed during dissection, and the tissue was homogenized with a Sorval Omni-Mixer. All parts of the Sorval Omni-Mixer were cleaned between samples following the same protocol as described above for the glassware. Following homogenization, samples were subsampled for PEs analysis and moisture and lipid contents where applicable. Sediment samples were also homogenized but not sieved prior to extraction. Approximately 2 g of sediment or 5 g of biota sample was weighed, spiked with the suite of surrogate internal standards described above, blended with 15−20 g of prebaked Na_2SO_4, and ground with mortar and pestle to a free-flowing powder. The homogenate was placed in a flask, extracted with 50 mL of 1:1 (v/v) DCM/Hex in a Branson 5210 ultrasonic water-bath (Branson Ultrasonics Co., CT) for 10 min, and shaken on a shaker table (Eberbach Co., MI) also for 10 min. Once the suspended particles settled, the supernatant was removed. The extraction was repeated two more times with fresh solvent. The combined extracts were concentrated to ca. 5 mL with a gentle stream of high-purity nitrogen. The concentrate was quantitatively transferred onto a 350 mm × 10 mm i.d. glass column packed with 15 g of deactivated alumina (15% HPLC water, w/w) and capped with 1−2 cm of anhydrous Na_2SO_4. To prepare samples for analysis by LC/ESI-MS, the column was eluted first with 30 mL of hexane, which was discarded, followed with 50 mL of 1:1 (v/v) DCM/Hex that contained the PEs of interest. The DCM/Hex fraction was evaporated to dryness under a stream of nitrogen; the residue was reconstituted in 2 mL of methanol, spiked with the surrogate performance standards as described above, and analyzed by LC/ESI-MS. To prepare samples for GC/MS analysis, the alumina column was eluted with three 30-mL fractions of (i) hexane; (ii) 1:9 DCM/Hex; (iii) 1:1 DCM/Hex. The third fraction was further cleaned by loading it onto a Florisil column and eluting the analytes of interest with 30 mL of pure dichloromethane followed by 30 mL of 5% acetone in dichloromethane. The eluent was then concentrated to 0.5 mL under a stream of nitrogen and spiked with the surrogate performance standards before GC/MS analysis.

Moisture content of the sediment samples was determined by drying a second aliquot of the sample in a 105 °C vented oven for 48 h and weighing the sample before and after drying.

LC/ESI-MS and LC/ESI-MS/MS. The LC system used was a Beckman model 126 programmable solvent system controlled by a Beckman System Gold software (version 8.1) (Beckman, Fullerton, CA). Separations were performed on a 250 mm × 2 mm i.d. stainless steel analytical column packed with Spherclone 5 μm C8 (Phenomenex, Torrance, CA). An OPTI-SOLV Mini-Filter (Chromatographic Specialties Inc., Brockville, ON) was used as a guard column. All analytes of interest, individual phthalate congeners, and the isomeric mixtures were eluted from the column in a single chromatographic run using a gradient elution program. Mobile phase A was 90:10 methanol/water with 0.5 mM sodium acetate, and mobile phase B was 100% water also with 0.5 mM sodium acetate. The gradient program was: 83% A and 17% B held for 1 min, then linearly increased to 100% A over 3 min, and then returned to initial conditions (83% A, 17% B) over 28 min. The injection volume was 3 μL, and the mobile phase flow rate was 0.22 mL/min. To be compatible with conditions required for optimum electrospray ionization, a splitter was used to feed about 20 μL of eluent/min into the electrospray probe. The flow split ratio was regulated by adjusting the length and diameter of two capillary tubes. Mass analysis was performed using a VG Quattro tandem mass spectrometer (MS/MS) equipped with a pneumatically assisted electrospray source (Micromass, Manchester, U.K.) and operated in either the single or tandem MS mode depending on the experiment. The source temperature was 150 °C, and nitrogen was used as the bath and nebulizing gas (250 and 16 L/h, respectively). Typical electrospray ionization conditions were as follows: electrospray capillary voltage, 3.7 kV; high-voltage lens (counter electrode), 150V; skimmer cone voltage, 27 V; focus (second skimmer) voltage, 20 V. Prior to LC/ESI-MS analyses, the instrument response was optimized by performing flow-injection analysis of a solution of the stable isotope-labeled benzyl butyl phthalate (ring-d_4). The mass spectrometer was operated in the positive ion mode. Full-scan mass spectra were obtained with the MS scanned in the m/z range of 50−500 at the rate of 5 s/scan and an inter-scan delay of 10 ms. For quantitative LC/MS determinations, the mass spectrometer was operated in the selected ion monitoring (SIM) mode, monitoring m/z of 357, 385, 413, 441, and 469 for C6−C10, respectively, as well as m/z 417 for the internal standard DnOP-d_4; the dwell time was 200 ms per mass window. Data were processed using the Masslynx software. Peak areas were obtained from the Masslynx data system by interactive processing, and peak baselines were operator defined.

The ESI-MS response of PEs was explored in both positive and negative ionization modes. Best results, detection sensitivity, and long-term stability were obtained in the positive ion-mode with the PEs detected as the sodiated adducts. Optimization of ESI-MS parameters was carried out by flow injection analysis (FIA) experiments. For FIA/ESI-MS, a 20-μL injection of the phthalate standard solution was directly injected into the flow of the mobile phase at a flow rate of 20 μL/min, and the MS was operated in full-scan positive ion mode covering the mass range m/z 50−500. After preliminary tuning and signal optimization with FIA, final optimization was also accomplished with the LC column in place because, under chromatographic conditions, system performance depends on the presence and condition of the LC column, ionic strength, modifiers added to the mobile phase, and variable solvent compositions from gradient elution. The quantitative linearity of the LC/ESI-MS method was tested in the concentration range of 0.0028−42.8 ng/μL for individual phthalates and 0.0428−55.1 ng/μL for PE isomeric mixtures, respectively.

The presence of PEs, the isomeric mixtures in particular, in all samples was confirmed by LC/ESI-MS/MS experiments. Initially, fragment ion experiments were performed to identify characteristic fragments, and subsequently, multiple reaction monitoring (MRM) experiments were designed for the quantitative determinations. With the MRM experiments, we were able to separate the target analytes from coeluting and/or isobaric interferences arising from the sample matrix. For all MS/MS experiments, argon was used as collision gas with a pressure of about 2×10^{-5} mbar (measured on the analyzer Penning gauge). Other conditions were as follows: collision energy, 19 eV; cone voltage, 51 V; capillary, 3.4 kV; and HV lens, 320 V. The MRM transitions monitored were m/z 469−329 and 469−171 for diisodecyl PE (i.e., C10); m/z 441−315 and 441−171 for diisononyl PE (i.e., C9); m/z 413−301 and 413−171 for diisooctyl (i.e., C8); m/z 385−287 and 385−171 for Jayflex 77 (i.e., C7); and m/z 357−273 and 357−171 for Jayflex DHP (i.e., C6).

GC/MS. GC/MS analyses were carried out on a Finnigan Voyager GC/MS system (Manchester, U.K.), which consisted of a Finnigan 8000 series gas chromatograph, a Finigan Voyager quadrupole mass spectrometer (1000 amu mass range), and a CTC A200S autosampler. Instrument control, data acquisition, and data processing were performed using the Finigan Masslab software. The mass spectrometer was operated in the positive EI mode with an electron energy of 70 eV. Data were acquired in the single-ion monitoring mode (m/z 149 for all phthalates except 163 for DMP) with a dwell time of 100 ms and a delay time of 10 ms. A J&W DB-5 fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used for separation. Splitless injections of 1

μL of sample extract and 0.5 μL of air were made, and the carrier gas used was helium at a flow rate of 1 mL/min. The GC temperature program was 70 °C (hold 1 min) to 180 at 12 °C/min, to 240 °C at 5 °C/min, and to 300 °C at 5 °C/ min (hold 10 min). The injection port was at 260 °C, the GC/MS interface was at 250 °C, and the ion source was at 200 °C.

Quantitation and QA/QC Measures. Quantification was based on five-point calibration curves generated for each analyte. The calibration standards prepared covered the range from 2.8 to 4280 pg/μL for individual phthalates and from 4.28 to 5510 pg/μL for phthalate isomeric mixtures. The concentrations of the surrogate internal and performance standards were kept constant in all calibration solutions at the 0.5 ng/μL level. Calibration curves were constructed by plotting the peak area ratio of the analyte to internal standard versus analyte concentration. Sample concentrations were calculated from the weighted least-squares regression analysis of the standard curve.

The surrogate internal and performance standards were added to all the samples, and the final volume of the sample extract was adjusted so that the concentrations of these standards were the same as in the calibration solutions, 0.5 ng/μL. The performance standards were used to adjust for instrument fluctuations over time and to determine the amount of IS lost throughout the sample preparation process. The analyte concentrations reported 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 compounds in the calibration standards; and (c) the signal-to-noise ratio of the representative ion had to be ≥3. The retention time, the identity of the analyte through its confirmation ions, and the signal-to-noise (for both the ion transitions monitored for each analyte) quantitation criteria were also used for the MRM experiments. MRM calibration curves were established using the most intense ion transition of the two monitored.

Samples were processed in batches of 7, which contained 2 procedural blanks, 4 real samples, and 1 PE native-spiked sample. The PEs standard mixture, with PE concentrations at the middle of the concentration range, was run on the LC/ESI-MS at the beginning and at the end of each batch of 8 samples to ensure that the calibration curve under use was holding and that there were no large drifts in calibration throughout the analysis.

Results and Discussion

LC/ESI-MS. The ESI-MS response was optimized by FIA experiments using PE authentic standards. Early in our work, we realized that when methanol−water was used as the mobile phase PEs presented a weakly protonated ion $[M + H]^+$ and an intense Na adduct ion $[M + Na]^+$ without adding any sodium to the solvents. Na is ubiquitous and can come from a number of sources: impurities in methanol and other solvents used, contaminated stainless steel solvent transfer lines, electrospray probe, and ionization chamber. Also, Na leaches from the walls of glassware, and most importantly it is present in most environmental matrixes. These difficult to control Na sources introduce large $[M + Na]^+$ signal fluctuations as the amount of Na varies from one experiment to the next. Similarly as with our nonylphenol ethoxylate work (*26*), to control this experimental variable small quantities of NaOAc were added to all sample extracts and to HPLC solvents used. By adding small amounts of Na to the electrosprayed analyte solution, the $[M + H]^+$ ion was completely suppressed and the $[M + Na]^+$ adduct ion became the base peak in the mass spectra of all the PEs examined. The amount of NaOAc added to the electrosprayed solution

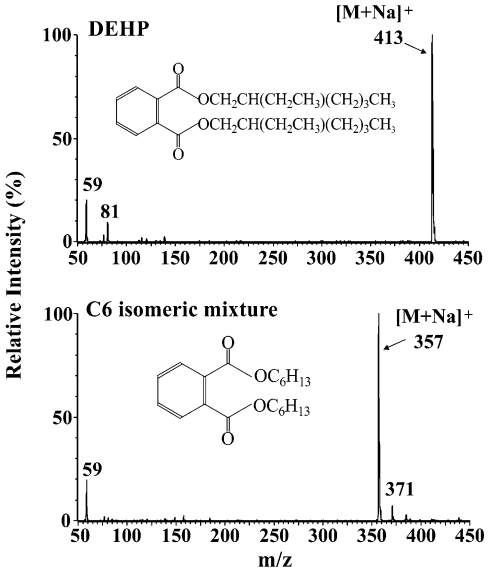

FIGURE 2. Background-subtracted ESI mass spectra obtained from the flow injection analysis of two selected phthalate esters, DEHP (∼3 ng injected) and the C6 isomeric mixture (∼8 ng injected).

was optimized as to obtain maximum $[M + Na]^+$ signal intensity without effecting the efficiency of electrospray ionization, which depends on total electrolyte concentration and solution ionic strength (*27*, *28*). Optimum conditions were achieved when the sample solution and the mobile phase were spiked with NaOAc to produce an overall concentration of 0.5 mM. The temperature of the ionization chamber and the interface voltages were also optimized as to minimize fragmentation and to focus the entire ionization onto a single ion, i.e., the $[M + Na]^+$ adduct. Best ion intensity was obtained with the ionization chamber at 150 °C, skimmer cone voltage, 27 V; focus (second skimmer) voltage, 20 V. During the optimization experiments, it was realized that the ion source temperature effected significantly the signal intensity of all the target analytes, thus a set of experiments was designed to systematically explore this variable. By increasing the ion source temperature from 50 to 150 °C, the $[M + Na]^+$ signal intensity increased 4-fold for C10 and more than 20-fold for C6. The results of ion source temperature dependence experiments are summarized in Supporting Information Figure 1.

Using these experimental conditions, we were able to improve substantially the detection limits and the specificity of the method. All target analyes formed intense molecular adduct ion of $[M + Na]^+$ and exhibited little or no fragmentation; typical mass spectra obtained are shown in Figure 2 for two selected phthalates. To achieve maximum sensitivity possible, the $[M + Na]^+$ ion was used as the quantitation ion and the mass spectrometer was operated in the SIM mode. The sodium adduct ion is characteristic for individual PEs and each isomeric group [i.e., m/z is 357 (for C6 isomers), 385 (for C7), 413 (for C8), 441 (for C9), and 469 (for C10)]. The detection of isomeric mixtures by their own characteristic ion provides a method to separate isomeric mixtures even when the separation on the LC column is incomplete as it is shown below.

Typical reconstructed ion chromatograms obtained from the LC/ESI-MS analysis of a cocktail of PEs is shown in Figure 3A,B. The Spherclone-C8 column, using the conditions described in the Experimental Section, provided good separation for the individual PEs (Figure 3A) and the PE isomeric mixtures (Figure 3B). Individual PEs eluted in less than 30 min and in the order of increasing chain length. Although the pairs BBP/DnBP and DEHP/DnOP were not

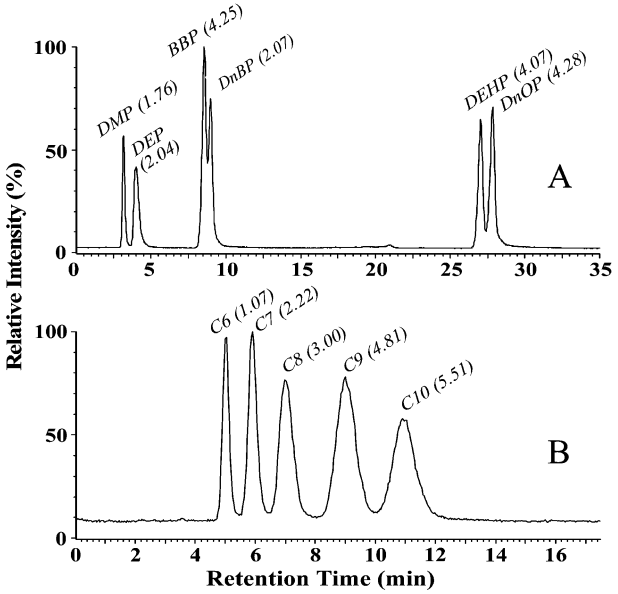

FIGURE 3. Detection of phthalate ester standards by LC/ESI-MS (positive ionization mode) under SIM conditions. The $[M + Na]^+$ ion was monitored for (A) six individual phthalates; (B) five phthalate isomeric mixtures. Numbers in parentheses indicate the solution concentration of the target analytes in ng/mL; 3 μL of sample was injected.

baseline separated, the separation obtained was sufficient for accurate quantification. The five isomeric mixtures (C6−C10) were baseline separated, and all isomers within each mixture merged into single envelopes. Although the LC/ESI-MS technique does not have the specificity to separate individual isomers within each mixture, it does have the capacity to separate isomeric mixtures from each other and to accurately quantify such constituents.

The dynamic range of the LC/ESI-MS technique was explored for all the target analytes. The instrument response was linear to the injected amounts of individual phthalates from 2.8 to 4280 pg/μL and from 4.28 to 5510 pg/μL for the isomeric mixtures (see Supporting Information Figure 2). The minimum detectable amounts (MDA), corresponding with the amount of chemical (in pg) injected onto the LC column in a volume of 3 μL standard solution that produced a 3:1 signal-to-noise ratio, ranged from 4 to 19 pg for individual PEs and from 8 to 213 pg for the five PE isomeric mixtures (Table 1). In general, the MDA increased with increasing molecular weight. This may be due to the decrease in polarity of the analytes with increasing alkyl chain length, which reduces the stability of the phthalate sodium adduct.

The overall performance of the analytical method, in terms of accuracy and precision, was measured using sediment and/or tissue samples spiked with a mixture of phthalates in acetone at various spiking levels of 0.1−5 μg/g depending on the individual compounds. The samples were then homogenized and stored at 4 °C for 24 h for equilibration. The procedural blanks consisted of 20 g of prebaked sodium sulfate. The recoveries from spiked sediments for all phthalates ranged from 71% to 106% (Table 1) by LC/ESI-MS. The relative standard deviation (RSD) for each analyte was less than 15%, demonstrating good method precision. Such method performance is compatible with typical U.S. EPA requirements for environmental analysis where average recoveries of 70−120% and a RSD of 20% or less is expected for spiked samples.

LC/ESI-MS versus GC/MS. The performance of both techniques was explored toward the analysis of individual PE isomers and isomeric mixtures. The GC/MS technique provided excellent separation and was much superior to that

TABLE 1. Comparison of Minimum Detectable Amounts (MDA) and Method Detection Limits (MDL) of Phthalate Esters Obtained by GC/MS and LC/ESI-MS[a]

	GC/MS		LC/ESI-MS		
	MDA (pg)	MDL (ng/g)	MDA (pg)	MDL (ng/g)	recovery (%), $n = 3$ (RSD,%)
Individual Phthalates					
DMP	0.5	1.1	11.2	1.8	71 (5)
DEP	0.5	0.3	3	1.6	87 (6)
BBP	0.6	0.5	14	0.5	106 (6)
DnBP	0.03[b]	0.5	18	4.2	94 (5)
DEHP	0.03[b]	3.3	30	4.2	96 (10)
DnOP	0.06	0.5	20	4.2	96 (8)
Phthalate Isomeric Mixtures					
C6	na[c]	na	8.3	3.0	102 (7)
C7	na	na	39	1.0	89 (8)
C8	na	na	35	1.1	100 (10)
C9	na	na	40	0.5	93 (11)
C10	na	na	50	0.5	93 (6)

[a] LC/ESI-MS method performance in terms of spiked recoveries and precision for phthalate esters using spiked sediment and fish tissue samples. [b] Overestimated detection limits due to the trace amounts of DnBP and DEHP in the solvents. [c] na, data not available due to difficulties associated in obtaining reliable and reproducible data for the isomeric mixtures using GC/MS.

obtained under LC/ESI-MS conditions not only for the six target individual PEs of interest but for a mixture of 18 different individual PEs (see Figure 4A). However, the technique exhibited very poor resolution when used to separate the commercial isomeric mixtures (Figure 4B). There were substantial retention time overlaps between all the isomeric groups (i.e., the C6 isomers overlapped with the C7, the C7 with the C8, etc.). The largest overlap was detected between the C9 and the C10 isomers, and this was confirmed from analyzing each isomeric mixture individually. The lack of specificity of the GC/MS method in handling the PE isomeric mixtures is due to limitations of both chromatographic and mass spectrometric techniques.

Although in mass spectrometric based techniques it is not necessary to have baseline chromatographic resolution for all the target analytes in a mixture, as the MS can provide the additional specificity required, in this application this is not possible. Such specificity cannot be obtained from the MS as the molecular ions of the PEs are of very small intensity (almost not detected) under positive ion electron impact ionization conditions. There were no distinct characteristic ions among the five groups that could be used for detection and quantification. The base peak in the GC/MS mass spectra of all the compounds in these five isomeric mixtures was m/z 149, and they also shared similar secondary ions. Selecting m/z 149 for one group of isomers (i.e., the C6s), a secondary ion for the next group (the C7s), then m/z 149 for the next group (the C8s), and so on proved not to be of great benefit. Even with this approach, using standards, the peaks within each group were not baseline resolved and the resolution was very poor when extracts of spiked samples were analyzed. Many of the smaller peaks detectable in the standard solutions were not detected in the spiked samples, and the overall method detection limits were poor.

Because of the limitations described above, the GC/MS technique could not be used reliably for the quantification of the C6−C10 isomeric mixtures in environmental samples. The LC/ESI-MS technique, however, provided the specificity required for quantitative work as each isomeric group was detected by a characteristic ion. For most samples there were no chromatographic and/or isobaric interferences for the C6−C9 isomers. However, for tissue samples, we recognized substantial inteferences with the detection of the C10 isomer.

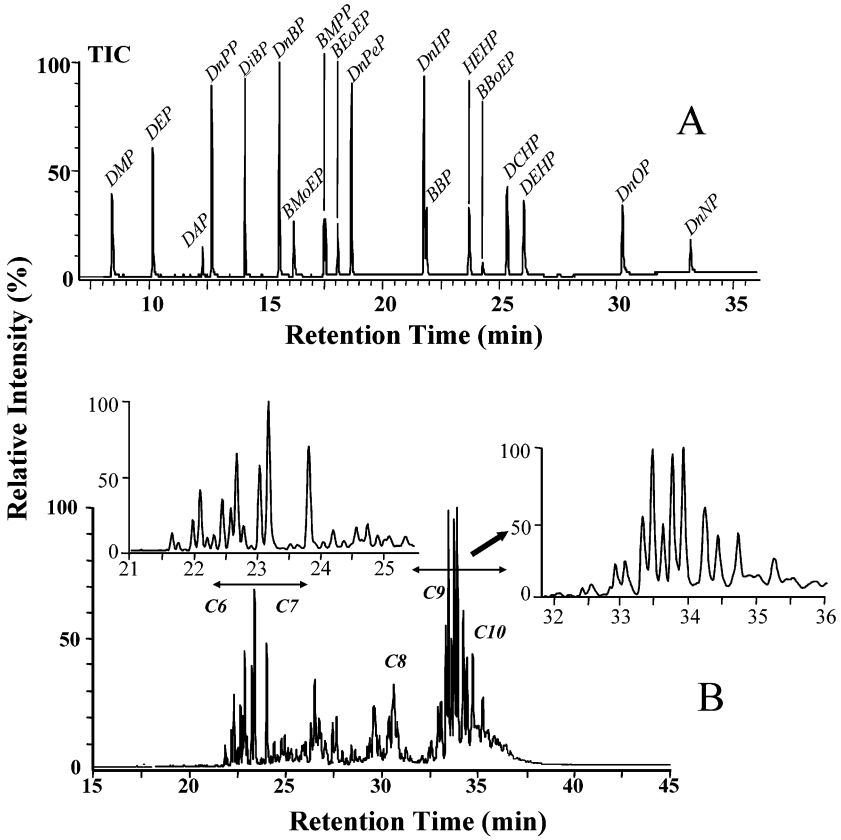

FIGURE 4. Detection of phthalate standards by GC/MS (positive EI mode) under SIM conditions. The ions monitored were $[C_6H_4(CO)_2OCH_3]^+$ (*m/z* 163) for DMP and $[C_6H_4(CO)_2OH]^+$ (*m/z* 149) for all other phthalate esters. The upper trace shows the ion chromatogram of the PE individual isomers, and the bottom trace shows that of the PE isomeric mixtures. The concentrations of the six target analytes are the same as those given in Figure 4, but only 1 μL of sample was injected. The concentrations of the remaining 12 individual PE isomers were between 4 and 5 ng/mL.

An isobaric compound that coeluted with the C10, possibly phospholipids, was difficult to separate using different chromatographic conditions. The C10 was resolved from the interfering compound(s) by performing MRM experiments. The characteristic fragment ions of the C10 isomers used in the MRM experiments were established from the analysis of the standard mixtures and operating the tandem mass spectrometer under fragment ion MS/MS conditions.

For the individual phthalates investigated in this study, the MDAs obtained by LC/ESI-MS were superior to those obtained by GC-FID (*12*, *13*), GC-ECD (*14*, *15*), LC−UV (18−22), and GC/MS (*16*, *17*). From the comparative experiments we performed in our lab, we observed that for the individual PEs the GC/MS technique provided superior MDAs to those obtained from the LC/ESI-MS technique (see Table 1). However, the method detection limits (MDL), defined as three times the standard deviation of procedural blanks ($n = 12$), of the GC/MS and LC/ESI-MS methods were comparable (Table 1). This is partially due to the fact that GC and LC methods we used had similar sample preparation procedures and thus have picked up similar amounts of background contamination. This result also suggests that the LC/ESI-MS technique is less prone to isobaric interferences that elevate the background signal and impact directly on the MDL. For the individual PEs, in our hands, the two methods performed equally well in terms of MDLs for all matrixes of interest (see Table 1). MDLs attained in this study, for the individual PEs, were much lower than those previously reported in the literature (e.g., 25−50 times lower for DEHP than what has been reported in refs *4*, *13*, and *21*). A comparison for the isomeric mixtures was not possible as the data obtained from the GC/MS analyses were not reliable nor reproducible. As reported in Table 1, for the matrixes of interest, the MDLs obtained by LC/ESI-MS were between 1.0 and 4.2 ppb (part per billion) for all the target analytes. The quantitation for two of the target analytes, C9 and C10, was impacted by isobaric interferences. We were able to resolve the interferences by employing MRM experiments. With the instrumentation used, the MDLs of these two compounds under LC/ESI-MS/MS conditions was about half that obtained under LC/ESI-MS conditions.

Application to Environmental Samples. The studies of which we report here are part of a larger, ongoing, comprehensive program where the aim has been to elucidate the current environmental distribution of PEs and to evaluate their bioaccumulation and biomagnification potential in aquatic and terrestrial food webs. We focused the first study of our program in an urbanized marine ecosystem, the False Creek of Vancouver's Inner Harbor (a residential /industrial location) in British Columbia, Canada. Marine surficial sediments and biota samples from four locations in False Creek were collected to assess the sources and distribution profiles of PEs in that ecosystem using the comprehensive analytical method described in this paper. To obtain maximum possible information from these samples and to cross-reference the quantitation between GC/MS and LC/ESI-MS, we analyzed all the extracts by both GC/MS and LC/ESI-MS. Of all the 18 individual congeners and the isomeric mixtrures targeted, we report here only the compounds that could be detected which were DMP, DEP, DiBP, DnBP, BBP, DEHP, DnOP, DNP, C6, C7, C8, C9, and C10. The LC/ESI-MS technique does have the capacity to resolve and reliable quantify all these compounds with the exception of DiBP and DnBP. These two coelute, and since they have the same

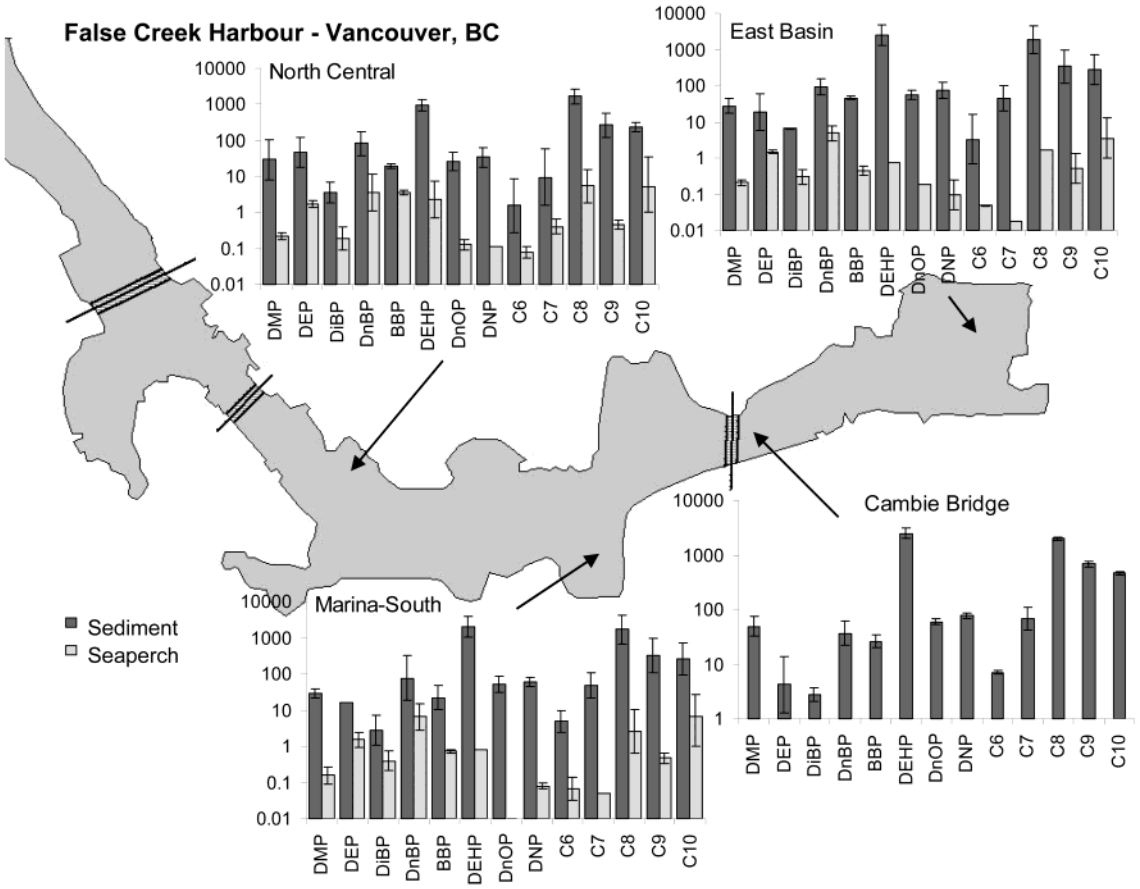

FIGURE 5. Concentrations (in ppb) of several individual phthalate ester congeners and isomeric mixtures in sediment and seaperch from several locations in False Creek, Vancouver, BC. Sediment concentrations are based on dry weight, and the biota samples are based on wet weight. Error bars indicate ± one standard deviation.

mass, the LC/ESI-MS technique can only provide a combined concentration of DBP ([DiBP] + [DnBP].

The LC/ESI-MS based analytical method, when applied to marine sediments, provided good separation for the most prevalent lower molecular weight PE congeners and for the higher molecular weight PE isomeric mixtures. Typical ion chromatograms for all the target analytes for a sediment sample of ppb concentrations are shown in Supporting Information Figure 3. The sediment concentrations measured in this ecosystem were relatively low as compared to other reported values but yet well above the MDLs achievable with this method. Even with the relatively simple extraction and cleanup procedure, the LC/ESI-MS technique has the capacity to selectively separate the phthalates from its sediment matrix. To ensure that all concentrations measured were interference free, all extracts were analyzed under MRM conditions as well. All target analytes showed no significant interferences with the exception of C9, which coeluted with an unidentified substance. The concentration of C9 was adjusted to account for the interference by MRM-based quantification. Since DiBP cannot be separated from DnBP by LC/ESI-MS, all sample extracts were also analyzed by GC/MS to assess the accuracy of determining DnBP by LC/ESI-MS. It was confirmed that DnBP concentrations obtained by LC/ESI-MS, which is in fact a combined concentration ([DnBP] + [DiBP]), were very close to those obtained by GC/MS. This was due to the fact that most environmental samples examined (see Figure 5) only contained trace amounts (low ppb) of DiBP while the concentration of DnBP was consistently 10 times or higher than that of DiBP.

The concentrations and the PE profiles measured in the marine sediments from the four locations in False Creek are given in Figure 5. Concentrations of all PE congeners combined [i.e., the sum of the lower molecular weight PEs (DMP and DEP, DiBP, DnBP, and BBP) and the higher molecular weight PEs (C6−C10)] ranged between 2.0 and 3.6 ppm on a dry weight basis. There were no statistically significant differences in the mean concentrations determined between the four stations in False Creek as determined by analyses of variance ($p > 0.05$). Diisooctyl phthalate (C8 isomers) comprised approximately 68% of the total PE concentrations in the sediments. The similarity of DEHP and diisooctyl phthalate concentrations indicate that the majority of the diisooctyl phthalate concentrations consist of DEHP. Diisononyl (C9 isomers) and diisodecyl phthalates (C10 isomers) made up respectively 14 and 11% of the total PE concentration in the sediments. Concentrations of the lower molecular weight PEs (including DMP, DEP, DiBP, DnBP, and BBP) ranged in the ppb levels and comprised approximately 5% of the total PE concentrations in the sediments. C6 and C7 PE isomers made up the remaining 2% of the total PE levels in the sediments. The simultaneous analysis of the majority of phthalates esters in current consumer goods makes it possible to compose a fingerprint of PEs. The PE fingerprint based on the full number of PE congeners and isomeric mixtures in the sediments can be established from the data presented in Figure 5. Using these data, a more simplified PE sediment fingerprint for the most commercially important PEs can be extracted, and such a profile is shown in Figure 6. Figure 6 shows that the PE composition in the sediment shares some similarities with the North American per capita PE consumption levels reported in ref *29*. Both sediment and consumption level fingerprints shows a predominance of the higher molecular

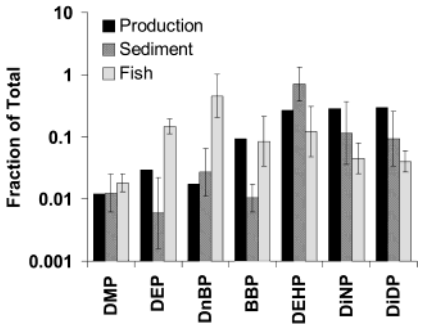

FIGURE 6. Phthalate ester fingerprints illustrating the composition of the most prevalent phthalate esters in North American consumer goods and marine sediments and in fish tissue from seaperch in an urbanized marine ecosystem. Error bars indicate ± one standard deviation.

weight PEs. This suggests that congener-specific sources of phthalates to False Creek are absent. It is also important to point out here that the major component of the PEs detected in the sediments were the isomeric mixtures (C6−10), and this signifies the necessity of a comprehensive analytical method to examine the environmental fate of these contaminants.

Similarly as with the sediment samples, the LC/ESI-MS technique provided good separation for the most prevalent lower molecular weight PE congeners as well for the higher molecular weight PE isomeric mixtures in fish tissue samples. Typical ion chromatograms for all the target analytes for a tissue sample are shown in Supporting Information Figure 3. Using MRM experiments, we confirmed that all target analytes in the tissue samples were interference free except the C10. This was a significant finding as most of the tissue samples analyzed had a major isobaric interference that also coeluted with the C10. With the MRM experiments, we were able to confirm that only 0.5−1.0% of the C10 detected under LC/ESI-MS conditions was actually the C10 substance. While lacking positive identification, it is believed that the majority of the C10 coeluting interference was a substance of phospholipid nature.

The concentrations and the PE profiles measured in the tissue samples of striped seaperch from three locations in False Creek are shown in Figure 5. The mean total wet weight based concentration of all PE congeners combined ranged between 4 and 54 ppb. There were no statistically significant differences between the mean concentrations determined at the three stations in False Creek as determined by analyses of variance ($p > 0.05$). The results indicate that while concentrations of DEHP and some of the other high molecular weight PEs can be relatively high (i.e., the ppm range) in sediments, the concentrations in fish can be substantially lower (i.e., 100−1000-fold). However, for the lower molecular weight PEs, the discrepancy between sediment and fish concentrations in this study was much smaller than for the higher molecular weight PEs. In fish, DnBP comprised approximately 42% of the total PE concentrations. Diisooctyl phthalate (i.e., C8) made up approximately 27% of the total PE concentration with DEHP contributing approximately half of the total C8 concentration. The other half of the C8 concentration represent C8 isomers other than DEHP. Data presented in Figures 5 and 6 show that the PE fingerprint in fish is substantially different from that in the sediments and per capita PE consumption. In contrast to the PE concentration in the sediments, which is comprised mainly (i.e., 95%) of higher molecular weight PEs (i.e., C8, C9, and C10), the fish tissue samples were mainly (i.e., 67%) comprised of the lower molecular weight PEs (DMP, DEP, DnBP, DiBP, and BBP). The results indicate that the higher molecular weight PEs are less biologically available than the lower molecular weight PEs. This is expected to be due to the high hydrophobicity of the higher molecular weight PEs, which favors sorption to particulate and dissolved matter in the water column and reduces the freely dissolved fraction of the chemical in the water, which can bioconcentrate in fish. A lack of biomagnification related to the often-reported metabolic transformation of PEs in fish may play an additional role in the prevalence of lower over higher molecular weight PEs in fish. These findings are consistent with those of Staples et al. (*2*), who after a review of laboratory-based bioaccumulation observations concluded that bioaccumulation of PEs are typically less than expected from their lipid−water partitioning properties. A more detailed analysis of the food-chain bioaccumulation of PEs will be presented in a forthcoming paper.

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Supporting Information Available

Three figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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