DISTRIBUTION OF PHTHALATE MONOESTERS IN AN AQUATIC FOOD WEB

by

Maggie L. McConnell
B.Sc., University of Prince Edward Island 2004

PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF RESOURCE MANAGEMENT

In the
School
of
Resource and Environmental Management

Report No. 426

SIMON FRASER UNIVERSITY

Spring 2007

APPROVAL

Name: **Maggie L. McConnell**

Degree: **Master of Resource Management**

Title of Research Project: **Distribution of Phthalate Monoesters in an Aquatic Food Web**

Report No.: **426**

Examining Committee:

_____

Dr. Frank A.P.C. Gobas
Senior Supervisor
Professor in the School of Resource and Environmental Management

_____

Dr. Margo Moore
Supervisor
Professor in the Department of Biology

Date Defended/Approved: _____

ABSTRACT

Dialkyl phthalate esters (DPEs) are a family of widely used industrial chemicals, mostly as additives to impart flexibility in plastics. The biodegradation of DPEs in the environment results in the formation of monoalkyl phthalate esters (MPEs). The environmental fate of MPEs is largely unknown but is important for the evaluation of DPEs. In this study, the presence, distribution, and bioaccumulation potential of MPEs in organisms of an aquatic food web were investigated. A field study was conducted in False Creek, Vancouver; sediment, seawater, and seven marine organisms were collected. The highest MPE concentrations (200ppb) were observed for M*n*BP in mussels. MPEs were not found to biomagnify in the food web. This indicates that MPEs are relatively quickly eliminated, possibly through gill water exchange and/or metabolic transformation. This study further suggests that the primary source of MPEs to the aquatic environment is via dietary DPE uptake and subsequent metabolism in biota.

Keywords: phthalate ester; phthalate monoester; aquatic food web; bioaccumulation; metabolism

Subject Terms: phthalate esters -- toxicology; food chains (ecology) -- British Columbia -- Vancouver -- False Creek; marine ecology -- British Columbia -- Vancouver -- False Creek

ACKNOWLEDGEMENTS

The work presented in this thesis would not have been possible without the support and collaboration of many colleagues, family members, and friends. I would like to thank my senior supervisor Frank Gobas for his vast and valuable knowledge, his enduring enthusiasm and positive attitude, and for the many opportunities I had to present this work at meetings and conferences. I would also like to thank Joel Blair from the Institute for Ocean Sciences (IOS) for his dedication and willingness to answer many questions about sample analysis, and for the countless hours working in the laboratory to generate the analytical data. Many thanks also to my second supervisor Margo Moore for her insightful comments on the project and thesis write-up. Thanks to all the members of the phthalate ester Environmental Research Task Group (ERTG) for providing comments throughout the project.

Several people were instrumental in assisting with the hands-on aspects of this project. I am appreciative to Linda White from IOS for conducting chemical analyses and to Shapna Mazumder from the University of Victoria for generating the isotope data. I am grateful to a group of people for their hard work while assisting with field collections: Adrian deBruyn, Alison Howell, Arianne Ransom-Hodges, Brad Penoff, Colm Condon, Doug Swanston, Elodie Nolf, Frank Gobas, Jeff Amos, Jill Mallory, Joel Blair, Laura MacLean, and Srinivas Sura.

I wish to thank the members of the Toxlab for enhancing my learning experience, sharing their knowledge, and for creating a positive environment. Special thanks to Adrian deBruyn for many insightful discussions and ideas. I have also greatly appreciated the support from the excellent staff at REM; thank you Bev Hunter and Laurence Lee. I would like to thank Natural Sciences and Engineering Research Council of Canada (NSERC) for scholarship funding and my friends in REM for making my experience at SFU an enjoyable one. Finally, I would like to thank my family for their constant support, motivation, and encouraging words.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

INTRODUCTION

The esters of 1,2-benzene dicarboxylic acid, commonly referred to as dialkyl phthalate esters (DPEs), are a family of chemicals which are widely used in consumer products (Stanley et al. 2003). Currently, over 5 million tonnes of DPEs are produced globally each year (Parkerton and Konkel 2000) and are commonly used to increase flexibility in polyvinylchloride (PVC) products (Staples et al. 1997). Most of the DPEs found in the environment are the result of slow releases of DPEs from plastics and other DPE-containing articles as they weather (Stanley et al. 2003). DPEs can be mobilized in the plastic polymer, removed at the surface of the product by a variety of physical processes (Stanley et al. 2003), and are then able to migrate into the aquatic environment. Thus, DPEs have become ubiquitous and have been observed in many environmental media (e.g., Parkerton and Konkel 2000, Morin 2003, Mackintosh et al. 2004). One field study in particular measured environmental concentrations of DPEs in an aquatic food web and found their levels to be 10-1000 times greater than PCBs in the same samples (Mackintosh et al. 2004).

The primary degradation products of DPEs are monoalkyl phthalate esters (MPEs) which are formed when one ester group is cleaved (Albro 1986) by hydrolysis from the DPE. MPE formation can occur through microbial processes in soil and sediment, both aerobically and anaerobically (Ejlertsson and

Svensson 1995), and in organisms by metabolic transformation (Webster 2003). MPEs can dissociate, and according to their estimated log acid dissociation constants of approximately 4.0 (Table 1), we would expect to see the MPEs mostly in their ionic form in the ecosystem of neutral pH. MPEs are not used commercially, and the only source of MPEs found in the environment is via the metabolism of DPEs. Laboratory studies have observed DPE metabolism and MPE production for some PE congeners in mammals and fish (Kluwe 1982, Barron et al. 1989, Barron et al. 1995, Webster 2003). Webster measured extensive DPE metabolism in the stomachs and intestines of fish, which created a pool of MPEs available for uptake or elimination (2003).

The octanol-water partition coefficients (K_{ow}s) increase for both DPEs and MPEs with increasing molecular weights (MWs) of the individual congeners. K_{ow}s of DPEs range from $10^{1.61}$ for dimethyl phthalate (DMP) to $10^{9.46}$ for di-*iso*-decyl phthalate (C10) (Cousins and Mackay 2000, Staples et al. 1997). The estimated K_{ow}s for MPEs are lower than those for DPEs and range from $10^{1.37}$ for monomethyl phthalate (MMP) to $10^{5.79}$ for monodecyl phthalate (MC10P) in the non-ionized form (Table 1) (Peterson and Parkerton 1999). Given these measures of hydrophobicity, there is the potential for certain DPEs to biomagnify in the food web (Staples et al. 1997), but no such potential exists for the majority of MPEs. However, because DPEs have been measured in relatively high concentrations in the aquatic food web (Mackintosh et al. 2004) and because they are known to transform into their respective MPEs we expect to observe similarly elevated concentrations of MPEs in the food web. Field studies to

confirm this do not exist. Very little is known with regards to abundance and distribution of MPEs in the various components of the environment (Suzuki et al. 2001). MPEs are potentially as widespread as their parent compounds (a more detailed literature review is included in Appendix 2).

This paper is the fifth in a series of the distribution of DPEs and MPEs in a marine environment. Previous studies focused on the analytical methodology for DPEs (Lin et al. 2003), the distribution of individual DPE congeners and commercial mixtures in the organisms of an aquatic food web (Mackintosh et al. 2004) and in water, suspended particulate matter, and sediment (Mackintosh et al. 2006), and the analytical methodology for MPEs (Blair et al. 2007 *in preparation*). In this paper we present a field study, which measures the environmental distribution of MPEs in a marine food web.

Objectives

The aim of this study is to:

1. Determine environmental concentrations and distribution of MPEs in a range of environmental media and organisms of the aquatic food web
2. Examine the parent-compound to metabolite concentration ratio
3. Investigate the relationship between MPE concentration in each species and its trophic level in food web

METHODS

Materials and Preparation

Due to their widespread use, DPEs are commonly found in both sampling and analytical equipment, as well as in laboratory air and reagents. Consequently, reducing and determining the background contamination of samples is crucial for ensuring that environmental data on DPEs are acceptable, accurate, and of high quality. Many preparatory steps for cleaning field equipment were undertaken and are described in detail in Lin et al. 2003[1]. HPLC grade solvents were used, hexane was doubly distilled and all glassware and aluminum foil used for collecting and storing samples were pre-cleaned following a detailed protocol before use.

[1] It is valuable to read the full text of Lin, Z., M.G. Ikonomou, J. Hongwu, C.E. Mackintosh, and F.A.P.C. Gobas. 2003. Determination of phthalate ester congeners and mixtures by LC/ESI-MS in sediments and biota of an urbanized marine inlet. *Environmental Science and Technology* 37(10): 2100-2108. Can be found at: http://pubs.acs.org/cgi-bin/article.cgi/esthag/2003/37/i10/pdf/es026361r.pdf

Sampling Site

The field sampling was conducted in False Creek Harbour (Figure 1) which has a mean depth of about 8m and is relatively well mixed (Mackintosh et al. 2004). False Creek is a small inlet of the Strait of Georgia, where the mean summer water temperature is 11°C, average salinity is 30ppt, and precipitation ranges from 90 to 200cm/year, and is located in downtown Vancouver, British Columbia, Canada (Mackintosh et al. 2006). The harbour is a heavily used area that encloses several marinas and is surrounded by urban infrastructure, both of which may act as sources of pollution into the water. PEs are closely associated with human use and as such we expect to observe elevated PE concentrations in False Creek Harbour.

Sample Collection

Water samples were collected in 4L amber glass bottles from mid-ocean depth (3-4m) using a 4m extendible stainless steel pole. Approximately 3ml of formic acid was added to each sample to reduce the pH of the water to 2.5. Ten samples were collected at random from False Creek. After collection, the bottles were sealed with a foil-lined lid, placed on ice, and then transferred to a 4°C refrigerator in the laboratory. The sample extraction occurred within 12 hours of collection.

Surficial sediment samples were collected using a petit Ponar grab sampler and transferred onto aluminum foil. The top layer (0.5 to 1.0cm) was removed with a metal spoon and transferred into100ml glass jars, covered with

aluminum foil, and sealed with a metal lid. Jars were immediately placed on ice and were then stored at -20°C in the dark prior to analysis. Ten samples were collected at random from False Creek.

Approximately 10 individual samples of 7 marine organisms from various trophic levels in the food web were collected (exact sample sizes and scientific names are shown in Table 2 and food web interactions are shown in Figure 2). A subset of species was collected to represent various trophic levels and taxonomic groups in the False Creek marine food web, as well as variety in feeding strategies, body sizes, and life histories. Green macroalgae, blue mussels, softshell and dark-mahogany clams, Dungeness crabs, shiner perch, white-spotted greenling, and spiny dogfish were collected.

Macroalgae, mussels, and clams were collected from intertidal regions during periods of low tide. Shiner perch were collected as juveniles using a gill net of ½ inch mesh size. Stainless steel crab and prawn traps with bait were used to collect crabs and white-spotted greenling. Dogfish were collected using a longline fishing system during incoming tides with help from a local fisherman. All biota samples were wrapped in aluminum foil or collected in jars and placed on ice in the field. The samples were stored frozen at -20°C in the lab prior to analysis. Bivalves and juvenile shiner perch were combined to obtain samples of 5-10g. All samples were collected between July and September 2005.

Sample Extraction and Analysis

Each sample was analyzed for MPEs and DPEs. A detailed description of the methods used for the analysis of DPEs is provided in Lin et al. 2003[1] and Mackintosh et al. 2004[2] and for MPEs in Blair et al. 2007[3] *in preparation*. Sample extracts were analyzed for DPEs by low-resolution gas chromatography mass spectrometry (GC/LR-MS) for the quantification of the individual phthalate esters (i.e., DMP, DEP, D*i*BP, D*n*BP, BBP, DEHP, D*n*OP, and D*n*NP). After GC/MS analysis, the extract was analyzed by liquid chromatography electrospray ionization mass spectrometry (LC/ESI-MS) to quantify the isomeric commercial mixtures of phthalate esters (i.e., C6, C7, C8, C9, and C10) in all samples.

Sample extracts were analyzed for MPEs by LC/ESI using tandem mass spectrometry (MS/MS) for the quantification of the individual phthalate monoesters (i.e., MMP, MEP, M*n*BP, MBzP, M*n*HP, MC7P, MEHP, M*n*OP, MC9P, MC10P) (full chemical names are listed in Table 1).

Quantitation and Quality Assurance and Control (QA/QC)

A detailed description of quantification using the internal standard isotope-dilution method for identifying concentrations of 10 MPEs and 13 DPEs is

[1] Can be found at: http://pubs.acs.org/cgi-bin/article.cgi/esthag/2003/37/i10/pdf/es026361r.pdf

[2] It is valuable to read the full text of Mackintosh, C.E., J. Maldonado, J. Hongwu, N. Hoover, A. Chong, M.G. Ikonomou, and F.A.P.C. Gobas. 2004. Distribution of phthalate esters in a marine aquatic food web: comparison to polychlorinated biphenyls. *Environmental Science and Technology* 38(7): 2011-2020. Can be found at: http://pubs.acs.org/cgi-bin/article.cgi/esthag/2004/38/i07/pdf/es034745r.pdf

[3] It is valuable to read the full text of Blair, J., M.G. Ikonomou, and F.A.P.C. Gobas. 2007. Determination of monoester phthalate metabolites by LC/ESI-MS in sea water, sediment, and biota in an urbanized marine inlet. *In preparation*. Until published, contact corresponding author for a copy of this article (gobas@sfu.ca).

provided in Mackintosh et al. 2004[1]. The recoveries of the PE isotope labeled surrogate internal standards were 72 to 94% (GC/MS analyses), and 71 to 96% (LC/MS analyses). Biological samples showed relatively low recoveries in some cases (e.g., crab hepatopancreas) and were re-analyzed to ensure accurate measurement of the concentration data. Minimum detectable amounts (MDAs), which correspond to the amount of chemical that produces a 3:1 signal-to-noise ratio, ranged from 0.09 to 2.0ng for individual MPEs, from 0.03 to 0.26ng for individual DPEs and DPE isomeric mixtures.

Procedural blanks were used throughout the entire extraction and analysis process to ensure that background contaminants would not contribute to the reported environmental concentrations. Procedural blanks were especially important for DPE analysis because these compounds are ubiquitous and extremely difficult to remove from solvents and glassware. Each sample batch consisted of 2-3 procedural blanks and 4-10 matrix samples. Procedural blanks consisted of 500mL of spring water for the water samples and 10-20g of Ottawa sand (MPE batches) and sodium sulphate (DPE batches) for the sediment and biota samples. For each MPE and DPE batch, sample concentrations were blank corrected and method detection limits (MDLs) were calculated as the mean + 2 standard deviations of the blanks (minimum and maximum MDLs for each matrix are in Table 3 for MPEs and in App.1 Table 1 for DPEs). Only the sample concentrations of DPEs and MPEs that exceeded the MDL in more than 30% of samples were included in further analyses and reporting. A value of ½MDL was

[1] Can be found at: http://pubs.acs.org/cgi-bin/article.cgi/esthag/2004/38/i07/pdf/es034745r.pdf

used to replace remaining non-detect (ND) values for the reportable data. Mean MPE amounts (i.e., average of MPEs across all the batches) in the procedural blanks ranged from 0.07ng for MC9P to 24ng for M*n*BP (n=27) (Table 4). Mean DPE amounts in the procedural blanks ranged from 0.15ng for D*n*NP to 17ng for DEHP for GC/MS analysis (n=36) and from 4.8ng for C6 to 150ng for C8 (n=50) for LC/MS (App.1 Table 2).

Table 4 shows that the percentage of samples with detectable concentrations that met the method detection limit in sediment ranged from 0% for MC7P and MEHP to 100% for MMP and MBzP, and in biota ranged from 3% for MC9P to 78% for M*n*BP (organisms are shown separately in App.1 Table 3). App.1 Table 2 shows that the percentage of samples with detectable concentrations that met the method detection limit in sediment is 100% for all DPE congeners and in biota ranged from 40% for D*n*NP to 84% for BBP and from 31% for C6 to 77% for C8 (organisms are shown separately in App.1 Table 4).

Trophic Position Calculation

Nitrogen and carbon stable isotopes (i.e., $\delta^{15}N$ and $\delta^{13}C$) were also measured in the sediment and biota samples. These signature ^{15}N and ^{13}C isotopes are calculated as the concentration ratio of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ expressed relative to a standard. The $\delta^{15}N$ and $\delta^{13}C$ can be used to estimate trophic position because the $\delta^{15}N$ of a consumer is typically enriched by 3-4‰ relative to its diet due to the preferential excretion of the lighter nitrogen isotope

(DeNiro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987) and the $\delta^{13}C$ changes very little as carbon moves through a food web (Rounick and Winterbourn 1986, Peterson and Fry 1987, France and Peters 1997). To analyze for nitrogen and carbon stable isotopes, approximately 35mg of freeze-dried surficial sediment (n = 4) and 1mg (3mg for algae samples) of freeze-dried biota tissue (n = 4 for each species) were finely ground using an acid-washed mortar and pestle and were enclosed in 8 x 5mm tin capsules from Costech Technologies (Valencia, CA). The samples used for isotope analysis were the same as those analyzed for DPEs and MPEs. Samples were analyzed for natural abundance of stable nitrogen and carbon isotopes on a Costech 4010 Elemental Analyser coupled to a Thermo Delta Plus Advantage stable isotope ratio mass spectrometer. Details on the calculation of $\delta^{15}N$ and $\delta^{13}C$ are presented in Appendix 1 and measurements in sediment and all biota samples are reported in Table 5.

Organic Carbon Contents

Total organic carbon (TOC) was measured in sediment and all biota samples following Van Iperen and Helder (1985) and is reported in Table 5. Sediment and algae samples were oven dried at 50°C to a stable weight then homogenized with a mortar and pestle. Approximately 500mg of the dried samples were acidified in a clean crucible with 10ml of 1N HCL to remove carbonates. The acidified samples were then dried on a hot plate at 70°C overnight, followed by 2 hours in the oven at 105°C, and finally left open to room

temperature and humidity for 2 additional hours. Subsamples of approximately 3-10mg were weighed into tin cups for analysis on the Control Equipment Corporation 440 Elemental Analyzer. Acetanilide standards, containing 71.09% carbon, were included in the batches and sample duplicates were analyzed.

All other biota samples were oven dried at 50°C to a stable weight then homogenized with a mortar and pestle. Subsamples of approximately 5-8mg were weighed into tin cups for analysis on the Elemental Analyzer. Acetanilide standards, containing 71.09% carbon, were included in the sample batches and sample duplicates were analyzed. Organic carbon was expressed on a dry weight basis as g OC/g dry sample.

Lipid Contents

One to five grams of wet tissue from each biota sample were analyzed for lipid content and is reported in Table 5. Details of lipid content analysis can be found in Mackintosh et al. 2004[1]. Lipid content was expressed on a wet weight basis as g lipid/g of wet sample.

Lipid Equivalent Concentrations

In this paper we present the concentrations of MPEs and DPEs in each species normalized to lipid contents. Observed wet weight concentrations (C_{wet}, ng/g wet tissue) were converted to lipid weight concentrations (C_{lipid}, ng/g lipid)

[1] Can be found at: http://pubs.acs.org/cgi-bin/article.cgi/esthag/2004/38/i07/pdf/es034745r.pdf

using: $C_{lipid} = C_{wet}/[\Phi_L + (\Phi_{OC}*0.035)]$ for biota, $C_{lipid} = C_{wet}/ [\Phi_L + (\Phi_{OC}*0.35)]$ for algae, $C_{lipid} = C_{dry}/ (\Phi_{OC}*0.35)$ for sediment, and $C_{lipid} = C_{wet} * K_{ow}$ for water,

where Φ_L is the lipid fraction of the sampled tissue (g lipid/g wet tissue), Φ_{OC} is the fraction of non-lipid organic carbon (g OC/g wet tissue) calculated by subtracting the lipid fraction from the total organic carbon fraction (both on a wet weight basis), *0.035* and *0.35* are proportionality constants relating the sorption properties of organic carbon to those of octanol, and K_{ow} is the octanol-water partition coefficient.

Food Web Magnification Factors

FWMFs were calculated for each regression between log lipid MPE concentration and trophic level ($\delta^{15}N$) following the method used in Mackintosh et al. 2004[1].

Data Analysis and Statistics

All concentration data were tested for normality using the Kolmogorov-Smirnov normality test. Standard deviations are reported along with means, unless otherwise specified. Other statistical methods are reported in each section of the results.

[1] Can be found at: http://pubs.acs.org/cgi-bin/article.cgi/esthag/2004/38/i07/pdf/es034745r.pdf

RESULTS AND DISCUSSION

MPE and DPE Concentrations in the Marine Food Web

Kolmogorov-Smirnov normality tests revealed that both the MPE and DPE concentrations in the samples were log-normally distributed (results of normality tests can be found in App.1 Table 5 and App.1 Table 6 for MPEs and DPEs, respectively). Concentrations are presented in 10-based logarithm units in Table 5 and in App.1 Table 8.

Water

Concentrations of seven of the 10 MPE congeners were detected at levels above the MDL in the water samples whereas nine of the 13 DPE congeners were detected at levels above the MDL (Table 5). MPE concentrations in water ranged from 0.26ng/L for M*n*HP to 60ng/L for MEHP. MPE levels detected in water are comparable with those found by Suzuki et al. (2001) in the Tama River in Tokyo Japan (Table 6). Also, MPE concentrations in the water are approximately 6 orders of magnitude below available acute LC_{50}s (Scholz 2003) (Table 7).

DPEs were not measured in the water samples due to time constraints and to the high level of difficulty in accurately measuring these concentrations without contamination. For the purposes of comparison between MPE and DPE concentrations in the water samples, we refer to measurements of DPEs in water

samples from a similar field study (see data from Mackintosh et al. 2004 in Table 5). Although variability will occur, we believe the use of these data is justified for our purposes. Both studies took place at the same site and the field sampling and analytical techniques were identical for the two studies. However, we have not detected similarities or changes (increases or decreases) with any consistency between the two studies and have therefore attributed these observations to natural variability in the DPE concentrations over time.

Sediment

Concentrations of eight of the 10 MPE congeners were detected at levels above the MDL in the sediment samples whereas all of the 13 DPE congeners were detected at levels above the MDL (Table 5). Concentrations are presented on a wet weight basis (Table 5) and on dry weight basis (App.1 Table 8). MPE concentrations in sediment ranged from 0.04ng/g ww (0.10 ng/g dw) for M*n*HP to 4.0ng/g ww (11 ng/g dw) for M*n*BP.

DPE concentrations in sediments ranged from 1.1ng/g ww (2.9 ng/g dw) for C6 to 610ng/g ww (1600 ng/g dw) for C8. DPE concentrations in False Creek sediment are statistically less than (for DMP, D*n*BP, DEHP, C6, C7, C10), more than (forD*n*NP, C9), and the same as (for DEP, D*i*BP, BBP, D*n*OP, C8) DPE concentrations previously reported in False Creek sediment from a similar food web study (Mackintosh et al. 2004) (see results of statistical comparisons in Table 8). It is not clear whether sediment concentrations from the two studies are different from or equal to each other because there is not a consistent pattern among DPE congeners. These inconsistencies are most likely attributable to

natural variability and not to sampling error since the field sampling and analytical techniques were the same for both studies, as discussed above. Figure 4 provides a visual comparison of DPEs in sediment for the two studies; although some variability between sampling year is evident, concentrations for the same congeners never differ by more than one order of magnitude.

Organisms

Concentrations of the 10 MPE congeners were detected at levels above the MDL in less than a third of the biota samples whereas the 13 DPE congeners were detected at levels above the MDL in more than three quarters of the samples (Table 5). Concentrations are presented on a wet weight basis (Table 5) and on a lipid equivalent basis (App.1 Table 8). Certain congeners (i.e., high MW congeners: M*n*OP, MC9P, MC10P) were consistently non-detectable in biota samples and MPEs were never detected in dogfish muscle. MPE concentrations ranged from 0.05ng/g ww (2.9 ng/g lw) for M*n*HP in shiner perch to 200ng/g ww (39000 ng/g lw) for M*n*BP in mussels. Apart from M*n*BP, the MPEs that were present in the highest concentrations were MEP (7.5ng/g ww; 1300 ng/g lw) and MMP (6.9ng/g ww; 1300 ng/g lw) in mussels.

DPE concentrations ranged from 0.05ng/g ww for DMP in Dungeness crab to 2000ng/g ww for C8 in dogfish (Table 5) (distribution is shown in Figure 3). On a lipid weight basis, DPE concentrations ranged from 4.7ng/g lw for DMP in white-spotted greenling to 66000ng/g lw for C8 in algae (App.1 Table 8). Apart from C8, the DPEs that were present in the highest concentrations were D*n*BP, DEHP, C9, and C10 in various organisms. Similar to DPE concentrations in

sediment, DPEs in biota of False Creek show no consistent pattern when compared to DPE concentrations previously reported in False Creek biota from a similar food web study (Mackintosh et al. 2004). Figure 4 provides a visual comparison of DPE concentrations in clams and dogfish (as examples of biota), as well as in sediment, from the present study and from Mackintosh et al. 2004. This figure shows the range of variability in DPE concentrations that was detected; when all congeners are grouped together, sediments have significantly higher DPE concentrations in 1999 than in 2005, dogfish have significantly higher DPE concentrations in 2005 than in 1999, and clams are statisfically equal.

Parent Compound – Metabolite Relationship

To further investigate the presence of MPEs in the environment we present a comparison of the distribution of MPEs to DPEs in the various levels of the food web. Figure 5 illustrates the relative composition of MPEs and DPEs in water, sediment, mussels, and juvenile shiner perch. PEs are mainly in the diester form in water and in sediment whereas the monoester forms are more dominant in certain organisms, but only for lower MW congeners (Figure 5).DPE concentrations are significantly higher than MPE concentrations in the water except for the lowest MW congener (methyl) which is significantly higher for the MPE (results of statistical tests are in Table 9). Similarly, DPE concentrations are significantly higher than MPE concentrations in the sediment (Table 9). In contrast, with the exception of benzyl, MPE concentrations are significantly higher than DPE concentrations in the mussels at low MWs (e.g., methyl p =

<0.001) but the opposite is true for high molecular weight MPEs and DPEs (e.g., ethylhexyl p = <0.001) (Table 9). Similarly, in perch DPE concentrations are significantly higher than corresponding MPE concentrations at high MWs (e.g., ethylhexyl p = <0.001) but at low MWs only butyl (p = 0.001) has significantly higher MPE concentrations than DPE concentrations (Table 9).

We are not able to present useful patterns for the other organisms in the food web as well as for these two organisms, a result of the large number of NDs. However, upon examination of the MPE/DPE concentration ratios calculated for each parent-metabolite congener pair in each matrix, the remaining organisms seem to follow the same general pattern as the mussels and perch, where concentrations have been reported (i.e., decreasing MPE/DPE concentration ratios with increasing MW) (Table 10).

Similar to mussels and perch, an increase in the MPE/DPE ratio from water and sediment to biotic organisms continues through the remaining organisms of the food web and is more clearly demonstrated when we focus on a single congener (parent-metabolite pair). Figure 6 shows that D*n*BP is the dominant PE form in water, sediment, and algae whereas M*n*BP is this dominant form for the remaining organisms of the food web. This figure suggests that the organisms of the food web, specifically the consumer organisms (i.e., excluding algae), have metabolic capacities for degrading D*n*BP to M*n*BP. Other studies have reported rapid metabolism of DPEs to MPEs in biological organisms (e.g., Webster 2003, Kluwe 1982). Yet, it has been shown that mixed microbial populations, which are typically found in the environment, are capable of

completely mineralizing PEs (Kurane 1986). It is therefore possible that we observe relatively low MPE levels in water and sediment because the MPEs are further degraded to phthalic acid which can be used by the microbes as a carbon source.

However, this trend does not appear to hold true for all congeners. Figure 8 shows that the MEHP/DEHP composition in water samples is similar to that in organisms of the food web where MPE/DPE concentrations were detected. This result suggests that the organisms have decreased metabolic capacities for degrading DEHP to MEHP. Thus, we conclude that the organisms of the food web are capable of metabolizing low MW DPEs to MPEs but are not as capable at metabolizing the high MW DPE congeners. This discrepancy reflects the preferential degradation associated with low MW (and short, unbranched alkyl chain) compounds (e.g., D*n*BP vs. DEHP), which has been reported in various sediment and soil DPE biodegradation studies (e.g., Cartwright et al. 2000, Yuan et al. 2002, Staples et al. 1997). However, it is also possible that the high MW MPEs are further transformed from free monoesters into oxidized and glucoronidated forms by the organisms, which are not detected.

MPE Distribution in the Food Web

Linear regression analyses showed no statistically significant relationships between the lipid equivalent log concentrations for any of the MPEs and $\delta^{15}N$ (i.e., $p > 0.05$) (Table 11). Food web magnification factors (FWMFs) ranged from 0.22 (0.03-1.6; lower-upper 95% confidence limits) for MC7P to 1.6 (0.17-15) for

M*n*BP (Table 11). The FWMFs for some MPEs had large confidence intervals which are most likely due to the small number of points used to calculate the slopes, a result of removing ND data from analyses. Linear regressions for MEP, M*n*BP, MC7P, and MEHP are plotted in Figure 8; sample size is too small because of the large number of NDs to perform regressions for the other MPEs. Results of linear regression analyses for DPEs can be found in App.1 Table 7.

The regression lines appear to be flat for MEP, M*n*BP, and MEHP, an indication of little change in concentration throughout the food web and in fact their slopes are not significantly different from zero (MEP, $p = 0.69$; M*n*BP, $p = 0.63$; MEHP, $p = 0.91$). Although the regression line for MC7P appears to be declining with increasing trophic level or $\delta^{15}N$ (Figure 8), the slope of this line is not significantly different from zero either (MC7P, $p = 0.08$) (Table 11). These results indicate that MPEs do not biomagnify in the food web. This conclusion is consistent with our hypothesis that MPEs have no potential to biomagnify and those that have been previously proposed by other researchers (e.g., Peterson and Parkerton 1999, Scholz 2003).

Furthermore, we focus again on the consumer organisms of the food web, but with emphasis on the metabolism of MPEs instead of DPEs. Slopes resulting from linear regression between lipid equivalent log concentrations of each MPE and $\delta^{15}N$ are greater when analyses are performed only for the consumer organisms of the food web (Figure 9 and Table 11) as compared to the linear regressions that included sediment and algae samples (Figure 8). Negative slopes are greater for each of the three MPEs (e.g., MEP: slope changes from -

0.06 to -0.27, M*n*BP: from 0.06 to -0.28, MEHP: from 0.01 to -0.15) (Table 11). A negative slope significantly different from zero is indicative of trophic dilution, whereby concentrations of substances primarily absorbed via the diet decline with increasing trophic level, typically a result of metabolic transformation (Mackintosh et al. 2004). However, regression analysis indicates that apart from M*n*BP (p = 0.03), these correlations are not significantly different from zero (Table 11). Thus, there is no evidence of biomagnification or trophic dilution of MPEs in the food web, except for M*n*BP which shows trophic dilution for the consumer organisms.

The lack of MPE biomagnification is likely due to rapid MPE metabolism and/or efficient elimination. MPEs are relatively water soluble compounds (and have fairly low K_{ow}s; see Table 1) which means that they are able to eliminate to the water rather quickly. It is also possible that MPEs are being easily metabolized in the organisms. The combination of relatively rapid elimination and metabolic transformation negates any potential biomagnification.

CONCLUSIONS

1. We detected MPEs in most phases of the marine aquatic food web; almost all MPEs were found in water and sediment, and certain MPE congeners were found in all organisms of the food web, except the dogfish, which has the highest trophic status.

2. Variability among replicate samples was large and NDs were frequent, especially for high MW congeners (i.e., M*n*OP, MC9P, MC10P) and for high trophic level organisms (i.e, white-spotted greenling, dogfish).

3. The highest detectable concentrations of MPEs were observed at 200 ppb wet weight for M*n*BP in mussels. High concentrations were also observed for MMP, MEP, and MEHP.

4. The relative composition of MPEs in PE concentrations is low in the water, sediment, and algae. This may indicate that microorgamisms in the water and sediment are completely degrading MPEs.

5. The relative composition of MPEs in PE concentrations is high for some consumer organisms in the food web, but only for low MW congeners; this suggests that the consumer organisms are able to metabolize DPEs to MPEs for these congeners.

6. The data show no correlation between the lipid equivalent log concentrations of each MPE and $\delta^{15}N$; this implies that MPEs do not biomagnify in the aquatic food web. MPEs do not biomagnify because they are relatively water soluble and are therefore rapidly metabolized in the organisms and/or efficiently eliminated.

7. MPEs do not show trophic dilution either, with the exception of M*n*BP. The data suggest that consumer organisms in the food web have increasingly efficient M*n*BP metabolism as trophic level increases and/or the concentrations of M*n*BP in their prey items have increasingly lower concentrations as trophic level increases.

8. MPEs are not commercially used and there is no source of MPEs to the environment. We detect much higher concentrations of DPEs than MPEs in the water and sediment although DPEs are very hydrophobic and MPEs are less hydrophobic and ionized. This result suggests that the primary source of MPEs to the environment is from dietary DPE uptake (from many DPE sources) and subsequent DPE metabolism to produce MPEs, as has been previously reported.

RECOMMENDATIONS AND FURTHER RESEARCH

This study is one component of a larger PE research project. The information learned from this MPE environmental distribution study will be used in a PE fate model that will provide the necessary tools to monitor and predict the behaviour of PEs in the environment and assess the costs and benefits of PE production, import, and use in Canada.

To date we have found that DPEs and now MPEs are readily transformed in the organisms of the aquatic food web and we have evidence suggesting that DPE metabolism is the main if not the only source of MPEs to the environment. Although DPEs and MPEs appear to be quickly eliminated, theoretically a steady state may be reached because of chronic and repetitive low level exposure resulting from dietary ingestion of DPEs which come from many commonly used products. In other words, a continual influx of DPEs could lead to a continuous production of MPEs in the environment. If production and release of DPEs into the environment increases substantially over years, concentrations of both DPEs and MPEs will increase in the various components of the food web.

The final model will be very useful in predicting the outcome of different production volume scenarios. However, we need to have a benchmark to which we can compare environmental concentrations in order to assess toxicological risk. For this reason, I recommend continued toxicity testing of MPEs (and other

PE metabolites) to generate reliable and useful toxic endpoints for a range of organisms because very few acute or chronic toxicity data are available for MPEs, as was previously discussed. Although MPEs and DPEs do not biomagnify, they are detected in various organisms of the food web, PE concentrations may become high enough to trigger certain toxic effects, and the total PE body burden (DPE+MPE) may also be of concern. In addition, with increased toxicity data, we could also establish meaningful water quality guidelines. We could use what we now know about DPEs and MPEs along with toxicity data to create these maximum threshold water concentrations set to protect the system at any desired level.

Regardless of the results of this study, we feel that metabolic compounds in general merit more attention than they receive, in terms of regulation. This study has helped to further our understanding of the parent compound-metabolite relationship for PEs and demonstrates that it is important to look beyond the parent chemical and to consider the metabolic forms in terms of their persistence, bioaccumulation potential, and toxicity. We recommend that metabolites be screened under *CEPA* following the same protocol as their parent compounds.

In conjunction with previous PE work and research that is currently taking place, this study has the potential to provide valuable information to PE manufacturers and help regulators (e.g., Environment Canada and Health Canada) address the environmental impacts of this class of industrial pollutants.

REFERENCE LIST

Albro, P.W. 1986. Absorption, metabolism, and excretion of di(2-ethylhexyl) phthalate by rats and mice. *Environmental Health Perspectives* 65: 293-298.

Anderson, W.A.C., L. Castle, M.J. Scotter, R.C. Massey, and C. Springall. 2001. A biomarker approach to measuring human dietary exposure to certain phthalate diesters. *Food Additives and Contaminants* 18(12): 1068-1074.

Barron, M.G., I.R. Schultz, and W.L. Hayton. 1989. Presystemic branchial metabolism limits di-2-ehtylhexyl phthalate accumulation in fish. *Toxicology and Applied Pharmacology* 98: 49-57.

Barron, M.G., P.W. Albro, and W.L. Hayton. 1995. Biotransformation of di(2-ehtylhexyl)ptththalate by rainbow trout. *Environmental Toxicology and Chemistry* 14(5): 873-876.

Blair, J., M.G. Ikonomou, and F.A.P.C. Gobas. 2007. Determination of monoester phthalate metabolites by LC/ESI-MS in sea water, sediment, and biota in an urbanized marine inlet. *In preparation*.

Cartwright, C.D., I.P. Thompson, and R.G. Burns. 2000. Degradation and impact of phthalate plasticizers on soil microbial communities. *Environmental Toxicology and Chemistry* 19(5): 1253-1261.

CEPA (Canadian Environmental Protection Act). 1999. Canada Gazette Part III: Vol. 22 No.3 November 4, 1999. Statutes of Canada, 1999, Chapters 33 and 34, Acts assented to from 18 June to 14 September, 1999. Ottawa, Canada: the Minister of Public Works and Government Services, the Queen's Printer for Canada.

Cousins, I., and D. Mackay. 2000. Correlating the physical-chemical properties of phthalate esters using the 'three solubility' approach. *Chemosphere* 41: 1389-1399.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45: 341–351.

Duty, S.M., A.M. Calafat, M.J. Silva, L. Ryan, and R. Hauser. 2005. Phthalate exposure and reproductive hormones in adult men. *Human Reproduction* 20(3): 604-610.

EC (Environment Canada). 2005. "Eighteen new substances added to the CEPA List of Toxic Substances," news releases–archives. Available at: http://www.ec.gc.ca/press/18psl_b_e.htm

Ejlertsson, J. and, B. Svensson 1995. A review of the possible degradation of polyvinyl chloride (PVC) plastics and its components phthalic acid esters and vinyl chloride under anaerobic conditions prevailing in landfills. Department of Water and Environmental Studies, Linkoping University, Sweden, 20pp.

France, R. L., and R. H. Peters. 1997. Ecosystem differences in the trophic enrichment of ^{13}C in aquatic food webs. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 1255–1258.

Kluwe, W.M. 1982. Overview of phthalate ester pharmacokinetics in mammalian species. *Environmental Health Perspectives* 45: 3-10.

Kurane, R. 1986. Microbial degradation of phthalate esters. *Microbiological Sciences* 3: 92-95.

Lin, Z., M.G. Ikonomou, J. Hongwu, C.E. Mackintosh, and F.A.P.C. Gobas. 2003. Determination of phthalate ester congeners and mixtures by LC/ESI-MS in sediments and biota of an urbanized marine inlet. *Environmental Science and Technology* 37(10): 2100–2108.

Mackintosh, C.E., J. Maldonado, J. Hongwu, N. Hoover, A. Chong, M.G. Ikonomou, and F.A.P.C. Gobas. 2004. Distribution of phthalate esters in a marine aquatic food web: comparison to polychlorinated biphenyls. *Environmental Science and Technology* 38(7): 2011-2020.

Mackintosh, C.E., J. Maldonado, M.G. Ikonomou, and F.A.P.C. Gobas. 2006. Sorption of phthalate esters and PCBs in a marine ecosystem. *Environmental Science and Technology* 40: 3481-3488.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between ^{15}N and animal age. *Geochimica et Cosmochimica Acta* 48:1135–1140.

Morin, A. 2003. Distribution of phthalate esters in a marine mammal food chain from Canada's Eastern Arctic. (Under supervision of Gobas). Master in Resource Management Thesis. Simon Fraser University.

Otton, S.V., S. Sura, J. Blair, M.G. Ikonomou, and F.A.P.C. Gobas. 2007. Biodegradation of phthalate monoesters in natural sediments. *In preparation.*

Parkerton, T. F., and W. J. Konkel (ExxonMobil Biomedical Services, Inc.). Evaluation of the production, consumption, end use, and potential emissions of phthalate esters; Report prepared for the American Chemistry Council [ACC], 1300 Wilson Ave., Arlington, VA, 2000.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18: 293–320.

Peterson, D.R., and T.F. Parkerton. 1999. The environmental fate and effects of phthalate monoesters. Report prepared for the CEFIC, Brussels, Belgium, 17pp.

PSL (Priority Substances List). 1993. Assessment Report of Di-*n*-Octyl Phthalate. *Canadian Environmental Protection Act*. Government of Canada, Environment Canada, Health Canada. Minister of Supply and Services, Canada. National Printers (Ottawa) Inc.

Rounick, J. S., and M. J. Winterbourn. 1986. Stable carbon isotopes and carbon flow in ecosystems. *BioScience* 36: 171–177.

Scholz, N. 2003. Ecotoxicity and biodegradation of phthalate monoesters. *Chemosphere* 53: 921-926.

Stanley, M.K., K.A. Robillard, and C.A. Staples. Introduction. In Handbook of Environmental Chemistry 3.Q. Phthalate Esters; Staples, C., Ed.; Springer:Berlin, 2003; pp. 1-7.

Staples, C.A., D.R. Peterson, T.F. Parkerton, and W.J. Adams. 1997. The environmental fate of phthalate esters: a literature review. *Chemosphere* 35: 667-749.

Suzuki, T., K. Yaguchi, S. Suzuki, and T. Suga. 2001. Monitoring of phthalic acid monoesters in river water by solid-phase extraction and GC/MS determination. *Environmental Science and Technology* 35: 3757-3763.

US EPA United States Environmental Protection Agency. Federal Registry. 49, 43305,12.

Van Iperen. J., and W. Helger. 1985. A method for the determination of organic carbon in calcareous marine sediments. *Marine Geology* 64: 179-187.

Webster, G. 2004. Dietary uptake and dietary biotransformation of phthalate esters in Staghorn Sculpin. (Under supervision of Gobas). Master in Resource Management Thesis. Simon Fraser University.

Yuan, S.Y., C. Lui, C.S. Liao, and B.V. Chang. 2002. Occurrence and microbial degradation of phthalate esters in Taiwan river sediments. *Chemosphere* 49: 1295-1299.

FIGURES AND TABLES

Table 1 **Molecular weight (g/mol), aqueous solubility (mg/L), log octanol-water partition coefficient (K_{ow}), and log acid dissociation constant (pK_a) of selected MPEs from Peterson and Parkerton (1999)**

Chemical		Molecular weight	Aqueous solubility	Log K_{ow}	Log acid dissociation
Monomethyl	MMP	180.2	3738	1.37	3.6
Monoethyl	MEP	194.2	1212	1.86	4.2
Mono-*n*-butyl	M*n*BP	222.2	126	2.84	4.2
Monobenzyl	MBzP	256.3	52	3.07	4.2
Mono-*n*-hexyl	M*n*HP	250.3	12.8	3.85	4.2
Monoheptyl	MC7P	NR	NR	NR	NR
Mono-2-ethylhexyl	MEHP	278.3	1.49	4.73	4.2
Mono-*n*-octyl	M*n*OP	NR	NR	NR	NR
Monononyl	MC9P	292.4	0.408	5.30	4.2
Monodecyl	MC10P	306.4	0.129	5.79	4.2

[1] Aqueous solubility, log K_{ow}, and log acid dissociation values are estimated using ASTER and EPIWIN models in Peterson and Parkerton 1999. Log K_{ow} is calculated for MPEs in the unionized form. NR = not reported.

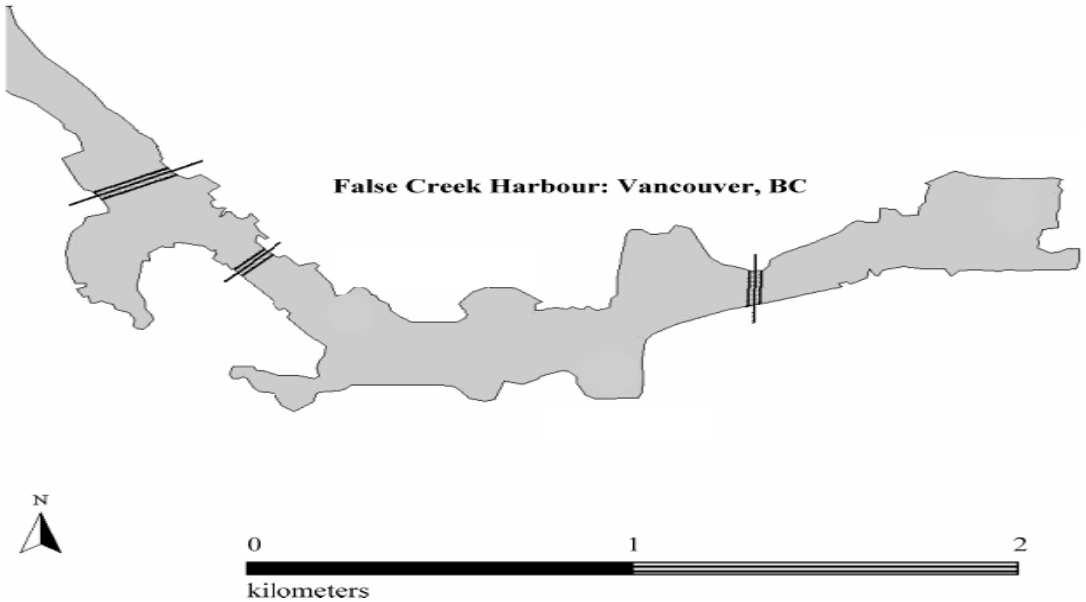


Figure 1 Map of field site: False Creek Harbour, Vancouver, BC[1]

[1] Modified from Mackintosh et al. 2004 by permission.

Table 2 **Description, scientific names, and the number of samples collected for each of the marine organisms sampled in False Creek Harbour, British Columbia**

Common Name	Description	Scientific Name	n
Green Alga	Primary producer	*Prasiola meridionalis*	8
Blue Mussel	Filter feeder	*Mytilus edulis*	10
Softshell Clam	Deposit feeder	*Mya arenaria*	10
(Dark-Mahogany Clam)		*(Nuttallia obscurata)*	
Dungeness Crab	Benthic invertebrate	*Cancer magister*	13
Shiner Perch	Forage fish	*Cymatogaster aggregata*	7
White-Spotted Greenling	Predatory fish	*Hexogrammos stelleri*	9
Spiny Dogfish	Slow-growing and long-lived omnivorous shark	*Squalus acanthias*	12

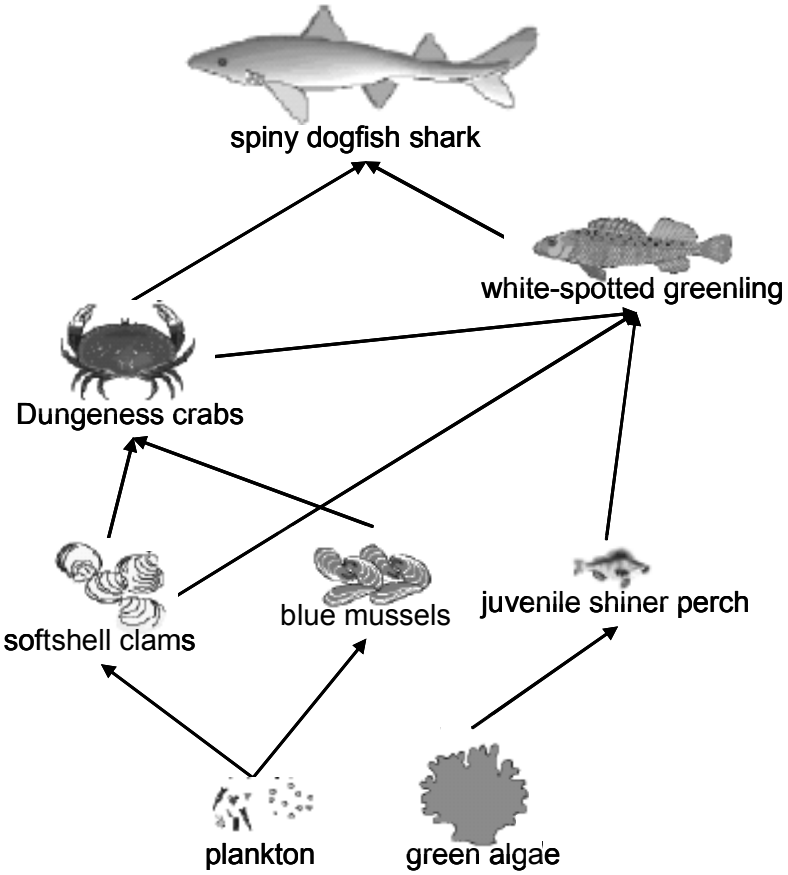


Figure 2 **Generalized trophic linkages among 8 marine organisms, 7 of which were collected from False Creek Harbour. Modified from Mackintosh et al. 2004 by permission**

Table 3 Minimum and maximum[1] MDLs for MPEs in water samples (ng/L), sediment samples (ng/g wet weight = *w*; dry weight = *d*), and organisms (ng/g wet weight)

	WT[2]	SD*w*	SD*d*	GA	BM	SC	DC*M*	DC*H*	jSP	WG	DF*M*	DF*L*
MMP	1.6	0.46	1.2	0.078-0.71	3.1	0.39-8.6	0.078-2.0	0.39-3.5	0.078-1.8	0.078-1.8	0.26-4.5	2.6-39
MEP	7.9	0.64	1.7	0.18-0.20	5.4	0.89-0.99	0.20-0.69	0.89-0.99	0.20-0.66	0.20-0.66	0.66-0.87	6.6-8.7
M*n*BP	67	5.5	14	2.6-4.2	7.9	13-21	3.1-4.2	13-21	2.7-3.8	3.1-3.8	7.9-12	89-130
MBzP	8.8	0.042	0.11	0.063-0.066	0.33	0.32-0.33	0.066	0.32-0.87	0.066-0.20	0.066	0.22-0.65	0.68-2.2
M*n*HP	0.22	0.032	0.084	0.011	0.65	0.056-0.88	0.011	0.056-0.19	0.011-0.020	0.011	0.037-0.068	0.37-1.3
MC7P	5.9	0.068	0.18	0.033	0.73	0.092-0.53	0.0072-0.018	0.092-0.17	0.018-0.040	0.018-0.033	0.11-0.13	1.1-41
MEHP	10	0.92	2.4	0.66-0.82	1.8	2.7-4.1	0.54-1.7	2.7-4.1	0.54-1.5	0.54-1.5	1.0-4.1	8.7-21
M*n*OP	9.2	0.093	0.24	0.027	0.49	0.14-0.81	0.027	0.14-0.83	0.027-0.26	0.027	0.091-0.87	0.91-9.4
MC9P	0.19	0.012	0.032	0.024	0.12	0.12	0.024	0.12	0.024-0.28	0.024	0.080-0.94	0.80-6.9
MC10P	0.18	0.015	0.039	0.073-0.13	0.67	0.67	0.13	0.67	0.13-0.21	0.13	0.45-0.69	4.5-39

[1] MDLs for each medium and each congener are presented as a range across all batches.
[2] Media: WT = water; SD = sediment; *w* = wet weight, *d* = dry weight; GA = green algae; BM = blue mussel; SC = softshell clam; DC = Dungeness crab; *M* = muscle, *H* = hepatopancreas; jSP = juvenile shiner perch; WG = white-spotted greenling; DF = spiny dogfish; *L* = liver.

Table 4 **Mean amount (ng) of MPEs in sodium sulphate procedural blanks for sediment and biota sample analyses, 2 standard deviations of the blanks, method detection limits defined as the mean blank amount + 2 standard deviations, and number of samples meeting method detection limits**

Type of machine analysis		Amount in blanks			Number of samples > MDL (%)	
		Mean (ng)	2 StD (ng)	MDL (ng)	Sediment	Biota
		(n=27)[1]			(n=10)[2]	(n=94)
LC/MS	MMP	4.5	8.4	12.9	10 (100%)	29 (31%)
	MEP	1.4	4.4	5.8	9 (90%)	39 (41%)
	M*n*BP	24	20	44	5 (50%)	73 (78%)
	MBzP	0.11	0.55	0.66	10 (100%)	16 (17%)
	M*n*HP	0.23	0.82	1.05	4 (40%)	20 (21%)
	MC7P	0.23	0.77	1.00	0 (0%)	50 (53%)
	MEHP	4.9	5.1	10.0	0 (0%)	39 (41%)
	M*n*OP	0.43	1.3	1.7	5 (50%)	4 (4%)
	MC9P	0.068	0.60	0.67	9 (90%)	3 (3%)
	MC10P	0.16	0.98	1.14	9 (90%)	5 (5%)

[1] n refers to the total number of blanks that were analysed for sediment and biota (all batches combined).
[2] n refers to the total number of sediment or biota (all grouped) samples that were analysed.

Table 5 **Mean biological parameters (length (cm), weight (g), tissue type, total organic carbon content (%), lipid content (%), $\delta^{15}N$ (‰), $\delta^{13}C$ (‰)) and the geometric mean phthalate monoester and diester concentrations (water: ng/L, sediment: ng/g wet weight, biota: ng/g wet weight) for water, sediment, and seven marine organisms collected from False Creek Harbour, Vancouver, British Columbia**

Biological Parameters											
Media[1]	WT	SD	GA	BM	SC	DC***M***	DC***H***	jSP	WG	DF***M***	DF***L***
Length	NA	NA	NA	NR	NR	15	NA	8	25	78	NA
Weight	NA	NA	NA	NR	NR	NR	NR	NR	NR	1900	NR
Tissue[2]	NA	NA	NA	W	W	M	H	W	M	M	L
%TOC	NR	2.9	26	41	38	423	48	38	48	55	72
StD		0.24	1.9	1.2	4.9	2.0	4.8	6.7	1.8	3.3	2.4
%lipid	NA	NA	0.20	0.47	0.27	0.13	7.5	1.6	1.5	9.0	68
StD		NA	0.10	0.31	0.15	0.12	4.6	1.2	0.43	3.4	8.1
$\delta^{15}N$‰	NA	4.3	5.5	7.9	8.3	11	NA	12	13	14	NA
StD		0.28	0.69	0.39	0.22	0.99		0.28	0.19	0.20	
$\delta^{13}C$‰	NA	-21	-16	-22	-21	-18	NA	-18	-17	-20	NA
StD		0.85	2.0	0.31	0.32	0.17		0.99	0.35	0.62	

[1] Media: WT = water; SD = sediment; GA = green algae; BM = blue mussel; SC = softshell clam; DC = Dungeness crab; *M* = muscle, *H* = hepatopancreas; jSP = juvenile shiner perch; WG = white-spotted greenling; DF = spiny dogfish; *L* = liver.

[2] Tissue types: W = whole body; M = muscle; H = hepatopancreas; L = liver. NA = not applicable; NR = not reported; ND = not detected; StD = standard deviation; TOC = total organic carbon.

	MPEs										
	WT	SD	GA	BM	SC	DC***M***	DC***H***	jSP	WG	DF***M***	DF***L***
MMP	9.4	1.8	0.90	6.9	ND	ND	ND	ND	ND	ND	8.5
StD	2.6	1.6	14	1.8							3.4
MEP	10	1.2	ND	7.5	ND	2.8	4.5	0.92	ND	ND	ND
StD	2.1	2.0		2.0		3.8	7.1	4.4			
M*n*BP	32	4.0	3.7	200	79	61	160	82	13	ND	ND
StD	1.6	1.8	1.9	2.1	3.0	2.1	3.1	2.9	2.6		
MBzP	ND	0.90	ND	0.64	ND	ND	ND	0.54	ND	ND	ND
StD		2.6		2.6				5.7			
M*n*HP	0.26	0.037	ND	0.73	ND	ND	ND	0.053	ND	ND	ND
StD	1.9	3.3		1.4				17			
MC7P	ND	ND	ND	1.9	0.43	0.20	0.46	0.79	ND	ND	ND
StD				1.3	2.9	6.3	4.5	2.3			
MEHP	60	ND	0.40	2.7	2.4	1.0	2.2	1.8	ND	ND	ND
StD	2.3		1.4	1.4	1.6	1.6	2.1	3.6			
M*n*OP	ND	0.072	ND	ND	ND	ND	ND	ND	ND	ND	ND
StD		1.6									
MC9P	0.65	0.13	ND	ND	ND	ND	ND	ND	ND	ND	ND
StD	13	5.8									
MC10P	3.8	0.050	ND	ND	ND	ND	ND	ND	ND	ND	ND
StD	1.5	2.5									

	DPEs										
	WT[1]	SD	GA	BM	SC	DC***M***	DC***H***	jSP	WG	DF***M***	DF***L***
DMP	3.7	3.9	0.33	0.14	0.19	0.051	0.47	0.26	0.071	ND	ND
StD	1.8	1.4	4.1	1.3	2.1	1.7	1.3	1.7	1.4		
DEP	120	8.2	ND	1.7	2.1	0.91	4.5	6.2	0.70	25	160
StD	3.1	2.7		1.9	2.4	2.6	1.3	1.9	1.6	3.1	2.2
D*i*BP	5.3	2.1	ND	0.78	0.46	0.24	2.0	1.8	0.35	6.9	ND
StD	1.8	1.4		2.1	2.0	1.6	1.4	2.5	1.6	2.1	
D*n*BP	110	15	20	3.4	2.3	1.7	15	11	2.1	29	ND
StD	1.8	1.2	1.3	1.5	2.4	1.5	1.4	1.9	1.4	2.6	
BBP	ND	15	9.9	1.4	3.1	1.1	10	7.9	1.3	16	180
StD		1.6	2.1	1.8	2.6	1.6	2.1	2.0	1.4	2.9	2.2
DEHP	250	310	260	25	42	7.9	45	43	7.3	58	1100
StD	1.3	1.3	2.9	2.8	2.5	1.7	1.4	1.6	1.6	4.4	3.5
D*n*OP	9.8	13	6.2	0.35	1.6	0.21	ND	1.2	ND	1.5	27
StD	2.4	1.6	4.4	1.9	3.8	8.1		4.8		6.9	5.8
D*n*NP	23	49	7.6	0.50	8.3	ND	ND	ND	ND	0.63	ND
StD	3.2	1.5	3.0	4.6	3.4					10	
C6	8.6	1.1	ND	0.21	ND	0.32	ND	5.3	1.1	12	ND
StD	1.7	1.5		2.7		3.0		1.3	1.9	1.9	
C7	ND	11	24	0.53	0.73	0.59	ND	44	3.0	55	550
StD		1.5	1.8	3.3	3.0	3.3		4.7	1.8	2.6	4.4
C8	ND	610	570	54	71	26	240	480	56	2000	37000
StD		1.3	1.6	2.2	2.7	1.7	2.9	4.1	2.7	1.9	3.0
C9	ND	400	330	12	48	ND	27	ND	4.9	270	1600
StD		1.2	2.3	1.5	2.1		3.5		1.9	2.4	4.4
C10	67	86	91	6.8	14	1.8	21	57	ND	150	ND
StD	1.8	1.3	2.6	2.2	2.1	3.9	3.6	4.0		3.2	

[1] DPE water data were not measured in current study. Source: Mackintosh et al. 2004.

Table 6 **Observed minimum and maximum MPE concentrations (ppb) in marine water from False Creek from the present study and in fresh water from the Tama River in Tokyo**

Study Site	MMP	MEP	M*n*BP	MEHP	M*n*OP
Tama River[1]	0.061-0.34	ND	0.012-0.48	0.021-1.3	ND
False Creek	0.0093-0.020	0.0056-0.035	0.011-0.051	0.035-0.56	ND

Table 7 **Comparison of water concentrations (ppm) from the present study with measured acute LC/EC_{50}s for several MPE congeners**

Study Site		M*n*BP	MEHP	MC9P
Lab test[2]	LC_{50} (96 hr)	133	62	40
	EC_{50} (72 hr)	134	NR	≥ 51
False Creek	Water Conc.	$1.1x10^{-05}$ – $5.1x10^{-05}$	$3.5x10^{-05}$ – $5.6x10^{-04}$	$9.3x10^{-08}$ – $2.9x10^{-05}$

Table 8 **Results of statistical tests between present study (P) and Mackintosh et al. 2004 (M) for DPEs in sediment samples**

t-test results	DMP	DEP	D*i*BP	D*n*BP	BBP	DEHP	D*n*OP	D*n*NP
p value	<0.001	0.49	0.05	<0.001	0.09	<0.001	0.08	<0.001
Interpretation	**M**>P	M=P	M=P	**M**>P	M=P	**M**>P	M=P	**P**>M
t-test results	**C6**	**C7**	**C8**	**C9**	**C10**			
p value	<0.001	<0.001	0.09	<0.001	<0.001			
Interpretation	**M**>P	**M**>P	M=P	**P**>M	**M**>P			

[1] Source: Suzuki et al. 2001. ND = non detect.
[2] LC_{50} data are for *Cyprinus carpio* (common carp) fish; EC_{50} data are for *Scenedesmus subspicatus* (alga species). Source: Scholz 2003. NR = not reported.

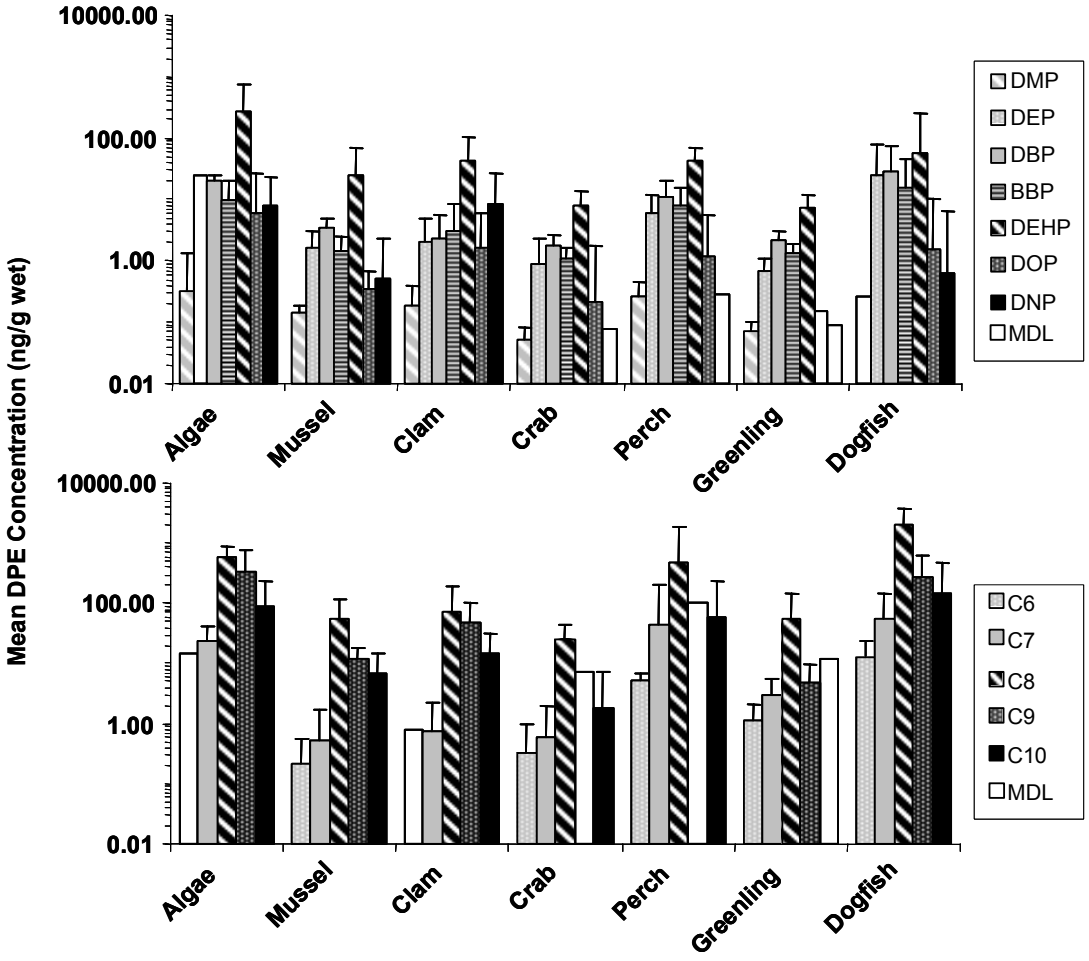


Figure 3 **Distribution of geometric mean (and standard deviation) DPE concentrations (ng/g wet weight) among organisms in False Creek (mean MDL levels are shown for non-detect DPEs as empty white cells)**

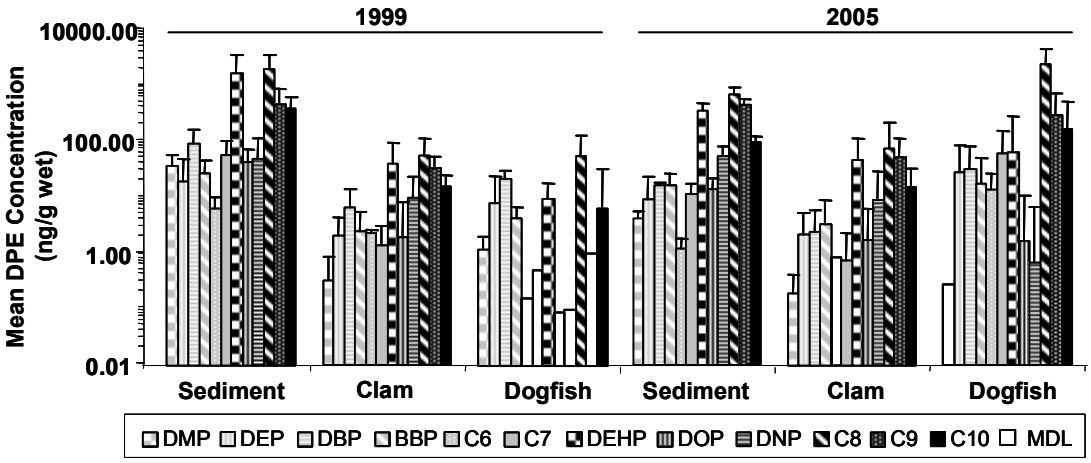


Figure 4 **Distribution of geometric mean (and standard deviation) DPE concentrations (ng/g wet weight) in sediment, clams, and dogfish from the present study (labelled 2005) and Mackintosh et al. 2004 (labelled 1999) (mean MDL levels are shown for non-detect DPEs as empty white cells)**

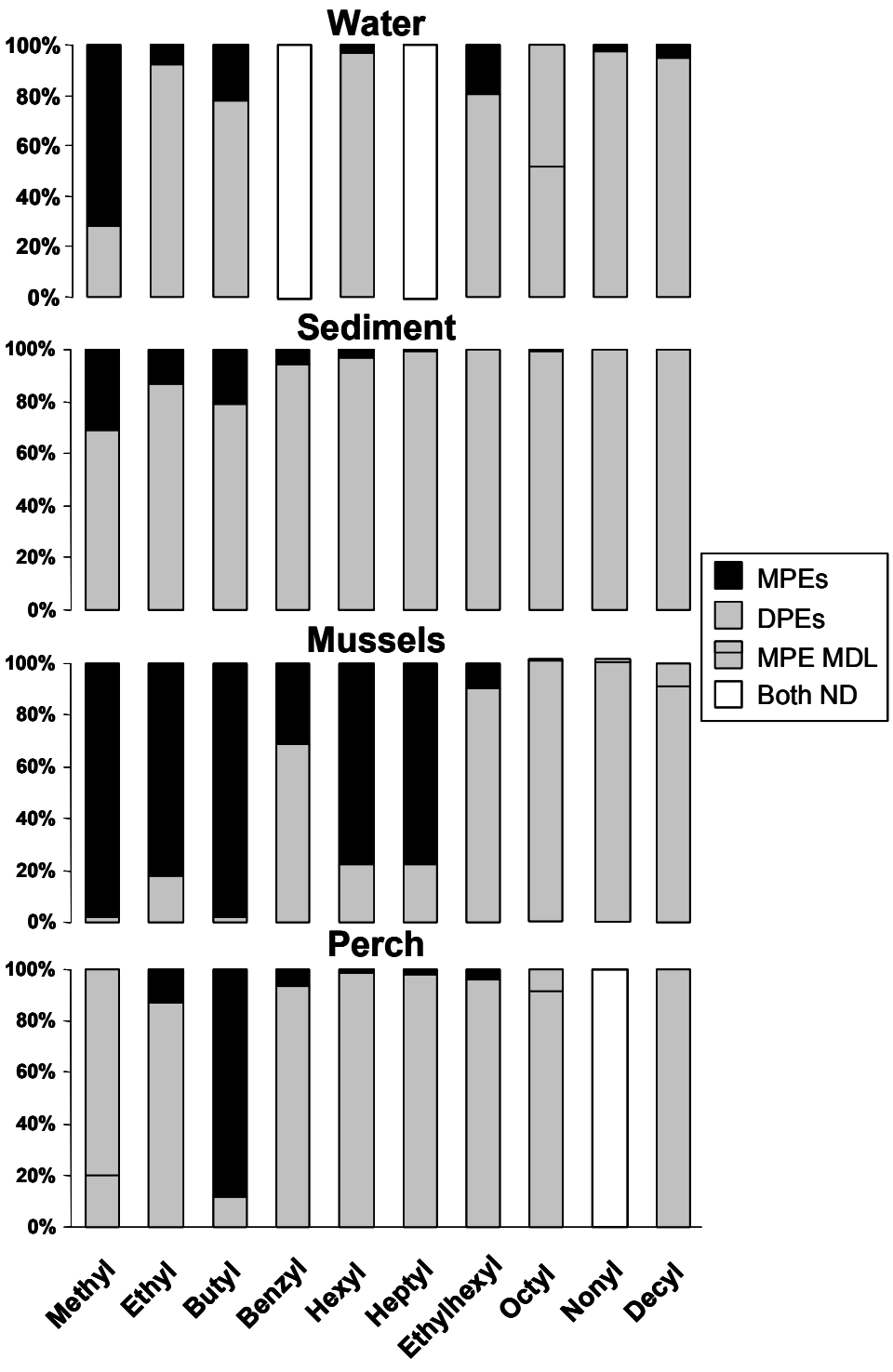


Figure 5 **Relative composition of MPEs (black) and DPEs (grey) for a range of PE congeners (increasing molecular weight from left to right) for water, sediment, mussel, and perch samples (MPE MDLs are shown for non-detect MPEs; empty cells indicate that both the MPE and DPE are non-detect)**

Table 9 **Results of statistical tests (two sample t-tests) testing whether MPE concentrations (M) are greater than or less than DPE concentrations (D) for individual congeners in water, sediment, mussels, and perch**

	Water		Sediment		Mussels		Perch	
	p value	result	*p* value	result	*p* value	result	*p* value	result
Methyl	0.012	**M**>D	<0.001	**D**>M	<0.001	**M**>D	ND	
Ethyl	<0.001	**D**>M	<0.001	**D**>M	<0.001	**M**>D	0.007	**D**>M
Butyl	<0.001	**D**>M	<0.001	**D**>M	<0.001	**M**>D	0.001	**M**>D
Benzyl	ND		<0.001	**D**>M	0.020	**D**>M	0.003	**D**>M
Hexyl	<0.001	**D**>M	<0.001	**D**>M	0.002	**M**>D	0.003	**D**>M
Heptyl	ND		ND		0.006	**M**>D	<0.001	**D**>M
Ethyl-Hexyl	<0.001	**D**>M	ND		<0.001	**D**>M	<0.001	**D**>M
Octyl	ND		<0.001	**D**>M	ND		ND	
Nonyl	0.001	**D**>M	<0.001	**D**>M	ND		ND	
Decyl	<0.001	**D**>M	<0.001	**D**>M	ND		ND	

Table 10 **Calculated MPE/DPE concentration ratios for a range of PE congeners in water, sediment, and organisms from False Creek Harbour; non-detect (ND) indicates cases where the MPE, DPE, or both are ND**

	WT[1]	SD	GA	BM	SC	DC***M***	DC***H***	jSP	WG	DF***M***	DF***L***
MMP/DMP	2.6	0.45	2.7	50	ND	ND	ND	ND	ND	ND	ND
MEP/DEP	0.089	0.15	ND	4.5	ND	3.0	1.0	0.15	ND	ND	ND
M*n*BP/D*n*BP	0.29	0.27	0.19	60	34	35	11	7.6	6.2	ND	ND
MBzP/BBP	ND	0.060	ND	0.45	ND	ND	ND	0.068	ND	ND	ND
M*n*HP/C6	0.030	0.033	ND	3.5	ND	ND	ND	0.010	ND	ND	ND
MC7P/C7	ND	ND	ND	3.5	0.59	0.33	ND	0.018	ND	ND	ND
MEHP/DEHP	0.24	ND	0.0015	0.11	0.057	0.13	0.049	0.042	ND	ND	ND
M*n*OP/D*n*OP	ND	0.0057	ND	ND	ND	ND	ND	ND	ND	ND	ND
M*n*OP/C8	ND	0.00012	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC9P/D*n*NP	0.028	0.0027	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC9P/C9	ND	0.00033	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC10P/C10	0.056	0.00058	ND	ND	ND	ND	ND	ND	ND	ND	ND

[1] Media: WT = water; SD = sediment; GA = green algae; BM = blue mussel; SC = softshell clam; DC = Dungeness crab; *M* = muscle, *H* = hepatopancreas; jSP = juvenile shiner perch; WG = white-spotted greenling; DF = spiny dogfish; *L* = liver.

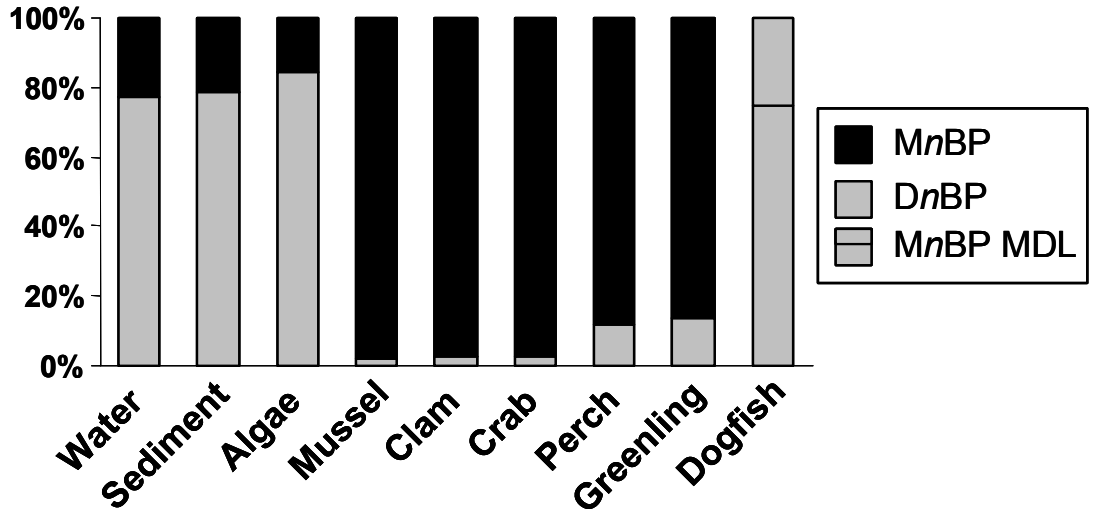


Figure 6 **Relative composition of MBP (black) and DBP (grey) for water, sediment, and collected organisms of the False Creek food web (increasing trophic level from left to right) (MPE MDLs are shown for non-detect MPEs)**

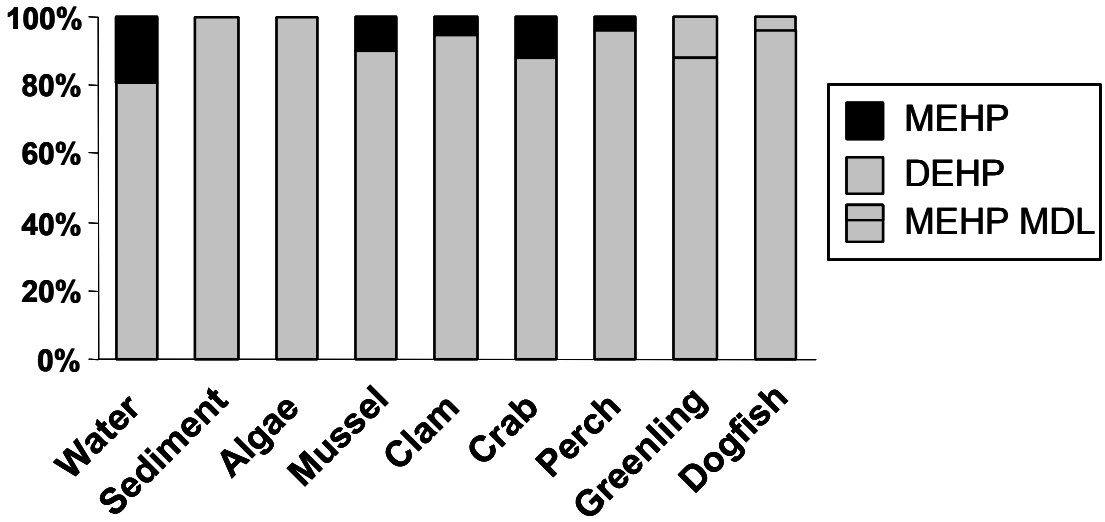


Figure 7 **Relative composition of MEHP (black) and DEHP (grey) for water, sediment, and collected organisms of the False Creek food web (increasing trophic level from left to right) (MPE MDLs are shown for non-detect MPEs)**

Table 11 Statistical results of regression analysis between log MPE concentration and $\delta^{15}N$ (i.e., slope, *p* value of slope, *Y*-intercept, and r^2) and food web magnification factors (FWMF) (lower – upper 95% confidence interval)[1]

	Log K_{ow}	*n*	Slope	*p* value	*Y* intercept	r^2	FWMF	Lower-Upper 95% CI
MEP	1.86	4	-0.06	0.69	6.0	0.10	0.65	0.01 – 36
		3[2]	-0.27	0.47	8.3	0.55	0.12	3E-12 – 5E9
M*n*BP	2.84	7	0.06	0.63	6.1	0.05	1.6	0.17 – 15
		5	-0.28	**0.03**	9.8	0.83	0.11	0.02 – 0.72
MC7P	NR	4	-0.19	0.08	6.9	0.85	0.22	0.03 – 1.6
MEHP	4.73	5	0.01	0.91	5.3	0.005	1.1	0.09 – 14
		4	-0.15	0.31	7.0	0.48	0.31	0.01 – 13

[1] *p* values in bold print represent statistically significant increases or decreases of the lipid equivalent concentration (i.e., <0.05).

[2] Second row of values for MEP, M*n*BP, and MEHP are results of regressions with algae and/or sediment samples removed.

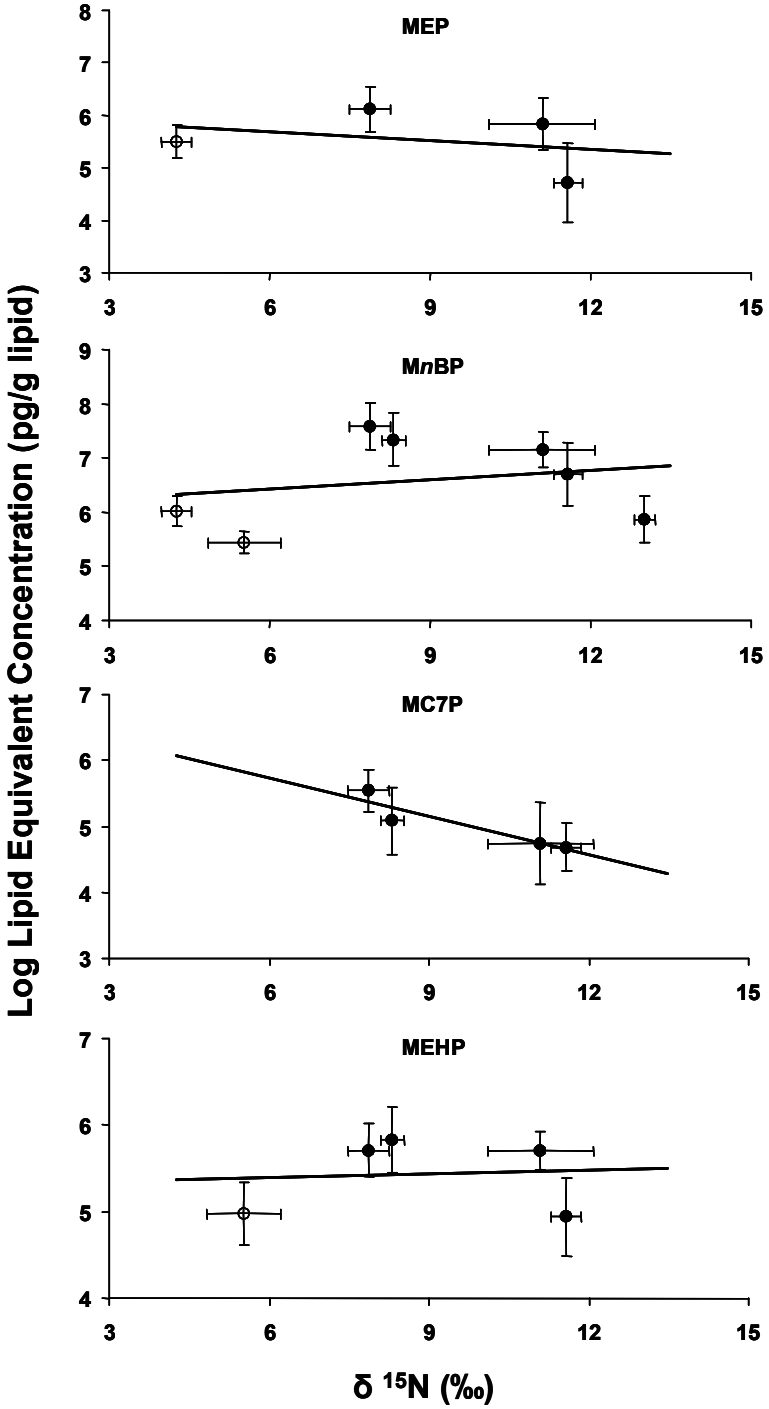


Figure 8 **Lipid equivalent log concentrations of MEP, MBP, MC7P, and MEHP in sediments and biota as a function of $\delta^{15}N$ (‰). Solid line indicates least sum of squares regression between lipid equivalent log concentration and $\delta^{15}N$. Open circles indicate sediment ($\delta^{15}N$ = 4.3) and algae ($\delta^{15}N$ = 5.5) samples**

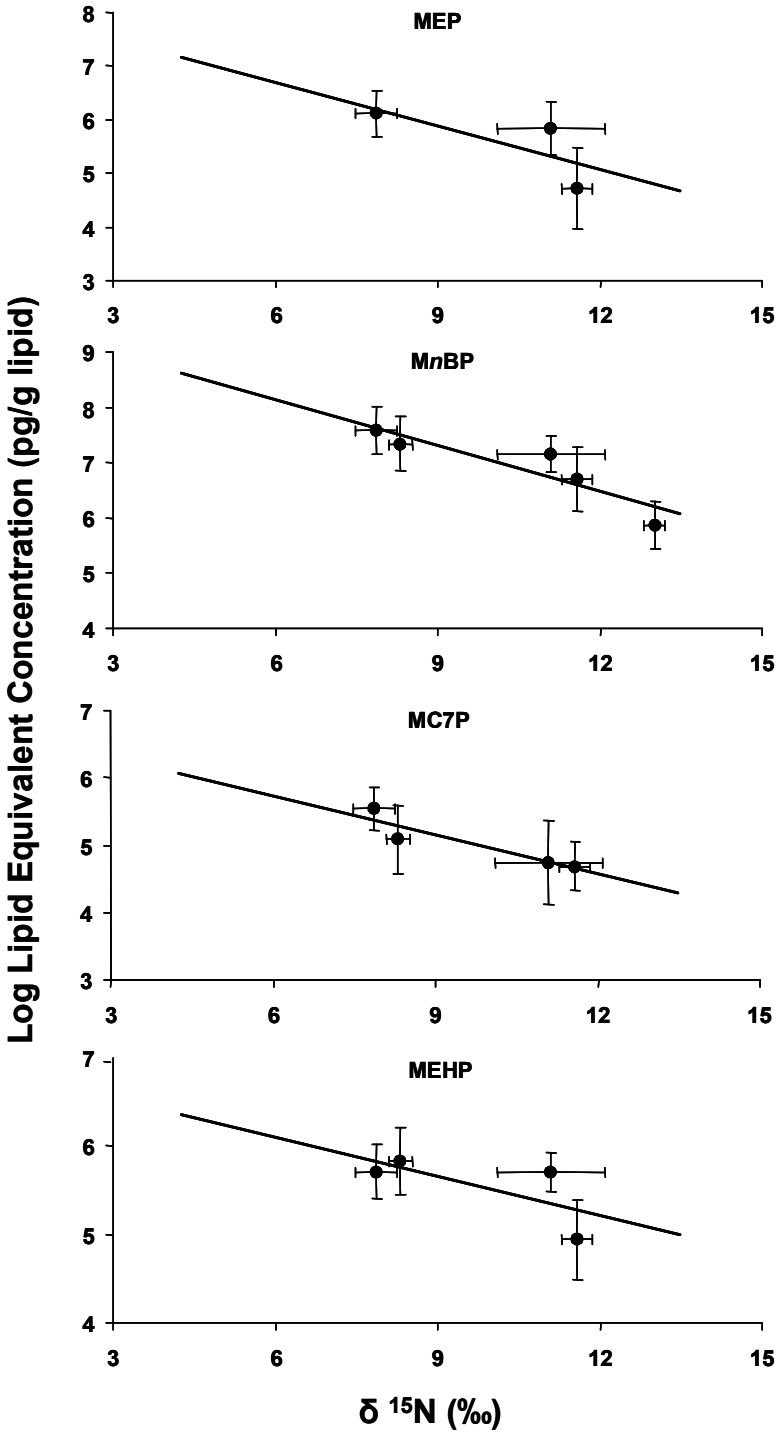


Figure 9 **Lipid equivalent log concentrations of MEP, MBP, MC7P, and MEHP in biota as a function of $\delta^{15}N$ (‰). Solid line indicates least sum of squares regression between lipid equivalent log concentration and $\delta^{15}N$. Sediment and algae samples have been removed**

APPENDICES

Appendix 1: Supporting Information

App.1 Table 1 Minimum and maximum[1] MDLs for DPEs in water samples (ng/L), sediment samples (ng/g wet weight = *w*; dry weight = *d*), and organisms (ng/g wet weight)

	WT[2]	SD***w***	SD***d***	GA	BM	SC	DC***M***	DC***H***	jSP	WG	DF***M***	DF***L***
DMP	3.8	0.20	0.52	0.26	0.026-0.072	0.072-0.13	0.026-0.13	0.26-1.0	0.26-0.64	0.026-0.13	0.26	4.4
DEP	46	2.8	7.3	24-27	0.51-1.6	0.51-1.4	0.39-1.1	3.9-8.3	3.9-27	0.39-1.1	24-27	190-220
D*i*BP	7.2	1.7	4.3	7.9-12	0.56-0.67	0.39-0.77	0.36-0.67	3.6-6.5	3.6-12	0.36-0.67	5.7-12	180-190
D*n*BP	200	10	27	40-44	2.4-5.2	2.4-5.4	2.6-5.2	24-33	26-44	2.6-5.2	31-44	800-1400
BBP	26	2.2	5.7	12-18	0.78-2.1	0.78-1.7	0.80-2.1	7.8-19	8.0-12	1.2-2.1	12-18	190-210
DEHP	470	5.7	15	40-110	8.7-9.8	4.5-9.0	3.2-14	32-138	32-140	3.9-14	40-110	420-440
D*n*OP	11	0.33	0.85	0.57	0.055-0.36	0.071-0.36	0.081-0.27	0.81-3.6	0.57-1.0	0.081-0.27	0.57	9.5
D*n*NP	20	0.16	0.42	0.20	0.020-0.17	0.019-0.13	0.020-0.17	0.20-0.47	0.20-0.47	0.020-0.17	0.20	ND
C6	15	2.6	6.7	10-20	0.83-1.3	0.48-0.98	0.48-1.3	4.8-11	4.8-11	0.48-1.6	9.5-20	260
C7	35	6.1	16	35-38	1.8-2.7	2.0-6.2	1.9-6.2	19-62	22-62	2.4-6.2	17-38	450
C8	690	430	1100	350-700	9.2-24	16-95	15-95	150-950	530-950	16-110	350-1100	5000
C9	370	66	170	75-80	2.6-5.8	4.9-17	3.7-17	51-170	60-170	3.7-17	80-99	1300
C10	75	15	40	53-99	0.70-6.2	5.0-37	2.0-37	50-370	51-370	2.0-37	47-99	1400

[1] MDLs for each medium and each congener are presented as a range across all batches.
[2] Media: WT = water; SD = sediment; *w* = wet weight, *d* = dry weight; GA = green algae; BM = blue mussel; SC = softshell clam; DC = Dungeness crab; *M* = muscle, *H* = hepatopancreas; jSP = juvenile shiner perch; WG = white-spotted greenling; DF = spiny dogfish; *L* = liver.

App.1 Table 2 Mean amount (ng) of DPEs in sodium sulphate procedural blanks for sediment and biota sample analyses, 2 standard deviations of the blanks, method detection limits defined as the mean blank amount + 2 standard deviations, and number of samples meeting method detection limits

Type of machine analysis		Amount in blanks			Number of samples > MDL (%)	
		Mean (ng)	2 StD (ng)	MDL (ng)	Sediment	Biota
GC/MS		(n=36)[1]			(n=10)[2]	(n=94)
	DMP	0.19	0.24	0.43	10 (100%)	56 (60%)
	DEP	3.7	4.9	8.7	10 (100%)	70 (74%)
	D*i*BP	2.2	1.7	3.9	10 (100%)	67 (71%)
	D*n*BP	13	9.8	23	10 (100%)	66 (70%)
	BBP	4.3	3.9	8.2	10 (100%)	79 (84%)
	DEHP	17	22	39	10 (100%)	73 (78%)
	D*n*OP	0.37	0.48	0.85	10 (100%)	47 (50%)
	D*n*NP	0.15	0.27	0.42	10 (100%)	38 (40%)
LC/MS		(n=50)			(n=10)	(n=88)
	C6	4.8	2.6	7.4	10 (100%)	27 (31%)
	C7	10	7.3	17	10 (100%)	55 (63%)
	C8	150	330	480	10 (100%)	68 (77%)
	C9	28	42	70	10 (100%)	59 (67%)
	C10	23	36	59	10 (100%)	52 (59%)

App.1 Table 3 Number of biota samples meeting method detection limits

	Number of samples > MDL								
	GA	BM	SC	DC*M*	DC*H*	jSP	WG	DF*M*	DF*L*
	n=8[3]	n=10	n=10	n=13	n=13	n=7	n=9	n=12	n=12
MMP	5	10	0	1	0	0	0	2	11
MEP	0	9	1	12	9	5	0	1	2
M*n*BP	8	10	10	13	13	7	9	2	1
MBzP	0	7	0	1	2	5	1	0	0
M*n*HP	0	9	1	2	3	3	0	1	1
MC7P	2	10	10	11	8	7	1	0	1
MEHP	3	10	10	5	8	3	0	0	0
M*n*OP	0	0	0	0	4	0	0	0	0
MC9P	0	0	0	0	3	0	0	0	0
MC10P	0	0	0	0	3	1	0	0	1

[1] n refers to the total number of blanks that were analysed for sediment and biota (all batches combined).
[2] n refers to the total number of sediment or biota (all grouped) samples that were analysed.
[3] n refers to the total number of biota samples that were analysed.

App.1 Table 4 Number of biota samples meeting method detection limits

	Number of samples > MDL								
	GA	**BM**	**SC**	**DC*M***	**DC*H***	**jSP**	**WG**	**DF*M***	**DF*L***
	n=8[1]	n=10	n=10	n=13	n=13	n=7	n=9	n=12	n=12
DMP	5	10	9	8	12	5	4	0	3
DEP	1	8	10	13	11	5	9	6	7
D*i*BP	2	10	6	10	12	6	9	11	1
D*n*BP	3	7	10	9	12	4	9	10	2
BBP	8	8	10	8	11	5	8	12	9
DEHP	7	9	9	11	11	4	5	8	9
D*n*OP	7	10	9	4	0	3	0	6	8
D*n*NP	8	9	10	4	0	2	0	5	0
	n=7	n=9	n=10	n=12	n=13	n=7	n=8	n=12	n=10
C6	0	6	2	4	3	3	4	4	1
C7	3	7	8	6	4	3	4	11	9
C8	5	9	9	8	6	2	7	12	10
C9	7	9	10	0	9	2	3	12	7
C10	7	9	10	6	8	3	0	8	1

Stable Isotope Ratio Calculations

Stable isotope ratios (parts per thousand, ‰) were calculated according to the first (nitrogen) and second (carbon) equations:

$$\delta^{15}N = [(^{15}N/^{14}N \text{ sample} - {}^{15}N/^{14}N \text{ standard}) / (^{15}N/^{14}N \text{ standard})] \times 1000$$

$$\delta^{13}C = [(^{13}C/^{12}C \text{ sample} - {}^{13}C/^{12}C \text{ standard}) / (^{13}C/^{12}C \text{ standard})] \times 1000$$

where the $\delta^{15}N$ standard is nitrogen in the air and the $\delta^{13}C$ standard is in Pee Dee Belomite limestone. Machine precision was assessed by analyzing 7 replicates of a prepared standard ($\delta^{15}N$ = 14.47‰ and $\delta^{13}C$ = -17.19‰) for which reproducibility was SD = 0.06‰ and 0.08‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively.

[1] n refers to the total number of biota samples that were analysed.

Normality tests on MPE and DPE Concentration Data

The results of the Kolmogorov-Smirnov normality tests on the two data sets (i.e., original and log-transformed) are presented in App.1 Table 5 for MPEs and App.1 Table 6 for DPEs. Results from the two tests were compared for each MPE and DPE to determine which distribution was more representative of a normal distribution. In order to simplify data analysis and other statistics, the decision was based on the outcome of all tests and not on a chemical and media specific basis. In general, the concentration data were log-normally distributed; thus, a log transformation was applied to all the data.

App.1 Table 5 Results of Kolmogorov-Smirnov normality test on original (C) and log-transformed (logC) MPE concentrations; (+) indicates a normal distribution, (-) indicates a non-normal distribution, and (ND) indicates a non-detect

		WT[1]	SD	GA	BM	SC	DC*M*	DC*H*	jSP	WG	DF*M*	DF*L*
MMP	C	+	+	+	+	ND	ND	ND	ND	ND	ND	-
	logC	+	+	+	+							+
MEP	C	+	+	ND	+	ND	+	+	+	ND	ND	ND
	logC	+	+		+		+	+	+			
M*n*BP	C	+	+	+	+	+	+	+	+	+	ND	ND
	logC	+	+	+	+	+	+	+	+	+		
MBzP	C	ND	+	ND	+	ND	ND	ND	+	ND	ND	ND
	logC		+		+				+			
M*n*HP	C	+	+	ND	+	ND	ND	ND	+	ND	ND	ND
	logC	+	+		+				+			
MC7P	C	ND	ND	ND	+	+	+	+	+	ND	ND	ND
	logC				+	+	+	+	+			
MEHP	C	-	ND	+	+	+	-	+	+	ND	ND	ND
	logC	+		+	+	+	+	+	+			

[1] Media: WT = water; SD = sediment; GA = green algae; BM = blue mussel; SC = softshell clam; DC = Dungeness crab; *M* = muscle, *H* = hepatopancreas; jSP = juvenile shiner perch; WG = white-spotted greenling; DF = spiny dogfish; *L* = liver.

		WT[1]	SD	GA	BM	SC	DC*M*	DC*H*	jSP	WG	DF*M*	DF*L*
M*n*OP	C	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND
	logC		+									
MC9P	C	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND
	logC	+	+									
MC10P	C	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND
	logC	+	+									

App.1 Table 6 Results of Kolmogorov-Smirnov normality test on original (C) and log-transformed (logC) DPE concentrations; (+) indicates a normal distribution, (-) indicates a non-normal distribution, and (ND) indicates a non-detect

		SD	GA	BM	SC	DC	DC*H*	jSP	WG	DF*M*	DF*L*
DMP	C	+	+	+	+	+	+	+	+	ND	ND
	logC	+	+	+	+	+	+	+	+		
DEP	C	+	ND	+	+	+	+	+	+	+	+
	logC	+		+	+	+	+	+	+	+	+
D*i*BP	C	+	ND	+	+	+	+	+	+	+	ND
	logC	+		+	+	+	+	+	+	+	
D*n*BP	C	+	+	+	+	+	+	+	+	+	ND
	logC	+	+	+	+	+	+	+	+	+	
BBP	C	+	+	+	+	+	+	+	+	+	+
	logC	+	+	+	+	+	+	+	+	+	+
DEHP	C	+	+	+	+	+	+	+	+	+	+
	logC	+	+	+	+	+	+	+	+	+	+
D*n*OP	C	+	+	+	+	-	ND	+	ND	+	+
	logC	+	+	+	+	+		+		+	+
D*n*NP	C	+	+	+	+	ND	ND	ND	ND	+	ND
	logC	+	+	+	+					+	

C6	C	+	ND	+	ND	+	ND	+	+	+	ND
	logC	+		+		+		+	+	+	
C7	C	+	+	+	-	+	ND	+	+	+	+
	logC	+	+	+	+	+		+	+	+	+
C8	C	+	+	+	+	+	-	+	+	+	+
	logC	+	+	+	+	+	+	+	+	+	+
C9	C	+	+	+	+	ND	+	ND	+	+	+
	logC	+	+	+	+		+		+	+	+
C10	C	+	+	+	+	+	-	+	ND	+	ND
	logC	+	+	+	+	+	+	+		+	

App.1 Table 7 Statistical results of regression analysis between log concentration and $\delta^{15}N$ (i.e., slope, *p* value of slope, *Y* intercept, and r^2) and food web magnification factors (FWMF) (lower – upper 95% confidence interval) for DPEs[1]

	Log K_{ow}	*n*	Slope	*p* value	*Y* intercept	r^2	FWMF	Lower-Upper 95% CI
DMP	1.80	7	-0.19	**0.01**	6.3	0.75	0.22	0.08 – 0.60
DEP	2.77	7	-0.13	**0.03**	6.8	0.66	0.37	0.16 – 0.84
D*i*BP	4.58	7	-0.12	**0.01**	6.2	0.76	0.40	0.23 – 0.72
D*n*BP	4.58	8	-0.14	**0.002**	7.2	0.81	0.33	0.20 – 0.57
BBP	5.03	8	-0.12	**0.01**	6.8	0.70	0.38	0.20 – 0.72
C6	6.39	6	-0.01	0.81	5.3	0.02	0.90	0.29 – 2.8
C7	7.30	8	-0.08	0.28	6.5	0.19	0.54	0.15 – 1.9
DEHP	8.20	8	-0.22	**<0.001**	8.7	0.95	0.18	0.12 – 0.27
D*n*OP	8.20	7	-0.21	**0.01**	7.1	0.81	0.20	0.08 – 0.50
C8	8.20	8	-0.11	**0.04**	8.4	0.51	0.43	0.18 – 0.98
D*n*NP	9.11	5	-0.30	0.07	8.0	0.73	0.09	0.01 – 1.4
C9	9.11	6	-0.20	**0.03**	8.5	0.71	0.21	0.05 – 0.83
C10	10.0	7	-0.11	0.05	7.5	0.56	0.42	0.17 – 1.0

[1] *p* values in bold print represent statistically significant increases or decreases of the lipid equivalent concentration (i.e., <0.05).

App.1 Table 8 Geometric mean phthalate monoester and diester concentrations (water: ng/L, sediment: ng/g dry weight, biota: ng/g lipid equivalent) for water, sediment, and seven marine organisms collected from False Creek Harbour, Vancouver, British Columbia

	MPEs										
Media[1]	**WT**	**SD**	**GA**	**BM**	**SC**	**DC*M***	**DC*H***	**jSP**	**WG**	**DF*M***	**DF*L***
MMP	9.4	4.5	130	1300	ND	ND	ND	ND	ND	ND	13
StD	2.6	1.7	4.9	2.4							3.5
MEP	10	3.2	ND	1300	ND	670	70	53	ND	ND	ND
StD	2.1	2.0		2.7		3.0	8.6	5.7			
M*n*BP	32	11	270	39000	22000	14000	2300	5000	750	ND	ND
StD	1.6	1.9	1.6	2.7	3.2	2.1	4.7	3.9	2.7		
MBzP	ND	2.3	ND	150	ND	ND	ND	37	ND	ND	ND
StD		2.6		3.2				8.0			
M*n*HP	0.26	0.10	ND	130	ND	ND	ND	2.9	ND	ND	ND
StD	1.9	3.3		1.9				0.15			
MC7P	ND	ND	ND	350	120	56	6.9	49	ND	ND	ND
StD				2.1	3.2	4.3	5.8	2.3			
MEHP	60	ND	96	510	690	510	36	89	ND	ND	ND
StD	2.3		2.3	2.0	2.4	1.7	2.6	2.8			
M*n*OP	ND	0.19	ND	ND	ND	ND	ND	ND	ND	ND	ND
StD		1.7									
MC9P	0.65	0.33	ND	ND	ND	ND	ND	ND	ND	ND	ND
StD	13	6.1									
MC10P	3.8	0.13	ND	ND	ND	ND	ND	ND	ND	ND	ND
StD	1.5	2.5									

[1] Media: WT = water; SD = sediment; GA = green algae; BM = blue mussel; SC = softshell clam; DC = Dungeness crab; *M* = muscle, *H* = hepatopancreas; jSP = juvenile shiner perch; WG = white-spotted greenling; DF = spiny dogfish; *L* = liver. ND = not detected; StD = standard deviation.

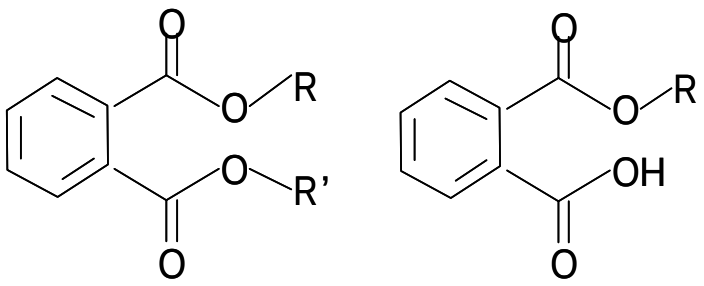
	DPEs										
	WT[1]	**SD**	**GA**	**BM**	**SC**	**DC*M***	**DC*H***	**jSP**	**WG**	**DF*M***	**DF*L***
DMP	3.7	10	46	26	55	18	8.8	17	4.7	ND	ND
StD	1.8	1.4	3.3	2.2	2.6	1.9	2.6	2.0	1.4		
DEP	120	21	ND	350	590	200	87	400	39	260	230
StD	3.1	2.6		2.3	3.0	2.3	2.5	2.1	1.7	2.5	2.3
D*i*BP	5.3	5.6	ND	150	160	71	33	110	20	76	ND
StD	1.8	1.6		2.5	2.5	2.4	2.4	2.3	1.4	2.3	
D*n*BP	110	39	4900	710	650	560	250	730	120	300	ND
StD	1.8	1.1	3.1	2.1	3.5	2.4	2.6	2.0	1.5	2.7	
BBP	ND	39	720	270	880	340	190	450	77	170	270
StD		1.7	2.4	2.4	2.8	2.1	3.1	2.2	1.7	3.0	2.3
DEHP	250	810	25000	4900	12000	2100	670	2200	440	640	1600
StD	1.3	1.3	2.0	3.1	3.0	1.7	2.0	1.9	1.4	4.6	3.6
D*n*OP	9.8	33	590	66	470	100	ND	67	ND	16	42
StD	2.4	1.7	2.3	2.7	5.2	6.4		4.6		6.7	6.0
D*n*NP	23	130	560	87	2300	ND	ND	ND	ND	7.3	ND
StD	3.2	1.6	2.8	5.1	4.9					11	
C6	8.6	2.9	ND	38	ND	160	ND	390	71	130	ND
StD	1.7	1.5		3.3		4.8		1.5	1.7	2.0	
C7	ND	27	4800	120	230	250	ND	2400	190	600	830
StD		1.6	2.4	3.2	3.4	5.2		4.7	1.7	2.8	4.4
C8	ND	1600	66000	10000	21000	8800	3200	30000	3300	22000	55000
StD		1.4	2.0	3.0	3.4	2.3	2.6	3.8	2.7	2.0	3.2
C9	ND	1000	24000	2300	13000	ND	320	ND	300	2900	2400
StD		1.4	1.9	2.4	2.6		3.4		2.2	2.6	4.5
C10	67	220	6500	1300	4100	720	270	3000	ND	1600	ND
StD	1.8	1.4	2.1	2.3	2.0	5.9	3.3	4.8		3.4	

[1] DPE water data were not measured in current study. Source: Mackintosh et al. 2004.

Appendix 2: Literature Review

Phthalate Ester Uses

PEs have various toxicological and chemical characteristics and a spectrum of industrial applications (Stanley et al. 2003) including food packaging and storage of human blood (Anderson et al. 1999). PEs are used as softeners of plastic, solvents in perfumes, and additives to hairsprays, lubricants, and insect repellents (Stanley et al. 2003). In the residential construction or automotive industries, several PEs are used in floorings, paints, carpet backings, adhesives, wood finishers, wallpaper, and in polyvinyl chloride (PVC) products (Stanley et al. 2003). They are commonly added to products to hold color or fragrance, provide a film or gloss, make certain plastics more flexible, and provide timed releases, such as with pharmaceuticals (Stanley et al. 2003). Di-2-ethylhexyl phthalate (DEHP) is the most widely used PE; it accounts for about 50% of all PEs produced (Li et al. 1998), and can constitute up to 40% of finished PVC products (Anderson et al. 1999). Generally, PEs (App.2 Figure 1) are successful because they offer desirable traits such as compatibility, permanence, efficiency, and processability at a reasonable cost (Stanley et al. 2003).



App.2 Figure 1 Generalized structure of a di- (left) and a mono- (right) alkyl phthalate ester

Phthalate Ester Sources and Exposure

PEs can be released into the environment at the manufacturing stage although release at this stage accounts for very little of the environmental concentrations (Stanley et al. 2003). PEs that are released in the production and processing stage are disposed of in wastewater which is then treated at treatment facilities where it is either biodegraded or adsorbed to sludge (Stanley et al. 2003). PEs used as plasticizers are just additives and thus they are not bound covalently to their products (Asai et al. 2000). Despite their low vapour pressures, PEs can also diffuse from solid surfaces into air under conditions of high surface exposure and warm temperatures (Stanley et al. 2003). As a result PEs have become well-known contaminants in environmental water, soil, and the atmosphere (Suzuki et al. 2001).

Given this ability to freely migrate from their products of origin, PEs have been detected in many media. They have been detected in sediments around the world (Parkerton and Konkel 2000) and they are found in water, aquatic invertebrates, fish, birds (Mackintosh et al. 2004), and marine mammals (Morin

2003). PEs are also present in high concentrations in residential indoor air and dust; concentrations can be especially high indoors where personal air concentrations are much higher than ambient concentrations because of PE-containing product use (Rudel et al. 2003, Clark et al. 2003). Finally, due to their widespread use, they have also been detected in all kinds of food, human breast milk (Clark et al. 2003), and urine (Kohn et al. 2000).

Phthalate Ester Degradation

The primary degradation products of PEs are monoalkyl phthalate esters (MPEs) (App.2 Figure 1). MPEs are of no commercial value, they exist only as a transient step during synthesis (Scholz 2003). MPEs have sometimes been suggested responsible for the toxicological and ecotoxicological properties of their corresponding DPEs (Barr et al. 2003, Gray and Gangolli 1986, Hoppin et al. 2002, Li and Heindel 1998, Jonsson and Baun 2003, Niino et al. 2003, Scholz 2003, Yagi et al. 1980).

Metabolic studies indicate that orally administered PEs are rapidly hydrolyzed to their corresponding monoesters by non-specific esterases (Kluwe 1982, Li et al. 1998) and lipases in the pancreas, blood, and wall of the small intestine (Niino et al. 2003). However, metabolism of PEs can also occur in the kidney and liver, where shorter chain DPEs (e.g., DMP, DBP) are more readily metabolized than longer chain DPEs, such as DEHP (Kluwe 1982), and in human serum and milk, which both contain DPE-metabolizing enzymes (Mortensen et al. 2005). Niino et al. reported that DBP and DEHP released from

PVC toys are hydrolyzed to their respective monoesters in human saliva (2001). In fish, the liver is believed to be the major site of biotransformation, due to high specific enzyme content (Lech and Bend 1980), but Barron et al. also found correspondingly high activity in the gills of trout (1989).

PEs are either hydrolyzed to their respective monoesters, or for some PEs, the monoester metabolite is further biotransformed to oxidative metabolites before being excreted in the urine and feces (Kluwe 1982, Hoppin et al. 2002). All of these metabolites can be glucoronidated which increases their water solubility and facilitates excretion (Kluwe 1982, Albro 1986, Silva et al. 2003). Typically, the more water soluble DPEs (e.g., DEP, DBP) are metabolized to and excreted as free monoesters (Albro and Moore 1974, Silva et al. 2003). Whereas, the more lipophilic DPEs (e.g., DEHP, DOP, DNP) are metabolized from their respective monoesters to more hydrophilic metabolites in a multistep oxidative pathway (Albro et al. 1973). PE absorption occurs almost entirely as corresponding monoesters (Lake et al. 1977).

Phthalate Monoester Exposure

As a result of the ubiquity of PEs and their tendency to be rapidly biotransformed into their corresponding monoesters, MPEs have also been detected in many environmental media. However, there are very few published reports that have analytical methods and monitoring data for MPEs in environmental samples (Suzuki et al. 2001). MPEs have been observed in the environment in landfill leachate water (Jonsson et al. 2003) and in river water

(Suzuki et al. 2001). For example, Suzuki et al. detected MMP, MBP, and MEHP in the Tama River in Tokyo at concentrations of 0.03-0.034, 0.01-0.48, and 0.01-1.3 μg/L, respectively (2001). They suggested that contamination by MPEs in the Tama River was attributable to the direct inflow of sewage and contaminated surface water containing the monoesters in urban areas or to biodegradation of DPEs in the river by some microorganisms (2001). MEHP has also been detected in intravenous solutions that were stored in medical grade PVC bags (Arbin and Östelius 1980). MBP and MEHP have been measured in serum and plasma products packed into plastic containers and in water from medical grade PVC tubing (Shintani 1985).

United States-based population studies indicate that human exposure to MPEs is common (Blount et al. 2000) and detectable levels have been measured in both breast milk and urine (Mortensen et al. 2005, Blount et al. 2000, Silva et al. 2004). In addition, even greater levels are detected in children and toddlers, possibly due to increased exposure during breast feeding (Mortensen et al. 2005). However, additional research is required to determine whether exposure to PEs at the levels found in people is a cause for health concern (Barr et al. 2003).

Phthalate Ester Toxicity and Effects

Concern about the toxic potential of PEs is increasing; PEs continue to be produced in large, mostly unregulated quantities and released into the environment where they can migrate into biological systems (Ema et al. 1995).

As a result, work is being done to determine the types of effects, modes of toxic action, and types of behaviour PEs are causing in environmental biota. Animal data suggests a broad spectrum of health outcomes associated with PE exposure: developmental toxicity, endocrine disruption, and carcinogenicity (Hoppin et al. 2002). Furthermore, these effects have been associated with exposure to some PEs at environmental levels (Duty et al. 2005).

Contradictory to the common view that metabolism of xenobiotics results in detoxification of the parent compound and facilitates excretion from the body (Silva et al. 2003), sources state that metabolism of PEs does not always detoxify them (Albro et al. 1989, Heindel and Powell 1992, Ema et al. 1995). Recently, some evidence has emerged which points to PE metabolites as the active compounds responsible for the observed toxicological effects of PEs (Barr et al. 2003, Gray and Gangolli 1986, Hoppin et al. 2002, Li and Heindel 1998, Jonsson and Baun 2003, Niino et al. 2003, Scholz 2003, Yagi et al. 1980).

For example, one study observed the same toxic effects in mice consuming DEHP and those consuming smaller doses of MEHP, suggesting the higher toxicity of MEHP (Yagi et al. 1980). Also, several studies have found that MEHP can activate the expression of several target genes associated with hepatocarcinogenic effects in rodents (Ward et al. 1998, Hurst and Waxman 2003, 2004). The responsiveness of the target receptor to activation by some MPEs suggests that MPEs may, in part, exhibit their endocrine disruptor activities by altering steroid hormone metabolism with potential adverse health effects in exposed individuals (Hurst and Waxman 2004).

Although there seems to be some evidence pointing to PEs metabolites as the cause of toxic effects associated with PEs, an opposing trend has also been reported. Scholz found the acute aquatic toxicity of a wide range monoesters to be considerably less pronounced than the parent compound and he states that degradation of diesters to monoesters and phthalic acid would reduce the risk of acute toxic effects in the aquatic environment (2003). Thus, Scholz suggests that based on these results we should not assume an immediate and unacceptable risk posed through PEs and/or their metabolites released into the environment (2003). However, he also says that we should be aware of possible hidden effects that could arise through longer term exposure to PEs, which could be partly ruled out with the results from long term tests (Scholz 2003). Long term studies allow internal concentrations to reach their maximum levels based on external exposure concentrations to the diesters, their uptake, and their subsequent turnover metabolic rates (Scholz 2003).

Thus, despite the rapid metabolism and elimination of most PEs (Kluwe 1982), theoretically a constant steady state may be reached because of chronic and repetitive, low level exposures from dietary ingestion and from many commonly used products (Duty et al. 2003). Hoppin et al. found no significant differences in urinary MPE levels in adult women from one day to the next suggesting that PE exposure was relatively stable over time (2002). Although MPEs are degradable (Scholz 2003), a continual influx of diesters would lead to a continuous production of MPEs, which could create relevant concentrations in the environment.

Phthalate Ester Bioaccumulation

PEs are hydrophobic chemicals and because of this it is believed that they have a high potential to bioaccumulate in biological organisms (Gobas et al. 2003). Staples et al. summarized several laboratory studies which measured the PE bioconcentration factors (BCFs) for a number of aquatic organisms (1997). BCFs for most PEs were lower than expected based on their hydrophobicity. This finding was explained by the presence of environmental artifacts, metabolic transformation, and low bioavailability (Gobas et al. 2003), which is lower for high MW PEs (Lin et al. 2003). Since then, both laboratory and field studies have investigated the biomagnification of PEs and determined that they do not biomagnify in aquatic food webs (Mackintosh et al. 2004, Webster 2003). The biological breakdown of PEs in aquatic systems, including some invertebrates, is considered to be rapid, and so no significant bioaccumulation occurs (Metcalf et al. 1973, van den Berg et al. 2003). Since humans rapidly metabolize PEs to their respective monoesters, phthalates do not bioaccumulate in humans either (Duty et al. 2005).

As for MPEs, some have suggested their bioaccumulation under environmentally realistic conditions to be highly unlikely (Scholz 2003). This reasoning is based on the rapidity of MPE metabolism in biological organisms (Anderson et al. 2001) and because they are more water soluble than DPEs, lending less cause for bioaccumulation. However, as previously discussed there is the potential for accumulation of MPEs in biota over time given a steady and

constant flux of PEs into the environment. There are currently no reports on the environmental behaviour of MPE with regards to bioaccumulation.

Phthalate Esters and Policy

The persistence (P), bioaccumulation potential (B), and inherent toxicity (iT) of PEs are important regulatory criteria. The 1999 UNEP (United Nations Environment Program) protocol on LRTAP (long range transport of atmospheric pollutants) and domestic legislation in several countries including Canada under the *Canadian Environmental Protection Act* (1999) have focused on P, B, and iT of commercially used PEs as well as many other industrial substances. If PEs are to be considered toxic, according to the law, they must first be P or B and iT, and then the substances will undergo a screening level risk assessment (CEPA 1999). Recently, DEHP was added to Canada's List of Toxic Substances (EC 2005) and DMP, DEP, DBP, BBP, and DOP were listed on the Priority Substances List in Canada (PSL 1993, US EPA, CEPA 1999). Currently, the metabolites of PE degradation, MPEs, are not screened or regulated.

Literature Cited

Albro, P.W., R.Thomas, L. Fishbein. 1973. Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of the urinary metabolites. *Journal of Chromatography* 76:321-330.

Albro, P.W., and B. Moore. 1974. Identification of the metabolites of simple phthalate diesters in rat urine. *Journal of Chromatography* 94: 209-218.

Albro, P.W. 1986. Absorption, metabolism, and excretion of di(2-ethylhexyl) phthalate by rats and mice. *Environmental Health Perspectives* 65: 293-298.

Albro, P.W., R.E. Chapin, J.T. Corbett, J. Schroeder, and J.L. Phelps. 1989. Mono-2-ethylhexyl phthalate, a metabolite of di-(2-ethylhexyl) phthalate, causally linked to testicular atrophy in rats. *Toxicology and Applied Pharmacology* 100: 193-200.

Anderson, D., T.-W. Yu, and F. Hinçal. 1999. Effect of some phthalate esters in human cells in the Comet Assay. *Teratogenesis, Carcinogenesis, and Mutagenesis* 19: 275-280.

Arbin, A., and J. Östelius. 1980. Determination by electron-capture gas chromatography of mono- and di-(2-ethylhexyl) phthalate in intravenous solutions stored in poly(vinyl chloride) bags. *Journal of Chromatography* 193: 405-412.

Asai, D., Y. Tahara, M. Nakai, Y. Yakabe, M. Takatsuki, T. Nose, T. Shinmyozu, and Y. Shimohigashi. 2000. Structural essentials of xenoestrogens dialkyl phthalates to bind to the estrogen receptors. *Toxicology Letters* 118: 1-8.

Barr, D.B., M.J. Silva, K. Kato, J.A. Reidy, N.A. Malek, D. Hurtz, M. Sadowski, L.L. Needham, A.M. Calafat. 2003. Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. *Environmental Health Perspectives* 111(9): 1148-1151.

Barron, M.G., I.R. Schultz, and W.L. Hayton. 1989. Presystemic branchial metabolism limits di-2-ehtylhexyl phthalate accumulation in fish. *Toxicology and Applied Pharmacology* 98: 49-57.

Blount, B.C., M.J. Silva, S.P. Caudill, L.L. Needham, J.L. Pirkle, E.J. Sampson, G.W. Lucier, R.J. Jackson, and J.W. Brock. 2000. Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108: 979-982.

CEPA (Canadian Environmental Protection Act). 1999. Canada Gazette Part III: Vol. 22 No.3 November 4, 1999. Statutes of Canada, 1999, Chapters 33 and 34, Acts assented to from 18 June to 14 September, 1999. Ottawa, Canada: the Minister of Public Works and Government Services, the Queen's Printer for Canada.

Clark, K., I.T. Cousins, D. Mackay, and K. Yamada. Observed concentrations in the environment. In Handbook of Environmental Chemistry 3.Q. Phthalate Esthers; Staples, C., Ed.; Springer:Berlin, 2003; pp. 125-177.

Duty, S.M., A.M. Calafat, M.J. Silva, L. Ryan, and R. Hauser. 2005. Phthalate exposure and reproductive hormones in adult men. *Human Reproduction* 20(3): 604-610.

EC (Environment Canada). 2005. “Eighteen new substances added to the CEPA List of Toxic Substances,” news releases–archives. Available at: http://www.ec.gc.ca/press/18psl_b_e.htm Duty, S.M., N.P. Singh, M.J.

Ema, M., R. Kurosaka, H. Amano, and Y. Ogawa. 1995. Development toxicity evaluation of mono-*n*-butyl phthalate in rats. *Toxicology Letters* 78: 101-106.

Gobas, F. A. P. C., C. E. Mackintosh, G. Webster, M. Ikonomou, T.F. Parkerton, and K. Robillard. Bioaccumulation of phthalate esters in aquatic food webs. In Handbook of Environmental Chemistry 3.Q. Phthalate Esthers; Staples, C., Ed.; Springer:Berlin, 2003; pp. 201-225.

Gray, T.J.B., and S.D. Gangolli. 1986. Aspects of the testicular toxicity of phthalate esters. *Environmental Health Perspectives* 65: 229-235.

Heindel, J.J., and C.J. Powell. 1992. Phthalate ester effects on rat sertoli cell function *in vitro*: effects of phthalate side chain and age of animal. *Toxicology and Applied Pharmacology* 115: 116-123.

Hoppin, J.A., J.W. Brock, B.J. Davis, and D.D. Baird. 2002. Reproducibility of urinary phthalate metabolites in first morning urine samples. *Environmental Health Perspectives* 110 (5): 515-518.

Hurst, C.H., and D.J. Waxman. 2003. Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. *Toxicological Sciences* 74: 297-308.

Hurst, C.H., and D.J. Waxman. 2004. Environmental phthalate monoestes activate pregnane X receptor-mediated transcription. *Toxicology and Applied Pharmacology* 199: 266-274.

Jonsson, S., and A. Baun. 2003. Toxicity of mono- and diesters of *o*-phthalic esters to a crustacean, a green alga, and a bacterium. *Environmental Toxicology and Chemistry* 22(12): 3037–3043.

Jonsson, S., J. Ejlertsson, A. Ledin, I. Mersiowsky, and B.H. Svensson. 2003. Mono- and diesters of *o*-phthalic acid in leachates from different European landfills. *Water Research* 37(3): 609-617.

Kluwe, W.M. 1982. Overview of phthalate ester pharmacokinetics in mammalian species. *Environmental Health Perspectives* 45: 3-10.

Kohn, M.C., F. Parham, S.A. Masten, C.J. Portier, M.D. Shelby, J.W. Brock, and L.L. Needham. 2000. Human exposure estimates for phthalates. *Environmental Health Perspectives* 108(10): A440-A442.

Lake, B.G., J.C. Phillips, J.C. Linnell, and S.D. Gangolli. 1977. The *in vitro* hydrolysis of some phthalate diesters by hepatic and intestinal preparations from various species. *Toxicology and Applied Pharmacology* 39: 239-248.

Lech, J.J., and J.R. Bend. 1980. Relationship between biotransformation and the toxicity and fate of xenobiotic chemicals in fish. *Environmental Health Perspectives* 34: 115-131.

Li, L.-H., and J.J. Heindel. 1998. Sertoli cell toxicants. In *Reproductive and Developmental Toxicology* (K. Korach, Ed.), pp. 657-693, Marcel Dekker, Inc., New York.

Li, L.-H., W.F. Jester Jr., and J.M. Orth. 1998. Effects of relatively low levels of mono-(2-ethylhexyl) phthalate on cocultured sertoli cells and gonocytes from neonatal rats. *Toxicology and Applied Pharmacology* 153: 258-265.

Mackintosh, C.E., J. Maldonado, J. Hongwu, N. Hoover, A. Chong, M.G. Ikonomou, and F.A.P.C. Gobas. 2004. Distribution of phthalate esters in a marine aquatic food web: comparison to polychlorinated biphenyls. *Environmental Science and Technology* 38(7): 2011-2020.

Metcalf, R.L., G.M. Booth, C.K. Schuth, D.J. Hansen, and P.Y. Lu. 1973. Uptake and fate of di-2-ethylhexyl phthalate in aquatic organisms and in a model ecosystem. *Environmental Health Perspectives* 4: 27–34.

Morin, A. 2003. Distribution of phthalate esters in a marine mammal food chain from Canada's Eastern Arctic. (Under supervision of Gobas). Master in Resource Management Thesis. Simon Fraser University.

Mortensen, G.K., K.M. Main, A.-M. Andersson, H. Leffers, and N.E. Skakkebæk. 2005. Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC/MS-MS). *Analytical Bioanalytical Chemistry* 382: 1084-1092.

Niino, T., T. Ishibashi, T. Itoh, T. Sakai, H. Ishiwata, T. Yamada, and S. Onodera. 2001. Monoester formation by hydrolysis of dialkyl phthalate migrating from polyvinyl chloride products in human saliva. *Journal of Health Science* 47(3): 318-322.

Niino, T., T. Ishibashi, H. Ishiwata, K. Takeda, and S. Onodera. 2003. Characterization of human salivary esterase in enzymatic hydrolysis of phthalate esters. *Journal of Health Science* 49: 76-81.

Parkerton, T. F., and W. J. Konkel (ExxonMobil Biomedical Services, Inc.). Evaluation of the production, consumption, end use, and potential emissions of phthalate esters; Report prepared for the American Chemistry Council [ACC], 1300 Wilson Ave., Arlington, VA, 2000.

PSL (Priority Substances List). 1993. Assessment Report of Di-*n*-Octyl Phthalate. *Canadian Environmental Protection Act*. Government of Canada, Environment Canada, Health Canada. Minister of Supply and Services, Canada. National Printers (Ottawa) Inc.

Rudel, R.A., D.E. Camann, J.D. Spengler, L.R. Korn, and J.G. Brody. 2003. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. *Environmental Science and Technology* 37(20): 4543-4553.

Scholz, N. 2003. Ecotoxicity and biodegradation of phthalate monoesters. *Chemosphere* 53: 921-926.

Shintani, H. 1985. Determination of phthalic-acid, mono-(2-ethylhexyl) phthalate and di-(2-ethylhexyl) phthalate in human-plasma and in blood products. *Journal of Chromatography* 337(2): 279-290.

Silva, M.J., D.B. Barr, J.A. Reidy, K. Kato, N.A. Malek, C.C. Hodge, D. Hurtz III, A.M. Calafat, L.L. Needham, and J.W. Brock. 2003. Glucoronidation patterns of common urinary and serum monoester phthalate metabolites. *Archives of Toxicology* 77: 561-567.

Silva, M.J., D.B. Barr, J.A. Reidy, N.A. Malek, C.C. Hodge, S.P. Caudill, J.W. Brock, L.L. Needham, and A.M. Calafat. 2004. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. *Environmental Health Perspectives* 112(3): 331-338.

Stanley, M.K., K.A. Robillard, and C.A. Staples. Introduction. In Handbook of Environmental Chemistry 3.Q. Phthalate Esters; Staples, C., Ed.; Springer:Berlin, 2003; pp. 1-7.

Staples, C.A., D.R. Peterson, T.F. Parkerton, and W.J. Adams. 1997. The environmental fate of phthalate esters: a literature review. *Chemosphere* 35: 667-749.

Suzuki, T., K. Yaguchi, S. Suzuki, and T. Suga. 2001. Monitoring of phthalic acid monoesters in river water by solid-phase extraction and GC/MS determination. *Environmental Science and Technology* 35: 3757-3763.

US EPA United States Environmental Protection Agency. Federal Registry. 49, 43305,12.

van den Berg, M., T. Sanderson, N. Kurihara, and A. Katayama. 2003. Role of metabolism in the endocrine-disrupting effects of chemicals in aquatic and terrestrial systems. *Pure Applied Chemistry* 75(11-12): 1917-1932.

Ward, J.M., J.M. Peters, C.M. Perella, and F.J. Gonzalez. 1998. Receptor and nonreceptor-mediated organ-specific toxicity of di(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferator-activated receptor alpha-null mice. *Toxicologic Pathology* 26: 240-246.

Webster, G. 2004. Dietary uptake and dietary biotransformation of phthalate esters in Staghorn Sculpin. (Under supervision of Gobas). Master in Resource Management Thesis. Simon Fraser University.

Yagi, Y., Y. Nakamura, I. Tomita, K. Tsuchikawa, and N. Shimoi. 1980. Teratogenic potential of di- and mono-(2-ethylhexyl)phthalate in mice. *Journal of Environmental Pathology and Toxicology* 4(2,3): 533-544.