DISTRIBUTION OF PHTHALATE MONOESTERS IN AN AQUATIC FOOD WEB

by

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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

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Degree: **Master of Resource Management**

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

Report No.: **426**

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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

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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

4 METHODS Materials and Preparation

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

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*

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%

used to replace remaining non-detect (ND) values for the reportable data. Mean

(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

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

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.

<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

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

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 frd2y 10.2((2)ero6( )]TJ-23987

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

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

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

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FIGURES AND TABLES

Table 1 **Molecular weight (g/mol), aqueous solubility (mg/L), log octanol-water partition coefficient (K_{ow}**

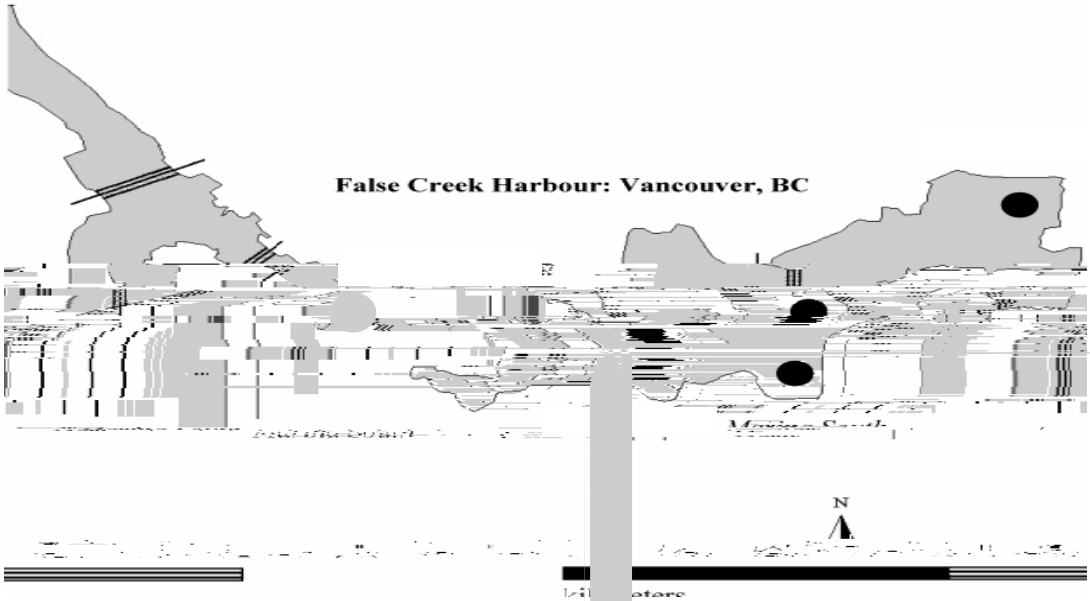

False Creek Harbour: Vancouver, BC
N

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 (Dark-Mahogany Clam)	Deposit feeder		

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*d

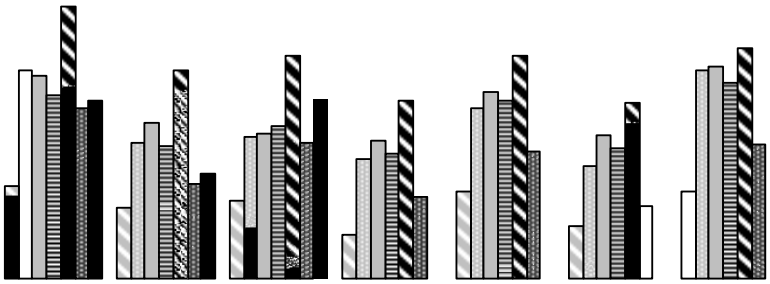
Table 4 **Mean amount (ng) of MPEs in sodium sulphate procedural blanks for sediment and biota sample analyses, 2 standard deviations of the blanks, method**

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:**

MPEs

	DPEs										
	WT[1]	SD	GA	BM	SC	DC***M***	DC***H***	jSP	WG	DF***M***	DF***L***

Table 6 Observed minimum and maximum MPE concentrations (ppb) in marine water



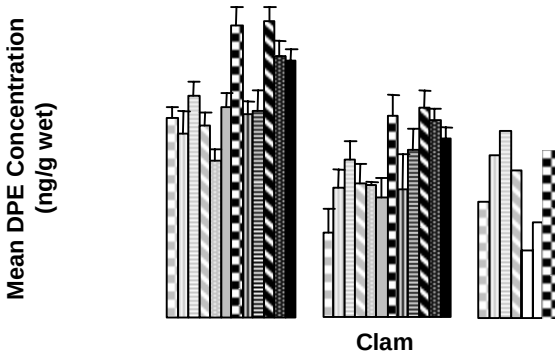

Mean DPE Concentration
(ng/g wet)
Clam

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 <

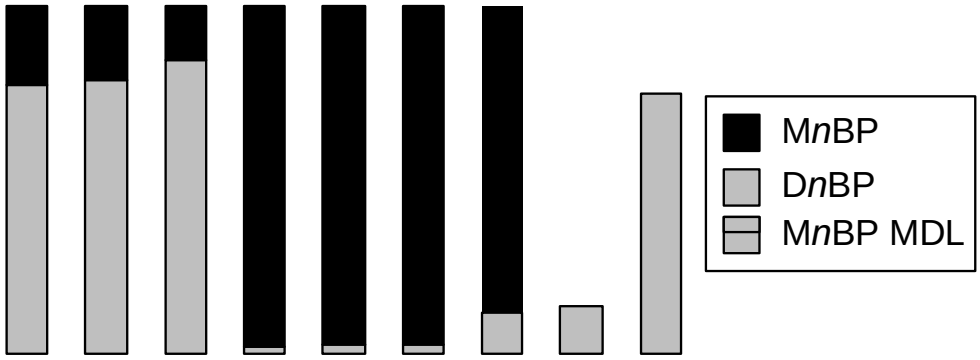
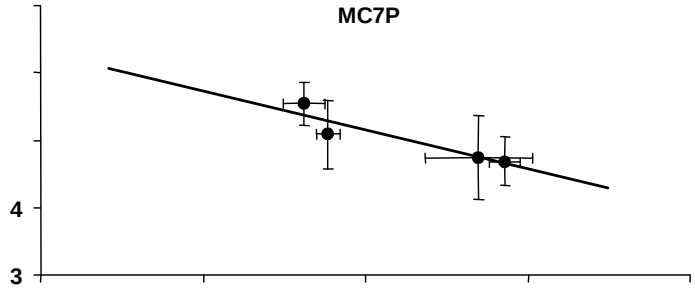

MnBP
DnBP
MnBP MDL

Table 11 Statistical results of regression analysis between log MPE concentration and $\delta^{15}N$ (i.e., slope, *p*


MC7P
4
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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,

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

Normality tests on MPE and DPE Concentration Data

		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

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							

	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	

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 insecs

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 2 l i r o

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

(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

Although there seems to be some evidence pointing to PEs metabolites as

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

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