Distribution of Phthalate Esters in a Marine Aquatic Food Web: Comparison to Polychlorinated Biphenyls

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Introduction

Dialkyl phthalate esters (DPEs) are widely used as plasticizers in poly(vinyl chloride), poly(vinyl acetate), cellulosics, and polyurethanes, and as nonplasticizers in products such as lubricating oils, automobile parts, paints, glues, insect repellents, photographic films, perfumes, and food packaging (e.g., paperboard and cardboard) (1). Current North American production of phthalate esters is approximately 0.65 million tonnes/year. The global production level is approximately 4.3 million tonnes/year (2, 3). DPEs have been detected throughout the world, particularly in sediments in North America and Western Europe (3). In several jurisdictions, DPEs are currently being evaluated for their ability to bioaccumulate, exert toxicity, and persist in the environment following the 1999 UNEP Protocol on Long-Range Transboundary Air Pollution (LRTAP).

Industrial formulations of phthalate esters include a large number of compounds, which vary in alkyl chain length and branching and range in molecular weight from 194 to over 600 g/mol. Phthalate esters exhibit a wide range of octanol-water partition coefficients (K_{ow}), extending from 10^{15} for dimethyl phthalate to 10^{ battling for di-isocetyl phthalate (Table 1) (4, 5). Because of their hydrophobicity, phthalate esters are often assumed to have a high potential to bioaccumulate in biological organisms. A number of laboratory studies, summarized by Staples et al. (4), have investigated the bioconcentration of phthalate esters in various aquatic species. With the exception of dimethyl and diethyl phthalate esters, reported bioconcentration factors (BCFs) of DPEs in fish and certain invertebrate species are less than expected based on their K_{ow} values. Experimental artifacts, metabolic transformation, and a low bioavailability have been proposed as reasons for the lower than expected BCFs of phthalate esters (6). The majority of bioaccumulation data refer to a small number of compounds. Data on DEHP are abundant, but similar data for other individual phthalates and commercial mixtures are sparse or nonexistent. In terms of trophic transfer, it has been suggested that phthalate esters do not biomagnify in food webs (4, 7, 8). Field studies to confirm this do not exist.

In this paper, we present a field study, which measures the trophodynamics of phthalate esters and polychlorinated biphenyls (PCBs) in a marine aquatic food web. The study involved the analysis of 8 individual DPEs (i.e., diethyl (DEP), di-isooctyl (DiOBP), di-n-butyl (DBP), butylbenzyl (BBP), di-2-ethylhexyl (DEHP), di-n-octyl (DnOP), and di-n- Nonyl (DnNP)), 5 isomeric DPE mixtures (i.e., di-isooctyl (C6), di-isooctyl (C7), di-isooctyl (C8), di-isooctyl (C9), and di-isooctyl (C10)) in a marine aquatic food web. DPE concentrations were determined in 18 marine species could be

results show that all DPEs tested did not biomagnify in the studied aquatic food web whereas PCBs did biomagnify.
TABLE 1. Molecular Weights (g/mol), Le Bas Molar Volumes (cm^3/mol), Aqueous Solubilities (mg/L), and Log Octanol-Water Partition Coefficients (Kow)* of Selected Phthalate Esters and Isomeric Mixtures (Data from ref 5) and Selected PCBs (Data from refs 9 and 10)

<table>
<thead>
<tr>
<th>chemical</th>
<th>molecular weight (g/mol)</th>
<th>Le Bas molar volume (cm^3/mol)</th>
<th>AQ solubility (mg/L)</th>
<th>log Kow (freshwater)</th>
<th>salinity-corrected log Kow</th>
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</thead>
<tbody>
<tr>
<td>dimethyl</td>
<td>194.2</td>
<td>206.4</td>
<td>5.22 x 10^3</td>
<td>1.61</td>
<td>1.80</td>
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<tr>
<td>diethyl</td>
<td>222.2</td>
<td>254.0</td>
<td>5.91 x 10^2</td>
<td>2.54</td>
<td>2.77</td>
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<tr>
<td>di-iso-butyl</td>
<td>278.4</td>
<td>342.8</td>
<td>9.90 x 10^1</td>
<td>4.27</td>
<td>4.58</td>
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<tr>
<td>di-n-butyl</td>
<td>278.4</td>
<td>342.8</td>
<td>9.90 x 10^1</td>
<td>4.27</td>
<td>4.58</td>
</tr>
<tr>
<td>butylbenzyl</td>
<td>312.4</td>
<td>364.8</td>
<td>3.80 x 10^2</td>
<td>4.70</td>
<td>5.03</td>
</tr>
<tr>
<td>di(2-ethylhexyl)</td>
<td>390.6</td>
<td>520.4</td>
<td>2.49 x 10^-3</td>
<td>7.73</td>
<td>8.20</td>
</tr>
<tr>
<td>di-n-octyl</td>
<td>390.6</td>
<td>520.4</td>
<td>2.49 x 10^-3</td>
<td>7.73</td>
<td>8.20</td>
</tr>
<tr>
<td>di-n-nonyl</td>
<td>418.6</td>
<td>564.8</td>
<td>3.08 x 10^-4</td>
<td>8.60</td>
<td>9.11</td>
</tr>
<tr>
<td>di-iso-hexyl</td>
<td>334.4</td>
<td>431.6</td>
<td>1.59 x 10^-1</td>
<td>6.00</td>
<td>6.39</td>
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<tr>
<td>di-iso-heptyl</td>
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<td>476.0</td>
<td>2.00 x 10^-2</td>
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<td>7.30</td>
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<tr>
<td>di-iso-octyl</td>
<td>390.6</td>
<td>520.4</td>
<td>2.49 x 10^-3</td>
<td>7.73</td>
<td>8.20</td>
</tr>
<tr>
<td>di-iso-nonyl</td>
<td>418.6</td>
<td>564.8</td>
<td>3.08 x 10^-4</td>
<td>8.60</td>
<td>9.11</td>
</tr>
<tr>
<td>di-iso-decyl</td>
<td>446.7</td>
<td>609.2</td>
<td>3.81 x 10^-5</td>
<td>9.46</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Polychlorinated Biphenyls

| C12                  | 257.5                   | 247.4                         | 1.38 x 10^-1         | 5.24                 | 5.46                      |
| C13                  | 293.5                   | 289.4                         | 6.22 x 10^-3         | 6.39                 | 6.65                      |
| C14                  | 326.5                   | 289.4                         | 9.86 x 10^-3         | 6.74                 | 7.00                      |
| C15                  | 351.5                   | 331.4                         | 3.07 x 10^-4         | 7.36                 | 7.66                      |
| C16                  | 390.6                   | 352.4                         | 7.64 x 10^-5         | 7.80                 | 8.12                      |
| C17                  | 430.0                   | 394.4                         | 1.51 x 10^-5         | 8.18                 | 8.53                      |
| C18                  | 499.0                   | 409.4                         | 1.36 x 10^-5         | 8.53                 | 8.85                      |

*Kow(water) is calculated as Kow = 0.0018 x C18 x β16, following ref 5 where Kow(water) is the salinity-corrected octanol—water partition coefficient, Kow is the standard octanol—water partition coefficient for freshwater, 0.0018 is a proportionality constant relating the solubility of seawater to that of freshwater (L/cm^3), C18 is the molar concentration of salt in seawater (0.5 mol/L), and β16 is the molar volume of the chemical (cm^3/mol).

compared on a common basis. Trophic positions in the food web were determined using trophic position model based on ref 14 and (ii) stable nitrogen isotopes (i.e., δ15N) (15, 16).

Methods

Sample Collection. A total of nine individual samples of 18 marine species were collected between June and September 1999 (Table 2). Three samples of each species were collected from each of three sampling stations: “North-Central” (49°16’13”N 123°07’40”W), “Marina-South” (49°16’09”N 123°07’15”W), and “East-Basin” (49°16’28”N 123°06’18”W), in False Creek harbor, a small (≏4.0 x 0.3 km), shallow (mean depth of ≏8 m) embayment of Burrard Inlet (Figure 1). The species were selected to represent (i) various trophic levels in the False Creek marine food web, (ii) benthic and pelagic based food webs, and (iii) a variety of feeding strategies, sizes, and life histories. Primary producers (e.g., plankton and macroalgae), filter feeders (e.g., blue mussels [Mytilus edulis]), and deposit feeders (e.g., geoduck clams [Panope abrupta]) were collected. Fish species that were collected included rapidly maturing, short-lived species with high fecundity rates such as the striped seaperch (Embioptera lateralis) (17) and slow-growing and long-lived species such as the spiny dogfish (Squalus acanthias), whose gestation period lasts 2 years and natural life expectancy exceeds 50 years (18). The selected species were “resident” or nonmigratory. The only exceptions were dogfish, which inhabit larger foraging areas and move inshore with the tide to forage (18), and surf scoters, a marine bird species which also occupies a larger foraging area and are more mobile (19). Plankton samples were collected using a 236-μm plankton tow net and were a composite of phytoplankton and zooplankton. Samples were transferred to precleaned 250-mL glass vials. Macroalgae, mussels, oysters, clams, and sea stars were collected from intertidal regions (e.g., shoreline and pilings) during periods of low tide. Crab and prawn traps were used to collect crabs, staghorn sculpins, and white-spotted greenlings. Small forage fish were collected using herring gill nets and beach seine nets. Dogfish were collected by longline fishing during incoming tides. Surf scoter liver samples were provided by Dr. Elliot of the Canadian Wildlife Service. Apart from plankton, all biota samples were wrapped in solvent-rinsed aluminum foil. All samples were placed on ice in the field and frozen at −20°C in the lab prior to analysis. Bivalves and shiner perch juveniles were combined to obtain samples of 5–10 g (Table 2).

Sample Extraction and Analysis. A detailed description of the methods used for the analysis of phthalate esters in the biota samples is provided in ref 20. A brief overview of the methods is included in the electronic supplement. Although the analytical methodology provided data for a large set of PCB congeners (see ref 21), only data for PCB-18, 99, 118, 180, 194, and 209 are reported in this study. These congeners were selected because they cover a large range in Kow and were representative of the bioaccumulation patterns of most of the other PCB congeners. A more detailed account of the trophodynamics of PCBs will be presented in a forthcoming paper.

Sample extracts containing the phthalate esters were first analyzed by low-resolution gas chromatography mass spectrometry (GC/LRMS) for the quantification of the individual phthalate esters (i.e., DMP, DEP, DIBP, DnBP, BBP, DEHP, DnOP, and DnNP) following ref 20. After GC-MS analysis, the extract was evaporated to near dryness, reconstituted in 100 μL of doubly distilled methanol, and analyzed by liquid chromatography electrospray ionization mass spectrometry (LC/ESI-MS). With this technique we were able to quantify the isomeric commercial mixtures of phthalate esters (i.e., DMP, DEP, DiBP, DnBP, BBP, DEHP, DnOP, and DnNP) following ref 20. During LC/ESI-MS determinations we recognized that for certain biological tissue samples the quantitation of the mixed isomers, C10 in particular, was impacted by chromatographic and/or isobaric interferences, which were difficult to separate using different chromatographic conditions. By employing multiple reaction monitoring experiments (MRM) on a tandem MS/MS mass spectrometer, we were able to resolve

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reaction monitor (MRM) mode to produce collision-induced dissociation (CID), using lithium ions (Li+) as the solvent modifier. Argon was used as collision gas, with a pressure of about 2 × 10⁻⁴ mbar in the analyzer vacuum. Typical

Table 2. Biological Parameters (Length (cm), Wet Weight (g), Tissue Type for Analysis, Lipid Content (%), Organic Carbon (OC), Trophic Position (TP), δ¹³C (‰), and δ¹⁵N (‰))

**Biomarker Parameters**

<table>
<thead>
<tr>
<th>Vol.</th>
<th>No.</th>
<th>Year</th>
<th>Species</th>
<th>Lipid</th>
<th>OC</th>
<th>TP</th>
<th>δ¹³C</th>
<th>δ¹⁵N</th>
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</thead>
<tbody>
<tr>
<td>38</td>
<td>7</td>
<td>2004</td>
<td>ENVS</td>
<td>0.3</td>
<td>0.2</td>
<td>1.7</td>
<td>-19</td>
<td>-0.1</td>
</tr>
<tr>
<td>2013</td>
<td>7</td>
<td>2004</td>
<td>ENVS</td>
<td>0.3</td>
<td>0.2</td>
<td>1.7</td>
<td>-19</td>
<td>-0.1</td>
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</tbody>
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**Metabolite Parameters**

<table>
<thead>
<tr>
<th>Vol.</th>
<th>No.</th>
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<th>Species</th>
<th>Metabolite</th>
<th>Concentration (µM)</th>
<th>SD</th>
<th>SD</th>
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<tbody>
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<td>2004</td>
<td>ENVS</td>
<td>DEHP</td>
<td>4.07</td>
<td>0.50</td>
<td>0.93</td>
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<tr>
<td>2013</td>
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<td>2004</td>
<td>ENVS</td>
<td>DEHP</td>
<td>4.07</td>
<td>0.50</td>
<td>0.93</td>
</tr>
</tbody>
</table>
conditions used for lithiated ions were as follows: collision energy 75 eV, cone voltage 33 V, and HV lens 320 V. Under MS-MS conditions, the Li+ adduct produced two major common daughter ions, that is, m/z 155 and 173, from the phthalate ester isomeric mixtures (C6, C7, C8, C9, and C10). Specific ions were monitored for each isomer: di-iso-octyl (C8) for m/z 453 (parent), 313 and 155 (daughters); di-iso-nonyl (C9) for m/z 425, 299, and 151; di-iso-decyl (C10) for m/z 397, 285, and 137; Jayflex 77 (C7) for m/z 369, 271, and 123; and Jayflex DHP (C6) for m/z 341, 257, and 109. The fraction of actual C10 in apparent C10 L/C/ESI-MS peaks was consistent within a species and ranged from approximately 70% of the mass response for green algae and plankton samples to approximately 0.1% in certain fish tissue samples. The latter illustrates the importance of the MRM confirmation for C10 analysis in fish tissue samples. For all samples analyzed, the presence of DPEs, particularly the isomeric mixtures, was confirmed by MRM experiments.

The methodology used for Quantitation and Quality Assurance Quality Control (QA/QC) of the samples, including the determination of method detection limits, is provided in the Supporting Information.

**Food-Web Characterization.** Two methods were employed to measure trophic position in the food web, that is, (i) a trophic position model and (ii) stable nitrogen isotope analysis. For the trophic position model, dietary preferences of the species were determined based on information documented in refs 17–19, 22, and 23. Figure 2 illustrates the generalized trophic linkages between the sampled species. The trophic position (TP) of each of the species was then calculated according to ref 14:

\[
TP_{\text{predator}} = \left( \sum_{i=1}^{n} TP_{\text{prey}} \times p_{\text{prey}} \right) + 1 \tag{1}
\]

where \(p_{\text{prey}}\) is the proportion of prey item \(i\) in the diet of the predator. Trophic positions and dietary information used to calculate trophic position are presented in Table 2 in the Supporting Information.

Nitrogen and carbon stable isotopes (i.e., \(\delta^{15}N\) and \(\delta^{13}C\)) were also measured in the biota samples. The concentration ratio of \(15N/14N\), expressed relative to a standard (i.e., \(\delta^{15}N\)), has been shown to increase with increasing trophic level due to the preferential excretion of the lighter nitrogen isotope (15, 16). As a result, it has been suggested to be a useful empirical measure of trophic status and used in several trophodynamic studies of persistent organic pollutants (e.g., 24, 25). The concentration ratio of \(13C/12C\) (i.e., \(\delta^{13}C\)) generally remains relatively constant with increasing trophic level (26), although a small degree of enrichment of \(\delta^{13}C\) from producers to consumers may occur in coastal marine systems (27). To analyze for nitrogen and carbon stable isotopes, approximately 30 mg of dried surficial sediment (\(n = 4\)) and 1 mg of dried biota tissue (\(n = 3\) for each species) were finely ground using an acid-washed mortar and pestle and were enclosed in 8 × 5 mm tin capsules from Costech Technologies (Valencia, CA). The biota tissues used for isotope analysis were the same as those analyzed for phthalate esters and PCBs. Samples were analyzed for natural abundance of stable nitrogen and carbon isotopes on a continuous flow Europa Scientific Hydra 20/20 Isotope Ratio Mass Spectrometer at the University of California at Davis stable isotope facility. Details on the calculation of \(\delta^{15}N\) and \(\delta^{13}C\) are presented in the Supporting Information.

**Organic Carbon Contents.** Total organic carbon (TOC) was measured in plankton and algae samples following ref 28 and reported in Table 2. Samples were dried at 60 °C to a stable weight, homogenized with a mortar and pestle, and acidified with 4% HCl to remove inorganic carbon. The homogenate was then transferred to a combusted 25-mm nuclepore filter, rinsed three times with ddH2O water to remove the acid, and oven-dried at 60 °C to a stable weight. A 2–3-mg dry weight sample was analyzed in Leeman’s 440 Elemental Analyzer, which was standardized with acetonitrile containing 71.09% carbon and 10.36% nitrogen.

**Lipid Contents.** Lipid contents were measured for all biota samples (Table 3). Five grams of wet tissue was homogenized with 100 g of anhydrous sodium sulfate in a glass mortar and transferred to a 30 × 30 cm glass column, which was packed with glass wool at the tip. The column was then eluted with 100 mL of 1:1 DCM/hexane, which was collected in the Turbovap below the column, and then reduced to 1 mL. The extract was quantitatively transferred with 1:1 DCM/hexane to a preweighed aluminum weigh boat and allowed to dry.

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**FIGURE 1.** Map of field study site: False Creek Harbor, Vancouver, British Columbia, showing locations of three biota sampling stations (●): “North Central”, “Marina–South”, and “East Basin”.
for several hours at 40 °C in a ventilated oven and then cooled completely in a desiccator. The sample weight was determined, and lipid content was calculated on a wet weight basis.

**Lipid Equivalent Concentrations.** To present the concentrations of phthalate esters in the various species on a common basis, observed wet weight concentrations (C_{wet}, ng/g of wet tissue) were expressed in terms of lipid equivalent concentrations (C_{lipid}).

![Diagram of trophic linkages among marine organisms](image)

**FIGURE 2.** Generalized trophic linkages among 18 marine organisms collected from False Creek Harbor, based on refs 17–19, 22, and 23.

**TABLE 3.** Statistical Results of Regression Analysis between log Concentration and δ^{15}N and Trophic Position (i.e., Slope, p-Value of Slope, and Y-Intercept), and Food-Web Magnification Factors (FWMF) (Lower–Upper 95% Confidence Interval) for Phthalate Esters and Polychlorinated Biphenyls

<table>
<thead>
<tr>
<th>Compound</th>
<th>K_{aw}</th>
<th>n slope p value</th>
<th>Y-intercept</th>
<th>r^2</th>
<th>FWMF</th>
<th>lower–upper 95% CI</th>
<th>n slope p value</th>
<th>Y-intercept</th>
<th>r^2</th>
<th>FWMF</th>
<th>lower–upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DMP</strong></td>
<td>1.8</td>
<td>17 -0.01 0.82</td>
<td>1.65</td>
<td>0.003</td>
<td>0.93</td>
<td>(0.49–1.77)</td>
<td>18 -0.11 0.28</td>
<td>1.84</td>
<td>0.07</td>
<td>0.77</td>
<td>(0.47–1.27)</td>
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<td>2.77</td>
<td>17 0.01 0.83</td>
<td>2.25</td>
<td>0.003</td>
<td>1.07</td>
<td>(0.55–2.08)</td>
<td>18 0.02 0.84</td>
<td>2.27</td>
<td>0.003</td>
<td>1.05</td>
<td>(0.63–1.76)</td>
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<tr>
<td><strong>DBP</strong></td>
<td>4.58</td>
<td>17 -0.02 0.57</td>
<td>1.93</td>
<td>0.02</td>
<td>0.86</td>
<td>(0.50–1.49)</td>
<td>18 -0.09 0.31</td>
<td>1.95</td>
<td>0.07</td>
<td>0.81</td>
<td>(0.52–1.24)</td>
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<td><strong>DDE</strong></td>
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<td>2.42</td>
<td>0.04</td>
<td>0.68</td>
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<td>(0.14–0.71)</td>
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<td>4.29</td>
<td>0.45</td>
<td>0.34</td>
<td>(0.18–0.64)</td>
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<td>16 -0.15 0.050</td>
<td>3.27</td>
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<td>(0.10–1.00)</td>
<td>16 -0.53 0.005</td>
<td>3.07</td>
<td>0.44</td>
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<td><strong>DE</strong></td>
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<td>3.37</td>
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<td>0.28</td>
<td>(0.11–0.71)</td>
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<td>0.47</td>
<td>0.34</td>
<td>(0.16–0.73)</td>
<td>13 -0.34 0.014</td>
<td>3.84</td>
<td>0.44</td>
<td>0.46</td>
<td>(0.25–0.82)</td>
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<td><strong>C10</strong></td>
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<td>15 -0.11 0.073</td>
<td>4.09</td>
<td>0.23</td>
<td>0.43</td>
<td>(0.17–1.10)</td>
<td>16 -0.35 0.040</td>
<td>3.74</td>
<td>0.27</td>
<td>0.44</td>
<td>(0.21–0.96)</td>
</tr>
</tbody>
</table>

*p values in bold print represent statistically significant increases or decreases of the lipid equivalent concentration (i.e., <0.05).
concentrations \( C_{\text{lipid},\text{ng/g of equivalent lipid}} \) to remove the effect of differences in lipid contents or sorbing matrices between organisms. Concentrations in the benthic invertebrates, fish, and birds were normalized on a sample specific basis according to

\[
C_{\text{lipid}} = \frac{C_{\text{wet}}}{L}
\]

where \( L \) is the lipid fraction of the sampled tissue (g of lipid/g of wet tissue).

Because algae and plankton contain low lipid contents (Table 2) and high organic carbon contents (Table 2), the organic carbon is the main energy and carbon source and an important site for chemical accumulation. Therefore, the lipid equivalent normalization for plankton and macroalgae incorporated both lipid and nonlipid organic carbon contents. Concentrations in plankton and algae were normalized on a sample specific basis according to

\[
C_{\text{lipid}} = \frac{C_{\text{wet}}}{[L + (\phi_{\text{OC}} \times 0.35)]}
\]

where \( \phi_{\text{OC}} \) is the fraction of nonlipid organic carbon (i.e., the lipid content has been subtracted from the total organic carbon content) (g of OC/g of wet sample) and 0.35 is a proportionality constant recommended in ref 29 to relate the sorption properties of organic carbon to those of octanol. It should be stressed that this proportionality constant includes considerable uncertainty (29). However, assuming that the lipid equivalent normalization applies to PCBs and DPEs in a similar fashion, errors in the normalization can be expected to affect DPEs and PCBs similarly, causing a minimal effect on the relative trophodynamic behavior of DPE and PCBs.

**Food-Web Magnification Factors (FWMFs).** The FWMF is a useful measure of the degree of biomagnification in the food web (30, 31). It represents the average increase in lipid equivalent chemical concentration for a 1.0 unit increase in trophic position or a 3.4% increase in stable nitrogen isotope ratio \( \delta^{15}N \). An increase in one trophic level is associated with an approximately 3.4% increase in \( \delta^{15}N \) (15, 16). FWMFs were determined as the antilog of the slope (m) of the log-linear regression between log PCB or DPE concentration and trophic level, defined as either 3.4 \( \delta^{15}N \) %o (i.e., FWMF \( m = 10^{0.939m} \)), or trophic position (i.e., FWMF \( m = 10^{m} \)). For example, a FWMF of 2 means that the lipid equivalent concentrations increase 2-fold for a 1-step increase in trophic position or an increase of 3.4% in \( \delta^{15}N \) by 3.4%. Conversely, a FWMF of 0.25 indicates that the concentration at a given trophic level is 25% of the concentration at the previous trophic level or that the concentration decreases by 75%, or a factor of \( \sqrt[4]{0.25} = 4 \), per trophic level step. A FWMF greater than 1.0 indicates chemical biomagnification in the food web. A FWMF less than 1.0 indicates trophic dilution.

**Results and Discussion**

**Food-Web Characterization.** Trophic positions and observed \( \delta^{15}N \) and \( \delta^{13}C \) ratios are summarized for all species in Table 2. Figure 3 reveals a general pattern of enrichment of both \( \delta^{15}N \) and \( \delta^{13}C \) from the sediment and moving through the food web to plankton, macroalgae, and bivalves, small forage fish, large fish, marine birds, dungeness crabs, and dogfish. This isotopic enrichment is consistent with the 3.4-%o increase per trophic level observed in some other food webs (15). Figure 4 illustrates a strong proportional relationship between TP and \( \delta^{13}N \) isotopic ratios (i.e., \( \delta^{13}N = 2.17TP + 5.33, r^2 = 0.65 \)), indicating that the two measures of trophic status are consistent. However, two exceptions exist. First, the \( \delta^{13}N \) ratio of the seastar was very low and appears to underestimate the actual trophic level of the seastar based on considerable evidence that the seastar primarily consumes mussel, whelk, and octopus (e.g., 32, 33). \( \delta^{13}C \) isotope ratios of the seastar and mussels also appeared unrelated. The differences in isotope ratios may be due to the unique calcareous ossicle skeletal tissue of the seastar (34) producing an isotopic signature that is incomparable to that of the fish and invertebrate muscle tissue. For this reason, the seastar was not included in the correlation between the lipid equivalent chemical concentration and \( \delta^{15}N \). Second, in the spiny dogfish samples, the \( \delta^{15}N \) ratio of the muscle tissue (11.0 ± 0.9%) was considerably lower than that of the liver (15.8 ± 0.9%) and embryo (16.8 ± 1.4%) tissues and appears to underestimate the trophic level of the species based on dietary literature evidence (18). Excretion of waste urea (containing higher levels of \( ^{15}N \)) to dogfish tissues (35) and a slow turnover rate of the dogfish muscle tissue relative to the liver and embryo (36, 37) may explain the difference in \( \delta^{15}N \) ratio between the tissues. Since our aim was to most accurately represent the current trophic status of the dogfish, we used the \( \delta^{15}N \) ratio of the dogfish liver to represent the trophic status of the dogfish. To ensure only independent data were used in the correlations of concentrations, TP and \( \delta^{15}N \), concentration data in dogfish embryo and liver were excluded.
as a function of $\delta^{15}$N in marine biota. Concentrations detected in this study were no statistically significant differences in PCB or DPE concentrations between the three sampling stations in False Creek. Kolmogorov-Smirnov and Shapiro-Wilk normality tests revealed that both the PCB and DPE concentrations in the blanks and marine species were log-normally distributed. Concentrations are presented in 10-based logarithm units ($\log_{10}$). Gray circles indicate biota data (i.e., for purple seastar and dogfish liver, and embryo) not included in the regression. Solid line indicates least sum of squares regression between lipid equivalent concentration and $\delta^{15}$N for the marine biota.

Both stable isotope and TP analyses indicate that the organisms collected represent approximately 4 trophic levels. This is sufficient to investigate the occurrence of biomagnification or trophic dilution of the DPEs and PCBs. Concentrations of the 6 target PCB congeners and 13 phthalate esters were detected at levels above the MDL in the majority of biota species (Table 2). ANOVA tests indicated that there were no statistically significant differences in PCB or DPE concentrations between the three sampling stations in False Creek. Kolmogorov-Smirnov and Shapiro-Wilk normality tests revealed that both the PCB and DPE concentrations in the blanks and marine species were log-normally distributed. Concentrations are presented in 10-based logarithm units ($\log_{10}$). Gray circles indicate biota data (i.e., for purple seastar and dogfish liver, and embryo) not included in the regression. Solid line indicates least sum of squares regression between lipid equivalent concentration and $\delta^{15}$N for the marine biota.

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FIGURE 7. Lipid equivalent concentrations of C8–C10 phthalate esters: di(Z-ethylhexyl) (DEHP), di-n-octyl (DnOP), di-iso-octyl (C8), di-n-nonyl (DnNP), di-iso-nonyl (C9), and di-iso-decyl (C10) in marine biota (●) as a function of δ15N (%). Gray circles indicate biota data (purple seastar and dogfish liver, and embryo) not included in the regression. Solid line indicates least sum of squares regression between lipid equivalent concentration and δ15N for the marine biota.

Lipid equivalent concentrations of DiBP, DnBP, BBP, C6, and C7 appear to decline slightly with increasing trophic position and δ15N in the food web (Figures 6 and 7, and Figures 2–4 in the Supporting Information). However, regression analysis indicates that this correlation is not statistically significant (p < 0.05). FWMFs based on δ15N ranged from 0.86 for DiBP to 0.68 for C6 (Table 3). Concentrations of C6 and C7 isomers were not detected in the top predator dogfish muscle tissue. The latter reduced the power to detect statistically significant trends in the food web. These results indicate that these DPEs do not biomagnify. A small degree of trophic dilution may take place for these DPEs, although this did not appear to be statistically significant. The lack of a statistically significant change in lipid equivalent concentration with trophic position suggests that the direct exchange and partitioning of these intermediate molecular weight DPEs between the organism and the water is an important route of exposure. This is in agreement with the bioaccumulation mechanisms of chemicals with similar Kow (e.g., chlorobenzenes), which also show tendency to partition between water and fish. The observation that lipid equivalent concentrations of the lower and medium molecular weight DPEs do not decline with increasing trophic position does not rule out the possibility that these DPEs are subject to metabolic transformation in biota. It is possible that these DPEs are metabolized but that the rate of metabolism is comparatively small to the rate of gill elimination. Gill elimination rates tend to drop with increasing Kow (49). Hence, metabolic transformation may have little effect on the distribution of DPEs between biota and water for the lower Kow substances, but become more important as Kow increases.

The lipid equivalent concentrations of the high molecular weight phthalates (i.e., DEHP, DnOP, DnNP, C8, C9, and C10) significantly declined with increasing trophic position and δ15N in the food web (p < 0.05 except for C10, where p = 0.073). FWMFs ranged from a factor of 0.48 for C8 to 0.25 for DnNP per 3.4½ increase of δ15N (Table 3); that is, equivalent concentrations of these DPEs decreased by 52–75% per trophic level step. This indicates that these high-molecular-weight phthalate esters do not biomagnify in the aquatic food web studied. Rather, they undergo trophic dilution. Trophic dilution occurs for substances that are predominantly absorbed via the diet and depurated at a rate greater than the elimination rate via fecal egestion and respiratory ventilation, typically as a result of metabolic transformation. Metabolic transformation of the chemical in the predator causes the predator to achieve a concentration lower than that in its prey. If this process is repeated at subsequent trophic interactions, the concentrations decline with increasing trophic status. The very high Kow of high-molecular-weight DPEs causes bioavailable concentrations in the water to be very low, resulting in dietary uptake being the predominant route of uptake. Their very high Kow also produces low respiratory and fecal elimination rates, as these rates are known to decline with increasing Kow (49–51).

Hence, even small rates of metabolic transformation of these high-molecular-weight DPEs can dominate the overall depuration rate. In addition, gill elimination rates drop with increasing organism body weight (49) and metabolic transformation rates have in some cases been observed to increase with trophic level. This causes metabolic transformation rates to have an even greater effect in larger size and higher trophic level organisms, which further contributes to the degree of trophic dilution. It is expected that metabolic transformation of the higher molecular weight DPEs is sufficiently large to cause a reduction of the internal chemical concentration in the predator compared to that in the prey, leading to an overall decline in concentration with increasing trophic level. The increase in importance of metabolic transformation in relation to gill elimination and fecal excretion as Kow increases is likely the main reason for the observed drop in FWMFs of the DPEs with increasing Kow (Figure 8).

Regulatory Implications. Because DPEs are high-production volume chemicals that are relatively stable and hydrophobic, their behavior in the environment has received considerable attention. Recently, the 1999 UNEP Protocol...
on LRTAP and domestic legislation in several countries including Canada under the Toxic Substance Management Policy of the Canadian Environmental Protection Act, 1999, have focused interests on the persistence (P), bioaccumulation (B), and inherent toxicity (iT) of DPEs as well as many other compounds. In Canada, the intent of the regulations regarding B is to identify substances that are "bioaccumulative", that is, increase in concentration with increasing trophic level in food webs or biomagnify as predator consumes prey. This study indicates that, in the marine aquatic food web studied, DPEs did not appear to biomagnify in this context. However, under UNEP LRTAP and Canadian legislation, substances are considered bioaccumulative if they exhibit a BAF (or BCF) > 5000 or a $K_{ow} > 10^5$. Food-Web Magnification Factors are not included as a criterion to identify bioaccumulative substances. This is unfortunate as neither $K_{ow}$ (a physical-chemical property) nor the BCF (a laboratory bioaccumulation experiment that does not include chemical exposure via the diet) provide direct insights into the biomagnification properties of chemical substances. The BAF (field measurements of bioaccumulation including dietary intake) is a better measure of biomagnification, although its numerical value is dominated by chemical exchange between organism and water rather than chemical transfer between organism and organism (e.g., predator and prey). In our view, the "bioaccumulative" nature of DPEs and perhaps many other commercial chemicals is most directly measured by FWMFs as performed in this study. If appropriate field data are available, the FWMFs should be treated as a key criterion to assess the biomagnification nature of chemical substances.

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Supporting Information Available

Accompanying text, tables, and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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