ENVIRONMENTAL PARTITIONING OF MONOPHTHALATE ESTERS

by

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Title of Project: Environmental Partitioning of Mono Phthalate Esters

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ABSTRACT

Di-phthalate esters are widespread industrial chemicals in the environment. Monophthalate esters are the primary metabolites of di-phthalate esters. The sorption coefficients of nine different monophthalate ester congeners in marine and fresh water sediments were determined in laboratory and field studies. Sorption of various monophthalate esters onto sediment reached equilibrium within 24 h. Organic carbon-normalized sorption coefficients for 9 monophthalate esters in laboratory experiments ranged between 38 and 603 L/kg for marine sediment and between 21 and 380 L/kg for fresh water sediment. Sorption coefficients measured in the field sediments were greater, and ranged between 200 and 46,000 L/kg. Sorption coefficient of MEHP increased with decreasing pH. Sorption coefficients of MPEs from laboratory studies were substantially lower than predicted based on octanol-water partition coefficient of the undissociated form. These results demonstrate that the dissociation of MPEs at environmental pH levels needs to be considered when assessing the fate of these substances in the aquatic environments.

Keywords: Monophthalate esters, di-phthalate esters, plasticizers, derivatization, sorption coefficients, ionization, partition.

Subject Terms: Toxicology, Chemistry, Environmental Fate of Chemicals, False Creek - Buntzen Lake - Vancouver - British Columbia.

To my beloved parents

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LIST OF ACRONYMS

ASTER:	Assessment Tools for the Evaluation of Risk
EPIWIN:	Estimations Program Interface for Windows
IOS:	Institute of Ocean Sciences
MPEs:	Mono-alkyl phthalate esters
DPEs:	Di-alkyl phthalate esters
MMP:	Mono-methyl phthalate
MEP:	Mono-ethyl phthalate
MBuP:	Mono-butyl phthalate
MEHP:	Mono-2-ethylhexyl phthalate
MC6:	Mono-hexyl phthlate isomer mixture
MC7:	Mono-heptyl phthalate isomer mixture
MC9:	Mono-nonyl phthalate isomer mixture
MC10:	Mono-decyl phthalate isomer mixture
OC:	Organic carbon
TOC:	Total organic carbon
GCMS:	Gas Chromatography Mass Spectrophotometry
DMP:	Di-methyl phthalate ester
DBP:	Di-butyl phthalate ester
DEP:	Di-ethyl phthalate ester
DIDP:	Di-iso-decyl phthalate ester
HPV:	High production volume
PVC:	Polyvinyl chloride
DEHP:	Di-2-ethylhexyl phthalate ester
DINP:	Di-iso-nonyl phthalate ester
DIDP:	Di-iso-decyl phthalate ester
DHP:	Dihexyl phthalate ester
DPP:	Dipentyl phthalate ester
DPrP:	Dipropyl phthalate ester
OECD:	Organization for Economic Cooperation and Development
US:	United States
EU:	European Union
LD_{50}:	Lethal dose of a chemical that kills 50% of the animals tested
pKa:	Symbol for acid dissociation constant
UV:	Ultra violet
C_S:	Concentration of chemical in sediment
C_W:	Concentration of chemical in water
K_{SW}:	Sediment-water partition coefficient
K_{OC}:	Organic-carbon normalized partition coefficient
TMSDM:	Tri-methylsilyl diazomethane

Φn:	The fraction of non-ionized form of MPEs
BCF:	Bio-concentration factor
BAF:	Bio-accumulation factor
DCM:	Dichloromethane

1 INTRODUCTION

1.1 Di-Phthalate Esters

Di-phthalate esters (DPEs) are widespread industrial chemicals that have been manufactured since the early 1900s (Houlihan and Wiles, 2000). DPEs are widely associated with industrial applications and consumer products. They are found in products such as home-building materials, automobiles, furniture, clothing materials, etc. The main sources of DPEs in the environment are anthropogenic. However, some di-esters, such as di-(2-ethylhexyl) phthalate, di-n-butyl phthalate and di-octyl phthalate are biosynthesized by some algal members and are not the result of contaminants in the water (Hayashi *et al.*, 1967; Marx, 1972; Stefanov *et al.*, 1988; Sastry and Rao, 1995).

1.1.1 Overview of DPE chemistry

Di-phthalate esters are products of esterification reactions. Ortho phthalate di-esters are the most widely used di-esters (Peterson and Parkerton, 1999; Staples, 2003), produced by the sequential addition of alcohols to phthalic anhydride in the presence of an acid catalyst. The esters of 1,2-benzene dicarboxylic acid, commonly called phthalate esters, (Figure 1), are a group of twenty-five compounds that are found in numerous commercial products mainly as additivies. The two alkyl side chains (R_1 and R_2) on the cyclohexatriene ring (benzene-dicarboxylic acid) characterizes a phthalate di-ester and also provides their name. Some of the congeners are listed with their chemical and physical properties in Table 1.

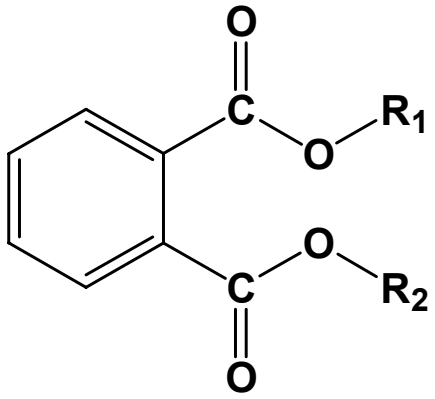


Figure 1: Generalized chemical structure of phthalate ester. R_1 and R_2 are alkyl side chains.

The length of the alkyl side chain determines the water solubility of the congener. Generally, the longer the alkyl side chain(s), the lower the water solubility of the congener. Di-methyl phthalate ester (DMP) is the most water soluble DPE while the long side chain isomers of Di-iso-decyl phthalate ester (DIDP) are the most hydrophobic and least water soluble. DPEs exist as non-ionic substances in water. The vapour pressures of DPEs range from 2.63×10^{-1} Pa for DMP to 7.45×10^{-6} Pa for C10 isomer (Cousins and Mackay, 2000). The viscosity of these isomer increases with the increase in the alkyl side chain. DPEs are hydrophobic in nature with octanol-seawater partition coefficients ranging between $10^{1.8}$ for DMP to $10^{10.6}$ for DIDP (Staples, 2003).

Table 1: Physio-chemical properties of selected phthalate di-esters. Source: (Cousins and Mackay, 2000; Staples, 2003).

Phthalate Ester Congener	Abbreviation	Molecular Weight (g/mol)	Water Solubility (mg/L)	Log K_{ow}
Dimethyl	DMP	194.2	5220	1.61
Diethyl	DEP	222.2	591	2.54
Di-iso-butyl	DIBP	278.4	9.9	4.27
Di-n-butyl	DBP	278.4	9.9	4.27
Butyl Benzyl	BBP	312.4	3.8	4.70
Di (2-ethylhexyl)	DEHP	390.6	0.0025	7.73
Di-n-octyl	DnOP	390.6	0.0025	7.73
Di-n-nonyl	DnNP	418.6	0.0006	8.60
Di-iso-hexyl	DIHxP	334.4	0.05	7.73
Di-iso-heptyl	DIHpP	362.4	0.011	6.87
Di-iso-octyl	DIOP	390.6	0.0025	7.73
Di-iso-nonyl	DINP	418.6	0.0006	8.60
Di-iso-decyl	DIDP	446.7	0.00013	9.46

1.1.2 Production and use

Di-alkyl Phthalates comprise of 90% of the plasticizer market and have a broad range of applications. As of 2000, Canada's annual production and consumption of total di-phthalate esters are estimated to be 40,000 and 70,000 tonnes/year, respectively (Parkerton and Konkel, 2000), while global production level is estimated to be 5.5 million tonnes/year as of 2006. There has been an enormous increase in the production

and consumption of these chemical during the last 2 decades world wide (NCEH, 2005). Due to their large scale production and usage, di-phthalates are categorized as high production volume (HPV) chemicals.

The length of the alkyl side chain determines their physiochemical properties and thus their usage. They can be broadly classified into three different categories based on their application: Polyvinyl chloride (PVC) plasticizers, non-PVC plasticizers and other minor applications. Lower molecular weight phthalates, those with alkyl side chains from carbon atoms 1 to 4, are included in a broad range of applications which include consumer products and pharmaceuticals. Lower molecular weight phthalates also provide plasticizer applications but only in some non-PVC products like acrylics, urethanes and cellulosics. The higher molecular weight phthalates provide plasticizer applications. Plasticizer applications include upholstery, wall covering, flooring and roofing, plastic clothing like foot wear and rain gears, pool lining, wire and cable coatings, shower curtains, paints, adhesives, rubbers, thermoplastics, cellulose plastics, garden hoses plastic toys, medical blood bags. These chemicals account for 85% of the production (Vitali *et al.*, 1997). Non-plasticizer application include inks, dyes, sealants, lubricating oils, photographic films, synthetic leathers, fixatives for perfumes (cosmetics), insect repellent, insecticides, and propellants (Peakall, 1975) tooth brushes, food packaging, personal-care products, such as soap, shampoo, deodorants, fragrances, hair spray, nail polish; and some medical pharmaceuticals like aspirin. Among the various phthalate congeners used in United States, DEHP, DINP, and DIDP account for 52.2 % of the chemicals, while linear phthalates account for 21.4 %, and the balance by other congeners (Staples, 2003).

Large-scale and widespread production combined with the fact that phthalate esters are not chemically bound to the polymeric matrix and are able to migrate from plastic products, provides the potential for this class of chemicals to enter the aquatic environment. Other point sources include leachates from landfills containing plastic waste and incineration of plastic waste.

1.1.3 Regulation

Because of their high production volumes and toxic potential, di-phthalate esters are subject to regulations world-wide to varying degrees. Environment Canada and Health Canada with the Canadian Environmental Protection Act (CEPA) in Canada, The Clean Water Act, Resource Conservation and Recovery Act (RCRA), Safe Drinking Water Act and the United States (US) Toxic Substance Control Act in US are some of the regulatory agencies that monitor, production, transportation, use, disposal, release into environment, and safe levels in water, and other environmental media.

DEHP, DBP and BBP are regulated differently from other DPEs in the European Union (Staples, 2003).

Di-(2-ethylhexyl) phthalate was added to 'List of Toxic Substances' under CEPA during 2006 revisions (EC, 2007b). Under this Act, substances in the 'List of Toxic Substances' are carefully monitored through the life cycle of the substance and shall be proposed for complete elimination (CEPA, 1999). Di-(2-ethylhexyl) phthalate, Dibutyl phthalate and Di-n-octyl phthalate were initially listed in 'first priority substance list' (PSL1) in the year 1989. Under CEPA, priority substance list identifies substances to be assessed on a priority basis to determine whether they are toxic (CEPA, 1999). Of the

other two phthalates in the PSL1, Dibutyl phthalate was considered to be not toxic under CEPA at the concentrations released into the environment, while with Di-n-octyl phthalate, it was concluded that there is not enough information to support it as a toxic substance under CEPA. Butylbenzyl phthalate was listed in PSL2 in the year 1995 (EC, 2007a).

The European Union (EU) recently banned the usage of DINP, DIDP and DNOP in plasticized toys or childcare articles or plasticized parts of toys and childcare articles which can be placed in the mouth by children (European Commission, 2006). EU has classified DEHP as a Category 2 reproductive toxicant for both fertility and developmental processes based on the evidence of effects seen in rodents. This is a precautionary categorization because the effects observed in rodents were in response to exposure concentrations hundreds of times higher than would normally be ingested by human beings (European Commission, 2001). The EU has classified DBP as a chemical causing possible risk of impaired fertility and potential of causing harm to the unborn child (European Commission, 2004).

All di-phthalate esters have undergone comprehensive environmental and human health risk assessments in Canada, United States, the European Union and the Organization for Economic Cooperation and Development (OECD, 2000).

1.1.4 Fate of di-esters in the environment

Only very small amounts of DPEs are released into the environment during their production and processing (Staples, 2003). However, due to non-covalent binding with polymers, DPEs are subject to easy migration and leaching into the environment from

plastics and other phthalate containing articles. Other point sources include leachates from landfills with the plastic wastes and incineration of plastic waste.

Various studies have reported detectable concentrations of DPEs in the air, water and fish since the 1970's. DEHP and DBP are the most frequently detected congeners in sediments, water bodies, plants and aquatic organisms (Atlas and Giam, 1981; Giam *et al.*, 1984). Atmospheric concentrations of DBP and DEHP during 1979 ranged from 0.9 to 18.5 ng/m^3 and 1.2 to 16.6 ng/m^3 respectively in atmosphere (sampled across Pacific, North American and North Atlantic regions). In precipitation samples, collected from the North Pacific Ocean, DBP and DEHP concentrations ranged from 2.6 to 72.5 ng/L and 5.3 to 213 ng/L respectively (Atlas and Giam, 1981). Latini and co-workers (Latini *et al.*, 2003) confirmed a significant and widespread presence of DEHP and its monoester metabolite, MEHP in newborn infants in an Italian hospital. Mean concentrations of DEHP and MEHP were 1.19 μg/mL and 0.52 μg/mL respectively.

Areas of industrial use and waster disposal can have the greatest DPE concentrations in soil and water. High concentrations have also been detected in landfill leachate (DMP – 300 μg/l; Phthalic acid – 18900 μg/l; Mono-methyl phthalate ester (MMP) – 6 μg/l) (Mersiowsky, 2002). Marine water concentrations in False Creek (a marine inlet in Vancouver, British Columbia, Canada) ranged from 3.5 ng/L for DMP and BBP to 275 ng/L for DEHP and C8 isomers while total di-ester concentrations were about 735 ng/L (Mackintosh, 2003). Despite these reports, Kiyomatsu *et al.,* (2001) suggested that DPE concentrations in the environment have been decreasing since 1980.

DPEs are subject to various processes such as aerobic and anaerobic microbial break down, hydrolysis and photo-oxidation. Biodegradation is the dominant loss

process in all compartments of environment except air where photo-oxidation dominates (Staples *et al.*, 1997b). Both aerobic and anaerobic biodegradation is initiated by ester hydrolysis to form the monoesters and corresponding alcohol.

In addition, di-esters are biodegraded to monoesters in living organisms (Heindel and Powell, 1992). DPEs are rapidly biodegraded to mono phthalate esters (MPEs) and other metabolites such as phthalic acids. Zeng *et al.*, (2002) found that the high molecular weight phthalate, DEHP, was biodegraded with a half life of 10 days by microorganisms found in activated sludge. The major metabolites found were the monoester, phthalic acid, benzoic acid, and phenol. They also suggested that the primary metabolite is the monoester.

Mackintosh (Mackintosh, 2003) found that DPEs do not biomagnify in the aquatic food web and that higher molecular weight phthalates are subject to trophic dilution. The same study also found that there is a sediment water disequilibrium with high concentrations in the sediment relative to the water concentrations.

1.1.5 Toxicological profile of di-esters

The ubiquitous distribution of DPEs in the environment and their potential carcinogenic and pseudoestrogenic properties (Giam *et al.*, 1984) have raised concern and interest in these chemicals.

Generally, the toxicity of DPEs is considered to be low to moderate (Staples *et al.*, 1997a). Laboratory studies on rats and mice have shown LD_{50}'s of DBP, DEHP, and DnOP in the range of 8 to 25 g/kg body weight (Chan and Meek, 1994a; Chan and Meek, 1994b; Meek and Chan, 1994).

Phthalate esters are classified as compounds commonly referred to as peroxisome proliferators (Woodward, 1988b). These chemical can cause an increase in the number and size of peroxisomes in liver tissue leading to condition known as hepatomegaly. Reddy and his co-workers were the first to show that DEHP induced peroxisome proliferation in rats and mice (Reddy *et al.*, 1976). DEHP was found to cause tumours in liver of rats and mice by non-DNA reactive mechanisms involving peroxisome proliferation (IARC, 2000). Peroxisome proliferators show their effects by activating the peroxisome proliferator-activated receptors (PPARs), members of the nuclear receptor superfamily (Lovekamp-Swan and Davis, 2003). Peroxisome proliferators administered to rodents resulted in not only increase in number and size of peroxisomes but also numerous alterations in gene transcription of proteins involved in lipid metabolism (Lampen *et al.*, 2003). This type of response is mediated by nuclear receptor transcription factors, the peroxisome proliferator-activated receptors (PPARs), which are activated by micromolar concentration of lipid or fatty acids (Issemann and Green, 1990; Krey *et al.*, 1997; Lampen *et al.*, 2001; Lampen *et al.*, 2003). Activation of one of the iso-forms of PPARs, PPARδ (PPAR-delta) was found to be associated with the teratogenic effects of antiepileptic drug valproic acid (Lampen *et al.*, 2001). DEHP and its metabolites, MEHP, mono-1-methyl-heptyl-phthalate, and 2-hexanoic acid, and butylbenzyl phthalate were found to activate PPARδ in invitro studies (Lampen *et al.*, 2003).

The toxicity of various di-phthalate ester congeners vary with the length of the side chain (Woodward, 1988a). Various di-phthalate ester congeners are found to effect the reproductive system of rats in both sexes (ATSDR, 1993; ATSDR, 1995; ATSDR,

1999; Lovekamp-Swan and Davis, 2003). However, based on the reproductive studies in mice, DEHP was found to be the most potent reproductive toxicant amoung the phthalates, followed by di-hexyl phthalate (DHP), di-pentyl phthalate (DPP), DBP, and di-propyl phthalate (DPrP) (Heindel *et al.*, 1989). Among the structurally related phthalates, DEHP, DBP, DPP, and DHP cause testicular atrophy and are both male and female reproductive toxicants in rodents (Heindel and Powell, 1992). Colon *et al.*, (2000) found association between higher levels of phthalates in blood of young girls and early development of breasts (abnormal reproductive development).

DEHP is categorized as non-carcinogen to human (IARC, 2000), but classified as a carcinogen to rats and mice by International Agency for Research on Cancer (IARC) (IARC, 2000). DEHP caused tumours in the liver of experimental rats and mice while no tumours were observed at other sites (IARC, 2000). Short-term mutagenicity bioassays have revealed negative results, indicating that these chemicals and their metabolites are not genotoxic (Barber *et al.*, 1994).

Toxicity tests conducted with Hyalella found 10-day (d) LC_{50}s for DMP, DEP, DBP, BBP to range from 0.46 mg/L to 28.1 mg/L in water without sediment (Call *et al.*, 2001a; Call *et al.*, 2001b). The Hyallela 10-d LC_{50} based on sediment concentrations for DBP were 17,400 mg/kg dry weight (with TOC 2.45%), 29,500 mg/kg dry weight (with TOC 4.8%) and 71,900 mg/kg dry weight (with TOC 14.1%).

1.2 Mono Phthalate Esters (MPEs)

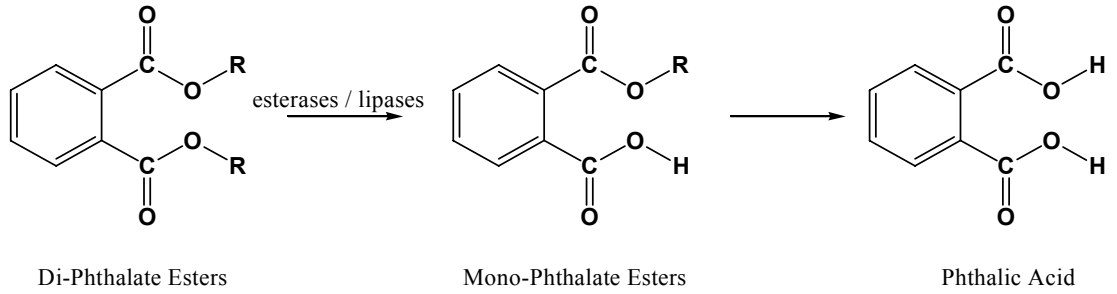


Figure 2: Hydrolysis of di-phthalate esters.

Monophthalate esters (MPEs) are the primary metabolites of DPEs (Figure 2). The main sources of these chemicals are their parent compounds, DPEs.

1.2.1 Over view of MPE chemistry

MPEs are clear oily liquids at room temperature. Monoester phthalates have no commercial applications at present. However, they are common intermediates in the production process of di-esters and are found as trace impurities in di-esters (Peterson and Parkerton, 1999).

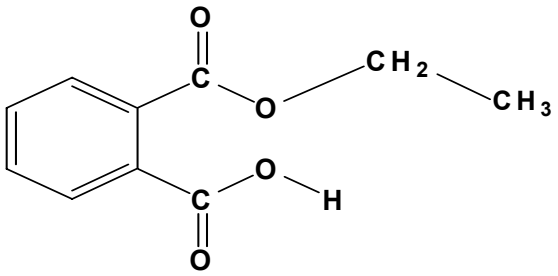
Pure monoesters are difficult to obtain synthetically (BASF). After sequential addition of either branched or normal alcohols to phthalic anhydride, the reaction temperature is gradually raised to reach 115 ^{o}C and monoesters are autocatalytically formed. This is an exothermic reaction which raises the temperature. High temperature favors the formation of di-esters thus, the reaction mixture is rapidly cooled in an ice bath. This prevents the excessive formation of di-esters.

There is very limited information on the physio-chemical properties of monoesters based on the experimental data; however, various models have been used to

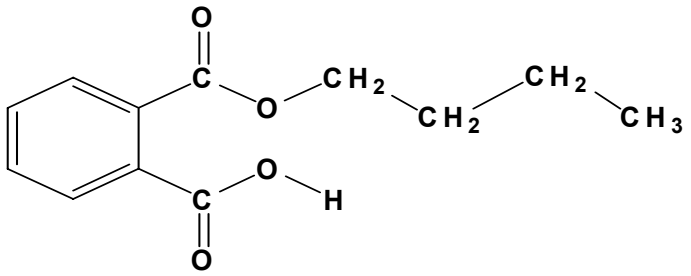
estimate some properties such as aqueous solubility and pKa. Some of the physiochemical properties of MPEs are shown in Table 2 and the chemical structures of some of phthalate monoesters are presented in Figure 3.

Table 2: Physio-chemical properties (molecular weight, water solubility, pKa, Low K_{OW} and Log K_{OC}) of Monoester Phthalates (Source: (Peterson and Parkerton, 1999). [1] is the measured water solubility or pKa (Chantooni and Kolthoff, 1975). Water solubility, pKa were estimated by ASTER program and Log K_{OW} estimated by EPIWIN program.

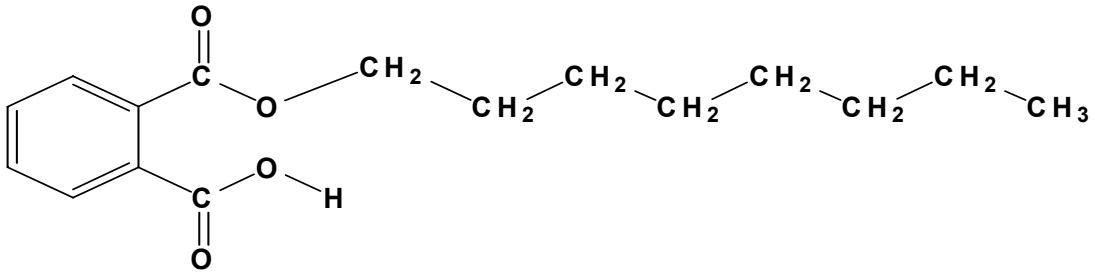
Phthalate Ester Congener	Abbre-viation	Molecular Weight (g/mol)	Water Solubility (mg/L)	Acid Dissociation pKa	Log K_{ow}
Monomethyl	MMP	180.2	37121[1]	3.18[1]	1.37
Monoethyl	MEP	194.2	7150	3.26[1]	1.86
Mono-n-butyl	MBuP	222.2	409	4.2	2.84
Mono-n-hexyl	MnHxP	250.3	23	4.2	3.85
Mono Benzyl	MBzP	256.3	282	4.2	3.07
Mono (2-ethylhexyl)	MEHP	278.3	1.85	4.2	4.73
Mono-2-methyl-octyl	MnOP	292.4	0.634	4.2	5.22
Mono-n-nonyl	MnNP	292.4	0.300	4.2	5.30
Mono-iso-heptyl	MIHpP	306.4	0.274	4.2	5.57
Mono-n-decyl	MnDP	306.4	0.070	4.2	5.79



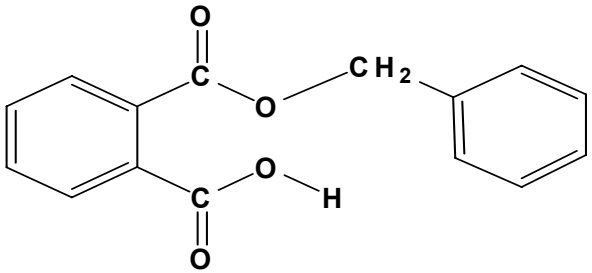
Monoethyl Phthalate (MEP)



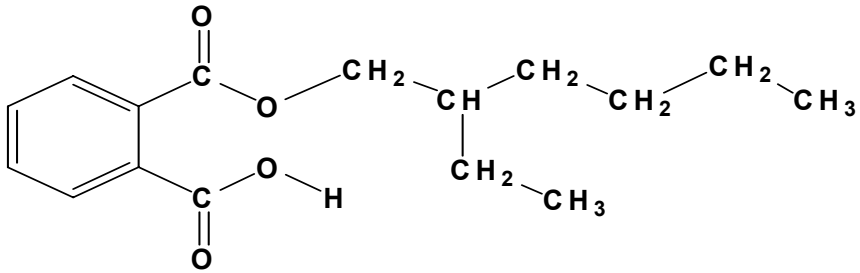
Mono-n-butyl Phthalate (MnBP)



Mono-n-Octyl Phthalate (MnOP)



Monobenzyl Phthalate (MBzP)



Mono (2-ethylhexyl) Phthalate (MEHP)

Figure 3: Chemical structures of five monophthalate esters.

MPEs are weak acids and, as such, are weak electrolytes. Unlike the DPEs, MPEs are ionizable compounds in aqueous solutions and their degree of dissociation will be influenced by pH. According to the Henderson-Hasselbalch equation (Equation 1), the pKa of an acid is the pH at which it is 50% dissociated. The pKa values available for the MPEs are estimated based on their solubility. At pH ≤ 4 MEHP (pKa of 4.32) will be ≤ 50% ionized while at neutral pH (6-7), > 90% of the compound will be ionized.

$$pKa = pH + \log \frac{[Unionized]}{[Ionized]} \qquad \textbf{Equation 1}$$

1.2.2 Production, use and regulation of MPEs

Unlike DPEs, MPEs are not used commercially nor are they used in manufacturing of commercial or consumer products (Peterson and Parkerton, 1999). They exist as a transient step during synthesis and are also the primary metabolites of di-esters. They are never isolated but always associated with their respective di-esters. Even during the synthesis of monoesters, di-esters formation is unavoidable.

Currently, there are no regulatory guidelines for MPEs, only the parent di-esters are regulated. As MPEs pose a more serious threat in terms of toxicity than their parent compounds (DPEs), it will be a useful guideline to consider the toxicity of both di-ester and monoester as total weight-age on toxicity of di-esters.

1.2.3 Toxicity of MPEs

Though monoesters are more hydrophilic than their parent compounds, di-esters, it is known that monoesters are more biologically active (Kato *et al.*, 2004) and are

responsible for the toxic effects of the phthalate esters. LC_{50} (96 h) for fish (*Cyprinus carpio*) was found to be 62 mg/L with MEHP and 125 mg/L with MBuP (Scholz, 2003). EC_{50} for daphnids *(Daphnia magna*) was found to be 73 mg/L with MEHP and 141 mg/L with MBuP ((Scholz, 2003).

Several MPE congeners including activated PPARα (PPAR-alpha) and PPARγ (PPAR-gamma) (see section 1.1.5 for introduction on PPARs). MMP and MEP did not activate any of the three PPAR isoforms while MEHP, mono-1-methyl-heptyl-phthalate, and MBzP activated PPARδ form (Lampen *et al.*, 2003; Lovekamp-Swan and Davis, 2003; Lovekamp-Swan *et al.*, 2003). MEHP activates both PPARα and PPARγ in the granulose cell resulting in the decreased transcription of aromatase, the primary mechanism of its female reproductive toxicity (Lovekamp-Swan and Davis, 2003).

Epidemiological studies report that detectable levels of MEP, MBuP, MBzP and MEHP were present in human urine in more than 75% of the population in U.S. (ATSDR, 1993; Blount *et al.*, 2000b; NTP-CERHR, 2000a; NTP-CERHR, 2000b). The highest level of MEHP reported was 192 μg/g creatinine (Ge *et al.*, 2007). Recent epidemiological studies also indicate that boys born to women exposed to phthalates during pregnancy have an increased incidence of congenital genital malformations and spermatogenic dysfunction (Ge *et al.*, 2007).

Phthalates and their metabolites have either weak or no estrogenic, anti-estrogenic, or androgenic activity *in vitro*, although not all metabolites have been tested (Jobling *et al.*, 1995; Coldham *et al.*, 1997; Okubo *et al.*, 2003), and have shown no estrogenic activity *in vivo* ((Milligan *et al.*, 1998; Zacharewski *et al.*, 1998). As stated earlier, exposure of monoesters from direct sources is very limited. The main route of

exposure is through the hydrolysis of di-esters. Absorption of MPEs by primates is much lower than in rodents (Rhodes *et al.*, 1986; Astill, 1989; Pugh *et al.*, 2000). Thus, it is unlikely that MPEs concentrations in primate liver achieve the levels as in rodent liver (Pugh *et al.*, 2000; Kamendulis *et al.*, 2002). However, further research is required to determine whether the internal human tissue concentrations reach the effective concentration levels to cause various toxic effects that are observed in invitro studies.

1.2.4 Metabolism of di-esters and monoesters in animals

Metabolic transformation of DPEs results in the metabolites which are more toxic than the parent compound. Generally, metabolic biotransformation of xenobiotics tends to result in metabolites that are more hydrophilic to facilitate their elimination but the metabolites are not necessarily less toxic.

Mono-esters are the primary metabolic products of di-esters (Rowland *et al.*, 1977; Kluwe, 1982; Peck and Albro, 1982; Woodward, 1988b; Niino *et al.*, 2001; Koch *et al.*, 2003c). Hydrolysis of primary and secondary ester linkages of phthalate esters result in mono-esters and phthalic acid. Hydrolysis of primary ester linkages are reported to occur more rapidly (Kluwe, 1982) than hydrolysis of both ester linkages. Esterases and lipases are responsible for hydrolysis. These enzymes are present in several mammalian tissues such as liver, lungs and kidneys (Carter *et al.*, 1974). Liver hydrolases are capable of hydrolyzing di-esters completely to phthalic acid (Albro and Thomas, 1973) and saliva (Niino *et al.*, 2002).

Di-esters migrate from PVC products and hydrolyse to mono-esters (Niino *et al.*, 2001). The various commercial uses of products containing phthalate esters form the

most common mode of exposure to humans primary through ingestion along with food or liquids, while medical uses of products containing di-esters form the other significant source of phthalate esters exposure via direct access into the circulatory system in human beings (Kluwe, 1982).

The general population is exposed to DPEs in various ways such as ingestion with food, dermal absorption through cosmetic application and inhalation of perfumes (ATSDR, 1993; Petersen and Breindahl, 2000).

Mono-esters are biomarkers of phthalate ester exposure (Blount *et al.*, 2000a; Kessler *et al.*, 2001; Anderson *et al.*, 2002), because mono-esters are the primary metabolites. Phthalic acid can be a general biomarker for di-ester exposure without specific indication to a particular phthalate congener (Koch *et al.*, 2003c). Mono-esters have been shown to form after oral ingestion in rodents (Rowland, 1974; Rowland *et al.*, 1977) and in fish (Webster, 2003). Other metabolic products are also known to exist in higher concentrations than monoesters. Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) are found to be 4.5 and 3.5 times respectively higher (Koch *et al.*, 2003c) to 10 times higher (Kato *et al.*, 2004) than MEHP in human urine samples after DEHP exposure. It is also found that these oxidative metabolites are excreted predominantly as glucuronide conjugates than free forms (Koch *et al.*, 2003c). Various metabolites are isolated in the metabolic pathway of DPEs (Figure 4).

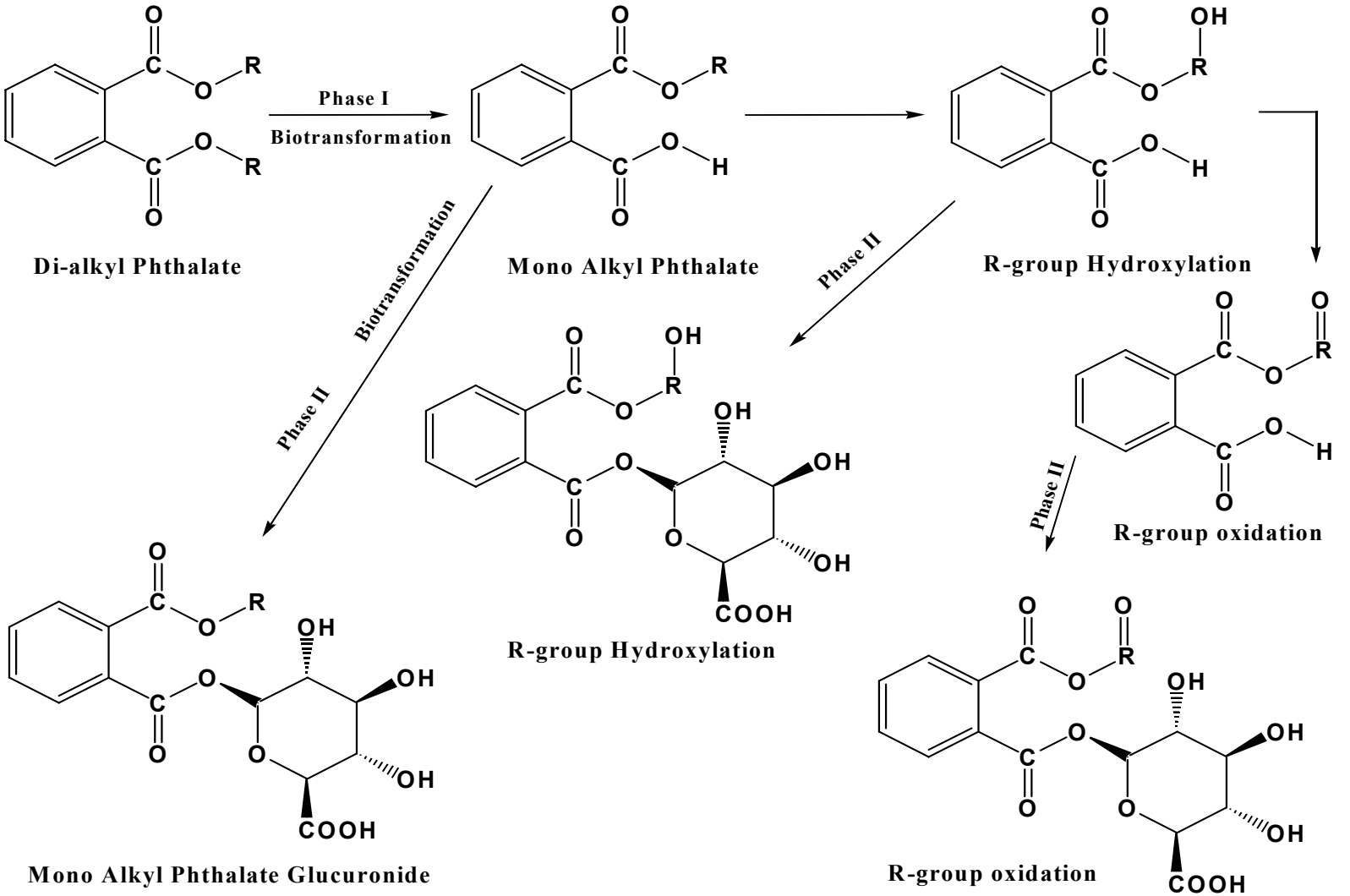


Figure 4: General metabolic pathways for phthalates. Source: (Heindel and Powell, 1992; Koch *et al.*, 2003a; Koch *et al.*, 2003b; Kato *et al.*, 2004).

1.2.5 Fate of di-esters and monoesters in the environment

The generalized biodegradation pathway of di-esters / monoesters is presented (Figure 5). DPEs are considered to be biodegradable and having a low to none bioaccumulation potential (Mackintosh *et al.*, 2004). However these chemicals are being constantly detected in the air, water and fish (Kiyomatsu *et al.*, 2001). Abiotic processes such as hydrolysis and photodegradation play a minor role in the natural environments (Staples et al., 1997). These reactions appear to be of less importance in the fate of phthalate esters than microbial degradation.

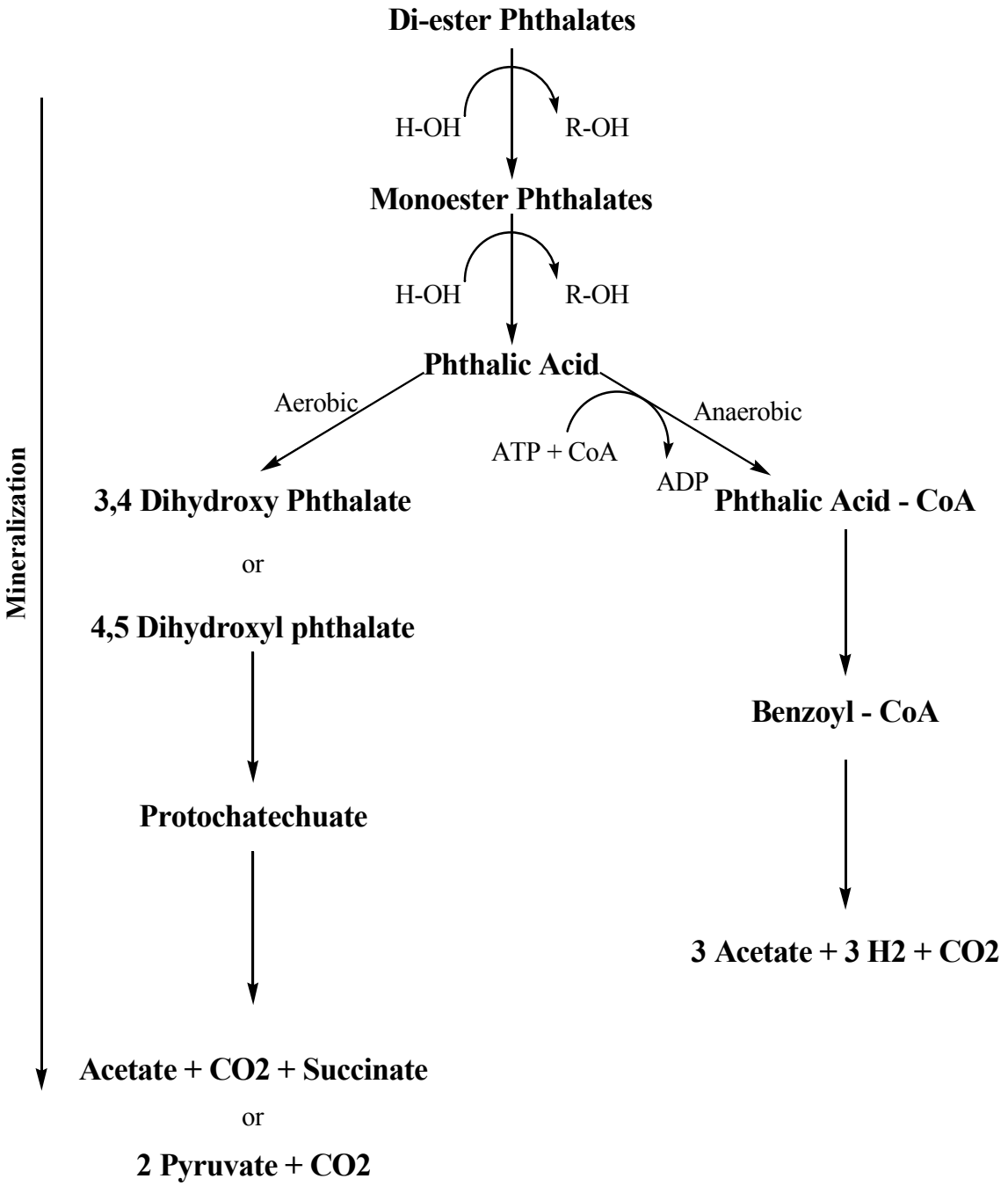


Figure 5: General biodegradation pathway of phthalate esters in the environment. Source: (Ejlertsson and Svensson, 1995; Staples *et al.*, 1997b).

1.2.5.1 Hydrolysis

Hydrolysis of phthalates is one abiotic process that occurs in the natural environment. The di-phthalate esters undergo a two-step hydrolysis which proceed at slow rates. The first hydrolysis of the di-ester results in a monoester and an alcohol. The

second hydrolysis is of the monoester which results in phthalic acid and another alcohol (Schwartzenbach *et al.*, 1993) (Figure 2). This process can be catalyzed either by a base, acid, anions, or organic materials. Base hydrolysis is a quicker process than acid hydrolysis; however, non-enzymatic hydrolysis is unlikely the main degradation of phthalate esters under natural conditions due to its comparatively slow rates (Schwartzenbach *et al.*, 1993). The rate of hydrolysis increases in the presence of soil or sediment as a result of a catalyzing effect of microorganisms in the soil or sediment (Furmidge and Osgerby, 1967; Burkhard and Guth, 1981).

1.2.5.2 Photodegradation

Photodegradation by ultraviolet (UV) radiation is another abiotic process. The mechanism of photolysis may be through direct absorption of UV radiation by natural substances such as water with the formation of activated species such as singlet oxygen or hydroxyl radicals that then react with phthalate esters or direct cleavage by UV radiation energy. Photodegradation of phthalate esters indirectly via free radicals is considered to be the most dominant degradation pathway in the atmosphere (Staples *et al.*, 1997b). Photo-oxidation half-lives in the atmosphere were estimated to be between 0.2-2 days for DEHP and 9.3 – 93 days for DMP (Staples *et al.*, 1997b). It was not evident in the study if the di-esters were completely mineralized or oxidized to various photodegradation intermediates.

1.2.5.3 Biodegradation

Biodegradation is the most critical process affecting the environmental fate of phthalate esters. Biodegradation in the natural environments is catalyzed by a diverse

group of microbes. Microorganisms can use these phthalate esters as a sole carbon source and energy and both aerobic and anaerobic pathways for degradation of phthalate esters exist. The general metabolic pathway for microbial biodegradation of phthalate is depicted in (Figure 5). The estimated half-lives of di-esters in natural waters and soils increase with increasing of alkyl side chain length: from 0.2-10 days for DMP, to 25 to 250 days (Staples *et al.*, 1997b) for DEHP. Half-lives in marine sediment range from 12 to 42 h for various monoesters (Otton *et al.*, In preparation).

1.3 Sorption of Organic Chemicals to Solids

Hydrophobic nature of di-esters may drive these chemicals to interact with sediment particles and the organic matter in the aquatic environment. This interaction affects the fate and distribution of di-esters and their metabolites in the environment. Adsorption, absorption and sorption are the terms used to describe the uptake of a solute from one phase by another phase. Adsorption describes the concentration of a solute at the interface of two phases, while absorption describes the process when a solute is transferred from the bulk state of one phase into the bulk phase of the other phase (Boethling and Mackay, 2000). In the sorption of an organic chemical to a sediment particle, the organic chemical is called the "sorbate" and the sediment particle which sorbs the organic chemical is called the "sorbent". The term sorption is used frequently in environmental situations to denote the uptake of a solute (chemical) by a solid (soil or sediment or component of soil or sediment) without reference to a specific mechanism, or when the mechanism is uncertain. The complex and heterogeneous nature of environmental solids makes it difficult, if not impossible, to identify specific sorption mechanisms for most solid-chemical combinations and in most situations, several

mechanisms like physical adsorption, hydrogen bonding, formation of coordination complexes, chemical adsorption may operate simultaneously (Site, 2001).

1.3.1 Water-sediment partitioning of organic chemicals

The distribution of an organic chemical between sediment and water is more than just an equilibrium partitioning, a physico-chemical property of the chemical, as it is also a result of enrichment of chemical concentrations in the sediment (Gobas and MacLean, 2003). Studies on environmental partitioning of organic pollutants helps in understanding the behaviour, mobility, distribution and bioavailability of chemical in the environment. The extent of sorption of an organic contaminants to sediment has a major influence on its transport and fate in the environment (Chiou *et al.*, 1998) and the risk of leaching and the extent of contamination of the chemical into the groundwater or to the surface waters (Schwartzenbach *et al.*, 1993; ter Laak *et al.*, 2006).

Water-sediment partitioning is often regarded as a partitioning process between the organic carbon content of the sediment and the water phase and is strongly dependent on both the amount (Chiou *et al.*, 1979) and the chemical nature of the organic matter (Grathwohl, 1990). Thus, sorption coefficients are often normalized to organic carbon content of the sediment to address the issue of difference in sorption between sediments. The composition and the structure of organic matter varies due to its origin and geological history and strongly influences the sorption affinity for organic compounds of organic matter (Grathwohl, 1990).

Partition coefficients alone are insufficient to describe sorption over large concentration ranges (Grathwohl, 1990). Linear isotherms are often observed for narrow

concentration ranges and low concentration levels, while non-linearity frequently appears over large concentration ranges. Such behaviour can be described by Freundlich isotherms or Langmuir isotherms.

1.3.2 Factors affecting sorption

Sorption of organic chemicals to soil or sediment is a complex process (Site, 2001), involving various processes between the sorbent and sorbate, which are influenced by the physio-chemical properties of the chemical. Sorption of polar or ionizable compounds depends on moisture content, the presence of exchangeable ions, electrolyte concentration, pH and water solubility.

When a non-ionic hydrophobic organic chemical is dissolved in water and the water is in direct contact with sediment, freely dissolved chemical will spontaneously diffuse from the water into the sorptive element(s) of sediment. The system will then establish an equilibrium where no net transfer of chemical between water and sediment phases occurs. Once equilibrium has been reached, the concentrations of the chemical in the two phases in contact are related through their partition coefficient, as stated by Nernst's Distribution Law (1891). Similarly, the sediment –water equilibrium partition coefficient, K_{SW} (mL/g or L/kg) is expressed as:

$$K_{SW} = \frac{C_S}{C_W} \qquad \textbf{Equation 2}$$

where C_S (µg/g) is the equilibrium concentration of the chemical in sediment and C_W (µg/mL) is the equilibrium concentration of the chemical in water. In abiotic

compartments like sediment and soil, hydrophobic chemicals are thought to partition primarily into the organic carbon fraction of these media, just as such chemicals in biotic compartments such as benthic invertebrates are associated predominantly with lipid. The "absorbing capacity" of sediment is thus closely related to the amount and quality of organic carbon (OC) contained in the sediment. For this reason, K_{SW} is usually expressed not in terms of bulk or dry weight sediment, but in terms of the weight of OC in the sediment. K_{SW} normalized for the fraction of OC in the sediment ($\phi_{OC,}$ in units of kg OC/kg sediment) gives the sediment OC-water partition coefficient (K_{OC}), which has units of L of water/kg of OC). K_{OC} is defined as:

$$K_{OC} = \frac{K_{SW}}{\phi_{OC}} \qquad \textbf{Equation 3}$$

1.3.2.1 pH effect

MPEs are known to be weak acids and partially dissociate or ionize in aqueous solution forming hydronium ions and the conjugate base. Thus, at any given pH, there is an equilibrium between ionic and non-ionic forms of MPEs and this is governed by acid dissociation constant, pKa. Henderson-Hasselbalch equation relates pH and pKa of a weak acid as described in section 1.2.1. Figure 6 depicts the various processes that are involved in the sorption of ionizable substances. A acidic compound can be considered almost ionized or completely dissociated when the pH is approximately 2 units above pKa (Site, 2001). An increase in sorption can be observed by decreasing the pH below the pKa assuming that only the non-ionized or neutral form participates in the sorption.

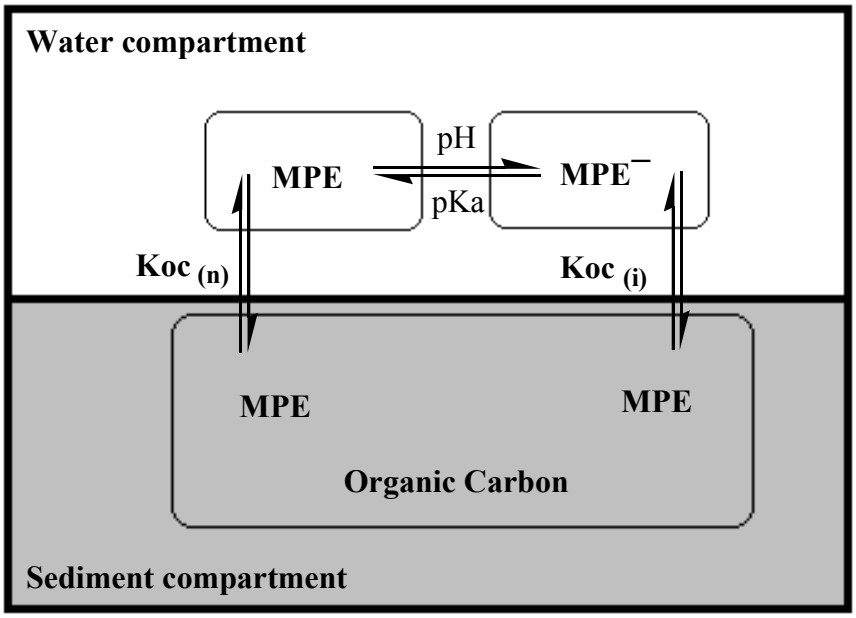


Figure 6: Graphic representation of effect of ionization on the partitioning of a chemical between sediment and water compartments. MPE is neutral form, MPE^- is the ionized form, $K_{OC\ (n)}$ and $K_{OC\ (i)}$ are the organic carbon partition coefficients for neutral and ionized forms of the MPEs respectively.

Thus for hydrophobic, ionizable organic compounds, like MPEs, the hydrophobicity alone may not be sufficient for estimating the sorption coefficients. Lee *et al.,* (1990) derived an equation that can account for sorption due to both ionic and neutral forms of the weak organic acids like MPEs.

$$K_{OC\ (t)} = K_{OC\ (n)} . \Phi_n + K_{OC\ (i)} . (1 - \Phi_n) \qquad \textbf{Equation 4}$$

where $K_{OC(t)}$ is the partition coefficient of both ionized and un-ionized form, $K_{OC(n)}$ is the partition coefficient of non-ionized form, $K_{OC(i)}$ is the partition coefficient of the ionized form and Φ_n is the fraction of non-ionized for of MPEs

The fraction of neutral MPEs in the solvent (water) can be represented as follows:

$$\Phi_n = \frac{[MPE\ ]}{([MPE\ ] + [MPE^-])}$$ **Equation 5**

where Φ_n is the fraction of non-ionized form of MPEs, where MPE represents the neutral form, MPE^- represents the ionized form.

Since pH = -log $[H^+]$ and pKa = -logKa, the above equation can be rearranged as follows:

$$\Phi_n = \left[1 + 10^{pH - pKa}\right]^{-1}$$ **Equation 6**

The sediment-water partitioning coefficient K_{SW} for the neutral form of MPEs can be written as follows:

$$K_{SW(n)} = \frac{[MPE\ ]_S}{[MPE\ ]_W}$$ **Equation 7**

and for the ionized form as follows:

$$K_{SW(i)} = \frac{[MPE^-]_S}{[MPE^-]_W}$$ **Equation 8**

Therefore, the total K_{SW} for neutral and ionized forms is:

$$K_{SW(t)} = \frac{[MPE]_S + [MPE^-]_S}{[MPE]_W + [MPE^-]_W}$$ **Equation 9**

where, $K_{SW(n)}$, $K_{SW(i)}$ and $K_{SW(t)}$ are the sorption coefficients of MPEs in neutral, ionized and both forms respectively, $[MPE]_S$ and $[MPE^-]_S$ are the concentrations of neutral and ionized forms of MPE in the sediment and $MPE]_W$ and $[MPE^-]_W$ are the concentrations of neutral and ionized forms of MPEs in water phase.

1.3.2.2 Organic carbon

In marine environments, microorganisms including phytoplankton and zooplankton are the most abundant source of the organic carbon in the sediment (Aiken *et al.*, 1985). Ultimately, biopolymers such as carbohydrates, lipids, proteins, lignins, tannins, pigments are degraded and condensed to form geopolymers such as fulvic acid, humic acid, humin, kerogen (Grathwohl, 1990). Natural waters contain various concentrations of high molecular weight organic substances like humic acid, fulvic acid, and humin, which are referred to as dissolved organic matter (DOM) or dissolved organic carbon (DOC) (Site, 2001) which may bind organic chemicals. It is known that DOC and or DOM enhance the water solubilities of relatively water insoluble organic solutes (Grathwohl, 1990).

1.3.2.3 Sediment mineralization / diagenesis / carbonation

The changes in the composition of the organic carbon during burial and weathering result in a decrease or increase of the relative amounts of oxygen-containing functional groups like carboxyl, hydroxyl and this change in the oxygen-containing

groups may change the binding properties of organic chemicals (Grathwohl, 1990). High amounts of oxygen-containing functional groups can result in an increase in the overall polarity of organic polymers composing natural organic matter and thus in a lower affinity for non-ionic compounds thereby decreasing the sorption capacity of the substance (Grathwohl, 1990). Organic matter is commonly characterized by the H/C and O/C atomic ratios. Similarly, the H/O atomic ratios can be used as an index of the degree of oxidation of the organic matter. A high H/O ratio indicates relatively low amounts of oxygen containing functional groups, corresponding to a low polarity and high hydrophobicity and therefore a high sorption affinity of non-ionic organic compounds by organic matter.

It has been observed that sediment-water distribution coefficients of organic chemicals are considerably larger than those estimated based on the octanol-water partition coefficient and solubility. Laboratory derived experimental values also differ from those obtained from field observations. Gobas and MacLean (2003) proposed an organic carbon mineralization model to explain the discrepancies. They speculated that there is a degree of disequilibrium between the sediment and water and this is linked to the organic carbon breakdown during sediment diagenesis. This disequilibrium may be due to the dead and decaying organic matter, which may carry their chemical load into the sediment. This phenomenon is possible when the rate of partition and equilibrium of chemical between the decaying organic matter and its surrounding water is slower than the rate of settling of decaying organic matter, especially during high productivity of phytoplankton.

1.3.3 Models predicting sorption coefficients

No measured data for soil or sediment adsorption coefficients of MPEs were found by Peterson and Parkerton (1999) therefore, they employed two models, ASTER (U.S.EPA, 1991) and EPIWIN (Meylan, 1999). ASTER (Assessment Tools for the Evaluation of Risk) uses a simple linear regression equation to estimate K_{OC} from K_{OW}. EPIWIN (Estimations Program Interface for Windows) uses a combined correlation with molecular connectivity index and with certain structural fragments.

1.4 Rationale for this Study

The fate of a chemical in the environment in terms of its mobility, distribution, concentration in biota, accumulation in the environmental compartments is very important in risk assessment of the effects of a chemical in the environment. At present very little information is available for the fate of MPEs in aquatic environments. Many characteristics of environmental behaviour can be predicted using environmental models such as those developed by Mackay (Mackay, 1979; Mackay and Paterson, 1981; Mackay, 1991). These models require a number of equilibrium parameters specific to the chemical of interest including water solubility, vapour pressure, Henry's Law constant, octanol-water partition coefficient (K_{OW}), sediment-water partition coefficient (K_{SW}), bioconcentration factor (BCF), bioaccumulation factor (BAF), etc. The persistence of a chemical in soil or sediment is estimated based on the half-lives in the respective matrices. Sorption coefficients can be used to predict and estimate the availability of chemical for degradation (OECD, 2000). Sorption is one of the most important physical interactions between chemical and sediment or soil as it influences the physical, chemical and biological interactions, thus its persistence, leaching and availability for degradation

(Furmidge and Osgerby, 1967). Without good quality data, it is extremely difficult to estimate environmental behaviour using these models and therefore, evaluate the risks of these chemicals in the environment. This research, which is part of a bigger project on environmental fate of monophthalate esters, attempts to measure the sorption behaviour of MPEs in marine and fresh water sediments.

1.5 Research Objectives

1. To determine the sorption kinetics of the nine different MPE congeners in sediment and overlaying waters in both the laboratory and field setting for both marine and fresh water sediments.
2. To compare the sediment water partitioning coefficients for the nine different MPE congeners.
3. To establish the influence of the sediment, organic carbon content, and low pH on the calculated K_{SW} coefficients.

2 MATERIALS AND METHODS

2.1 Field Sampling Methods

2.1.1 Study site and design

Sediment samples were collected at two different locations: False Creek Harbour, a marine water system, in downtown Vancouver, British Columbia, Canada (Figure 7), and Buntzen Lake, north of Port Moody, BC, Canada (Figure 7). False Creek Harbour is a residential / industrial embayment of the Straight of Georgia, where the mean summer temperature is approximately 11°C, average salinity is 30 ppt, and precipitation ranges from 90 to 200 cm/year. False Creek is shallow (i.e., mean depth is ~ 6m), and relatively well mixed. Within False Creek, two sampling stations were selected: "North-Central" (49°16'13"N 123°07'40"W) and "Marina-South" (49°16'09"N 123°07'15"W). From each station, ten independent samples of water and sediment were collected.

Buntzen Lake (Buntzen Lake Regional Park) is a fresh water system (49^{o}19'51"N 122^{o}51'37" W). Sediment samples were collected from the accessible points near the bridge (Figure 7).

Field concentrations of MPEs were determined only in the marine system (both water and sediment). Marine and fresh water sediments were used in the laboratory studies to determine sorption coefficients.

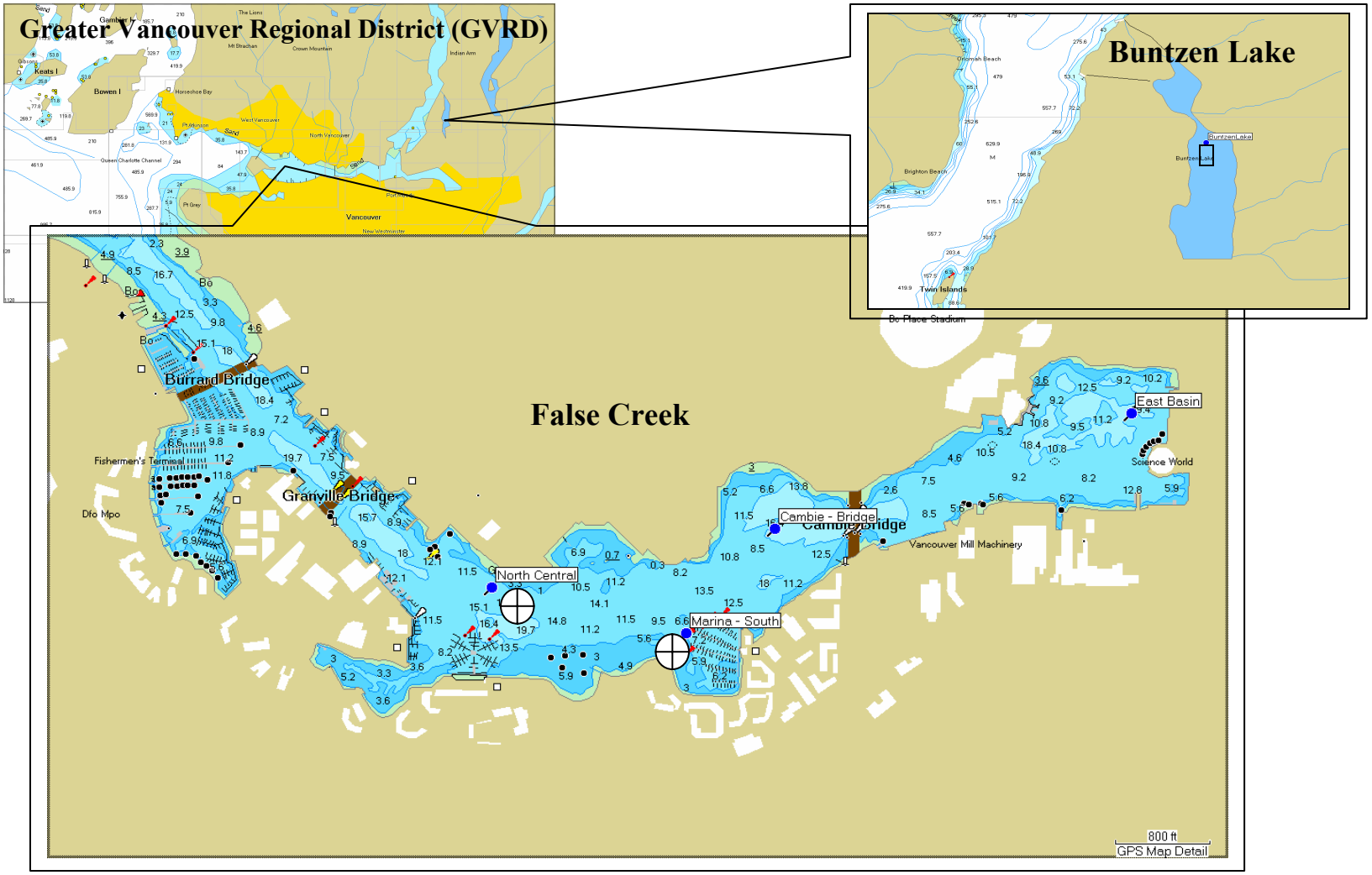


Figure 7: Map of field study sites used in this study: False Creek Harbour, Vancouver, British Columbia, showing locations of two sampling stations (⊕): "North Central", Marina – South". Buntzen lake, Port Moody, British Columbia, showing location of sampling station ( ).

2.1.2 Preparation of field sampling equipment

To ensure that measured concentrations of phthalate monoesters were a true reflection of environmental levels, care was taken to reduce contamination of samples by di-esters and monoesters from all possible sources. Several preparatory steps for cleaning field equipment were included in the protocol. All sampling equipment was made of glass or stainless steel. Glass jars used for storing sediment and water samples (125 mL, 250 mL, 4 L) were washed with lab grade detergent, rinsed twice with distilled hexane, iso-octane, and dichloromethane, and then heated in a muffler oven at 400°C for at least 10 hours. After baking, the jars were re-rinsed three times with distilled acetone, hexane, iso-octane, and dichloromethane, and then covered with solvent rinsed metal lids lined with cleaned aluminum foil. The aluminum foil was rinsed with distilled acetone and distilled hexane and then heated at 350°C for 10 hours. Stainless steel sampling tools (e.g., spoons, knives, trays, and buckets) were cleaned following the same procedure as for the glass jars, and were wrapped with aluminum foil prior to sampling. The Petit Ponar grab sampler was washed with lab-grade detergent and then rinsed three times with distilled acetone, hexane and dichloromethane.

2.1.3 Water sample collection

Water samples were collected in cleaned 4L amber glass bottles from mid-ocean depth (~3 - 3.5 m using a 4 m extendable stainless steel pole (Figure 8). Approximately 3 ml of formic acid was added to each sample to reduce the pH of the water to 2.5. 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.

2.1.4 Sediment sample collection

Superficial sediment samples were collected using a Petit Ponar grab sampler and transferred onto clean aluminum foil (Figure 9). The top 0.5 to 1.0 cm, representing the "active layer", was removed with a metal spoon and transferred into a pre-cleaned glass vial, which was covered with aluminum foil and sealed with a metal lid. Vials were immediately placed on ice and were then kept at – 20 °C in the dark prior to analysis.

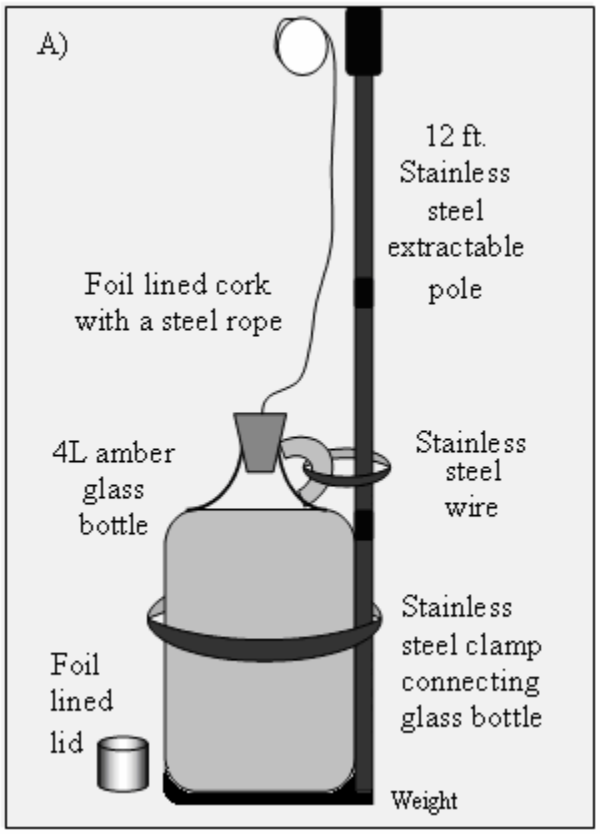


Figure 8: Field sampling equipment: Water Sampler.

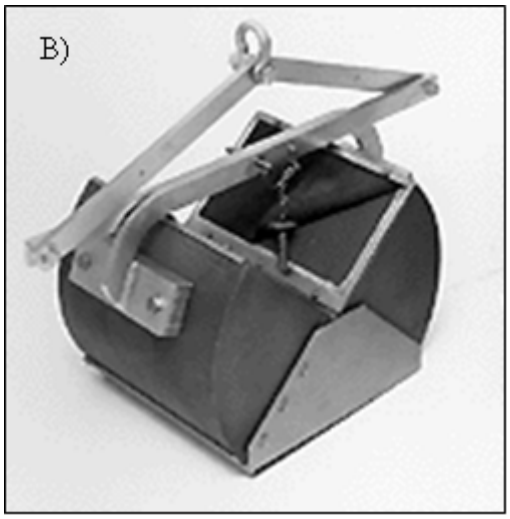


Figure 9: Field sampling equipment: Sediment Grab Sampler (Petit Ponar).

2.2 Materials and Methods (Laboratory Experiments)

2.2.1 Chemicals

2.2.1.1 Test chemicals

BASF Corporation, 4403 Laporte Road, Pasadena, TX 77501 prepared the monoesters of phthalic acid used for the Environmental Fate of Mono-Alkyl-Phthalate Esters research project at Simon Fraser University, Burnaby, BC, Canada and the Institute of Ocean Sciences, Dept. of Fisheries and Oceans, Sidney, BC, Canada. The structure, purity and state at room temperature of the nine mono-phthalate ester congeners used for the laboratory sorption experiments are shown in Table 3. The chemicals were used directly without any further purification process.

Table 3: List of MPEs, their abbreviation, R-group, purity, and physical appearance at room temperature used in the laboratory sorption experiments (Data provided by BASF Corporation).

MPE Congener / Abbreviation		R-group	Purity	Physical Appearance
Mono-ethyl phthalate	MEP	CH_2CH_3	93%	Pale orange liquid
Mono-butyl phthalate	MBuP	$(CH_2)_3CH_3$	98%	White solid (waxy)
Mono-benzyl phthalate	MBzP	Benzene ring	99%	Colorless liquid
Mono-hexyl phthalate	MC6	Mixture of isomers	95%	Colorless liquid
Mono-2-ethylhexyl phthalate	MEHP	$CH_2CH(C_2H_5)(CH_2)_3CH_3$	91%	Colorless liquid
Mono-n-octyl phthalate	MnOP	$CH_2(CH_2)_6CH_3$	97%	Colorless liquid (waxy)
Mono-heptyl) phthalate	MC7	Mixture of isomers	86%	Colorless liquid
Mono-nonyl phthalate	MC9	Mixture of isomers	97%	Pale orange liquid
Mono-decyl phthalate	MC10	Mixture of isomers	93%	Colorless liquid

2.2.1.2 Analytical reagents and solvents

All the chemical reagents and solvents used in the experiments are as follows with CAS no., chemical grade and vendor name in parenthesis. Hexanes (110-54-3, HPLC grade, Caledon Laboratory Inc., Ontario, Canada); Acetonitrile (75-05-8, distilled in glass, Caledon Laboratory Inc., Ontario, Canada); Toluene (108-88-3, Spectro-grade, Caledon Laboratory Inc., Ontario, Canada); Methanol (67-56-1, HR-GC grade, Merck KGaA, Germany); Trimethyl silyl diazomethane (TMSDM) (2.0M solution in hexanes,

Sigma-Aldrich Inc., USA); Ethyl acetate (141-78-6, distilled in glass, Caledon Laboratory Inc., Ontario, Canada); Mercuric chloride (Sigma-Aldrich Inc., USA).

2.2.2 Materials

2.2.2.1 Glassware

The water sampling apparatus (is described in section 2.1.3). 100 ml screw top glass vials with aluminum lined screw caps were used for sediment samples. 20 ml disposable glass scintillation vials with aluminum lined closures were from VWR International, Mississauga, Ontario, Canada. 2 ml amber silanized screw top glass vials with screw caps and teflon-lined septa were from Agilent Technologies, Mississauga, Ontario, Canada. 15 ml Pyrex glass centrifuge tubes for incubation and centrifugation and 5 ¾ inch disposable glass Pasteur pipets were from VWR International, Mississauga, Ontario, Canada. 5 µl, 10 µl, 25 µl, 50 µl, 100 µl, 1000 µl standard Pyrex glass syringes were from Agilent Technologies, Mississauga, Ontario, Canada. 5 ml, 10 ml, and 25 ml Pyrex glass pipettes were from VWR International, Mississauga, Ontario, Canada. 250 ml, 500 ml and 1000 ml volume Erlenmeyer flask were from VWR International, Mississauga, Ontario, Canada.

2.2.3 Methods

2.2.3.1 Glassware washing procedure

Glassware, aluminium foil and pipets were rinsed in acetone, toluene, hexane and DCM, then baked at a minimum of 350 °C for 6 to 8 h and again rinsed twice in iso-octane, once in hexane and DCM and twice in methanol. Once dry, glassware was covered with baked and solvent rinsed aluminium foil.

Mortars and pestles, spatulas and pipettes were cleaned similarly as glassware. They were dried completely under fume hood before baking for 10 h in oven (model 655F-iso-temp, Fisher Scientific) at 400 ^{o}C.

2.2.3.2 Preparation of stock solutions of MPEs:

All stock solutions were made in acetonitrile. Pure monoester was weighed using a weighing balance (model Mettler AJ50L, Mettler-Toledo AG, Switzerland) into 2 ml amber silanized glass vial and the volume adjusted to 2 ml with acetonitrile. All the monoesters stocks were prepared separately in individual vials. These stock solutions were used to prepare the working solutions which were dilutions of the stock in ACN in the appropriate spiking ranges. These working solutions were then used to spike the water phase that was in contact with the sediment during incubations. Stocks and stock dilution solution (in acetonitrile) were stored at 4 ^{o}C. The spiking was done each time at the start of the experiment.

2.2.3.3 Preparation of calibration standards

All the calibration standards were prepared using the stocks solutions. The calibration standards were matrix and method matched. To account for matrix (water or sediment) interferences in the final recovery of the monoesters, calibration standards were prepared in water matrix as standards for the water samples extracts, and in sediment matrix as standards for the sediment sample extracts.

2.2.3.4 Water phase

De-ionized water (Milli Q) was sterilized in an autoclave and stored at 4 ^{o}C until further use. The water phase was spiked with MPEs for sorption experiments as

described in the following section. For pH 4 experiments with MEHP, False Creek water was used. Approximately 2 mL of formic acid was added to 4 L of water to lower the pH to 4.

2.2.3.5 Preparation of working solution (water phase in contact with sediment)

The water phase was spiked with a single monoester (test chemical) at concentrations below water solubility for all the sorption experiments except MC7, MC9 and MC10. Sterilized water was used to prepare the working solutions. An appropriate amount of monoester stock (in acetonitrile) was transferred into 1000 ml Erlenmeyer flask and the final volume was made to 1000 ml using sterilized water. The volume of acetonitrile (organic solvent) added to spike the water was less than 0.1% v/v (OECD 106). The same spiked water was used for all the 15 ml glass (Pyrex) centrifuge tubes in the test to minimize variability between the tubes (instead of spiking individual centrifuge tubes with the test chemical). All the monoesters concentrations (test chemicals) were made to be below the reported water solubility of the respective chemicals.

2.2.3.6 Sediment phase:

2.2.3.6.1 Sediment processing

Sediment samples were thawed and air-dried at room temperature (20-22 ^{o}C) in a fume hood. Once completely dry, the sediment was gently homogenized using solvent-rinsed ceramic mortar and pestle to break any large sediment aggregates. Minimal force was used in an effort to ensure minimal changes in the original texture of the sediment. The sediment was then passed through a 2 mm sieve (mesh 60-80) to remove any larger particles and debris.

2.2.3.6.2 Sediment sterilization

The processed sediment was sterilized by autoclaving. To ensure complete sterilization, the sediment was autoclaved three times at 121 ^{o}C for 40 min alternating with 24 h room temperature (20-22 ^{o}C) incubation. Sterilized sediment was stored at -10 ^{o}C in solvent-rinsed glass containers until the start of the incubation process.

An alternate sediment sterilization method employed 30 µl of 1% mercuric chloride added to 2 g of processed sediment at the start of the incubation.

2.2.3.7 Characterization of sediment

2.2.3.7.1 Total organic carbon and nitrogen Analysis

Organic Carbon content analysis was carried out by Ms. Linda White at IOS according to Van Iperen and Helder (1985). Marine and fresh water sediment samples were oven dried at 50 ºC overnight to determine moisture content. The material was homogenized with a clean mortar and pestle to a fine textured mixture. Approximately 500 mg of sample was weighed into a clean pre-weighed crucible to determine the initial sample weight. Carbonates were removed by the addition of 10 ml of 1 N HCl, stirring gently until effervescence stopped. The samples were dried on a hot plate overnight at 70 ºC to evaporate water and excess HCl, and then oven dried another 2 h at 105 ºC. The samples were left open to room temperature and humidity for 2 h then re-weighed to determine the final weight. The decalcified samples were homogenised again using a clean glass rod and 3 to 10 mg sub-samples were weighed into clean tin cups for analysis in the Elemental Analyser.

The organic carbon was converted to CO_2 and the nitrogen oxides were reduced to N_2 gas. Both CO_2 gas and N_2 gas were measured by thermal conductivity on a Control Equipment Corporation (CEC) 440 Elemental Analyzer.

The organic carbon content (% w/w) for the initial sample (OC_i) was calculated from the organic carbon content of the acidified sample (OC_f) as follows:

$$OC_i = OC_f \times \frac{W_f}{W_i} \qquad \textbf{Equation 10}$$

where OC_i = Organic Carbon content of the original sample (non-acidified sample), OC_f = Organic Carbon content of the acidified sample, W_i = initial dry weight of the sediment, W_f = final dry weight of the sediment.

2.2.3.7.2 Determination of sediment moisture content

Moisture content of the processed sediment was determined. Approximately 1 g of sediment was weighed in a pre-cleaned aluminium weigh boat. Then the sediment was heated at 105 ^oC for 12 h, to attain a constant weight. After 12 h, the sediment was cooled in a dessicator over Drierite® absorbent to prevent absorption of moisture by the oven dried sediment during cooling. Once the sediment reached room temperature, it was weighed again. The moisture content of the sediment was calculated as follows:

$$MC\ \% = \frac{(W_f - W_i)}{W_i} \times 100 \qquad \textbf{Equation 11}$$

where MC % = Moisture Content of the sediment expressed in percentage, W_i = initial dry weight of the sediment, W_f = final dry weight of the sediment.

2.2.3.8 Measurement of culturable bacteria

To confirm sterilization of sediment by autoclaving, the sterilized sediment was assessed using Easicult® (Orion Diagnostica, Finland) culture technique. 1 g of sediment was weighed and diluted 1 g : 200 ml using sterilized deionized water. The Easicult dipslides were inoculated by dipping into the diluted sediment. The dipslides were sealed and incubated at 28 - 30 ^{o}C for 5 days. Growth of microorganisms was assessed visually.

2.2.3.9 Sediment / water ratio

Appropriate ratios of sediment to water for sorption studies depend on the distribution coefficient, K_d and the relative degree of adsorption. The sediment to water ratios used in these studies were based on pilot studies and 1:10 and 1:5 sediment to water ratios (w/v) were used to obtain more than 20 % sorption to solids and also not to limit proper mixing of solution in the test tube.

2.2.3.10 Incubation procedure

Each 15 ml glass (Pyrex) centrifuge tube received 1 g or 2 g (depending on the ratio of sediment to solution) of dried sterilized sediment. 10 ml of water spiked with a monoester was added to each tube at time zero. All the tubes were then wrapped in foil to avoid photolytic degradation of the monoester. The tubes were gently agitated on a rotating tilt-table at an inclination of 45^{o}, during the incubation at room temperature (22-25 ^{o}C). Periods of sediment:water / MPE contact were 4 h, 1 day, 3 days and 5 days. Experimental blanks were employed. Sediment blanks contained only sediment and water without any test substance. Reagent blanks during analysis were employed to

account for any contamination from the reagents. All time points / blanks were studied in triplicate samples.

2.2.3.11 Phase separation (by centrifugation)

At the end of each time interval, the incubations were stopped by centrifuging (model Centra CL-2, Thermo IEC, USA) at 1300g for 20 min. to separate the water and the sediment phases. Following centrifugation, the water phase was carefully decanted into clean 20 ml scintillation vials and stored at 4 ^{o}C until further analysis. The sediment pellet formed after centrifugation was retained in the centrifuge tube and stored at 4 ^{o}C until further analysis.

2.2.4 Analysis of MPEs (Extraction and Derivatization)

2.2.4.1 Water phase

The water phase was allowed to equilibrate to room temperature prior to analysis. 1 ml of water was transferred from the 10 ml water phase into a separate clean scintillation vial and 10 ml of acetonitrile was added. At this stage an internal standard was added to all the samples including the calibration standards. Mono-n-octyl phthalate ester (MnOP) was used as an internal standard for MEP, MBuP, MC6P and MEHP. MEHP was used as an internal standard for MnOP, MBzP. The mixture was vortexed for 10 seconds, sonicated (model 5510R-DTH Branson, USA) for 10 min,and dried in the fume hood. Once completely dry, it was re-suspended in 1.5 ml of ethyl acetate by vortexing and was transferred to 2 ml auto-sampler vial and evaporated to dryness at room temperature under N_2 gas. The residue was re-suspended again in 0.5 ml of ethylacetate.

2.2.4.2 Derivatization

Derivatization is the process of chemical modification of a compound to produce a new compound which has properties that are suitable for analysis using chromatographic methods, e.g., gas-chromatography (Blau and Halket, 1993). The modification of polar groups such as carboxylic acids, alcohols and amines with suitable reagents to produce non-polar derivatives, which are more stable and easier to separate and detect by gas chromatography. The hydroxyl group of the MPEs needs to be methylated (alkylated) to suit the chromatographic requirements. This can be achieved by a suitable derivatizing reagent. A suitable reagent should allow ease of derivatization reaction, generate quantitative reaction and reproducible yields, have no side reactions, and generate a stable product which has a higher volatility and which can be detected easily and in low concentrations (Blau and Halket, 1993). Trimethylsilyldiazomethane (TMSDM), a derivatization reagent is used to methylate the monoesters (Niino *et al.*, 2002). The following scheme shows the formation of methylated monoesters with TMSDM (Figure 10).

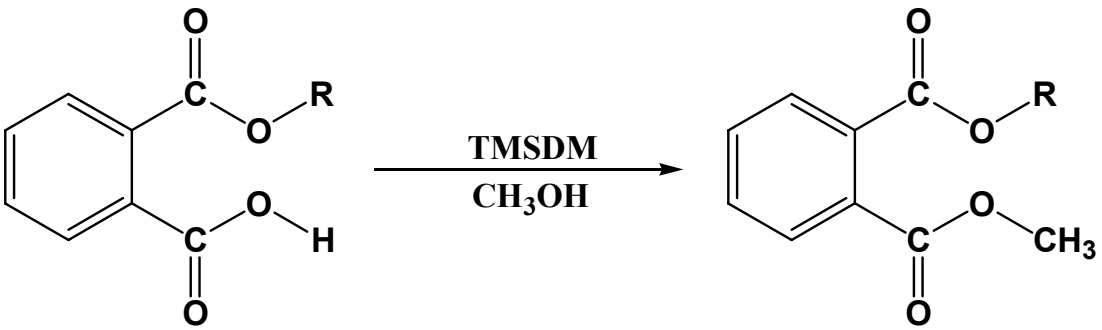


Figure 10: Methylation of monophthalate esters.

The derivatization process was carried out by adding 100 µl of MeOH and 25 µl of derivatization reagent, Trimethylsilyldiazomethane (TMSDM) (Niino *et al.*, 2002) to

the suspension and vortexed for 10 sec. The vials were gently rotated on a rotating table at room temperature for 30 min and then concentrated under N_2 gas. The residue was dissolved in 1 ml toluene and 1 μl of this solution was injected onto the GC column.

For MC7, MC9 and MC10, the water concentrations were obtained by an indirect method in which only one phase was analyzed and the other concentration was obtained by subtraction from the total spiked amount. In the present research, for the above mentioned MPEs, water concentration values were derived indirectly using sediment concentration data. Low concentrations of MPEs in water phase made it difficult to analyse and obtain the concentrations from water phase.

2.2.4.3 Sediment phase

The pelleted sediment samples stored at 4 ^{o}C were thawed to room temperature prior to analysis. Entire 2 g sediment pellets were resuspended in 10 ml of acetonitrile by vortexing. The mixture was sonicated for 15 min to extract MPEs into the acetonitrile and then centrifuged at 1300g for 10 min. The supernatant was decanted into a clean 20 ml scintillation vial and an internal standard (see section 2.2.4.1) was added to all the samples, including the calibration standards. From this stage onwards, the sediment phase samples were processed as described above (see section 2.2.4.1) for the water samples.

2.2.5 Quantitation of MPEs by gas chromatography mass spectrophotometry (GCMS)

2.2.5.1 Instrumentation

An Agilent GCMS (6890 GC connected to a 5973 series Mass Selective Detector fitted with 7683 series autosampler, connected to Enhanced Chemstation G1701CA version C.00.00 21-dec-1999 Agilent Technologies copyright 1989-1999) with a cool on-column inlet was fitted with an HP-5MS capillary column (30 m x 0.25 mm I.D., film thickness 0.25 μm) and a fused-silica deactivated guard column (5 m x 0.530 mm I.D.) was used for the analysis. Helium flow was 1.0 ml/min. The GC oven temperature was programmed (Figure 11) at 70 ºC for 4 min, rising at 30 ºC/min to 160 ºC, then 15 ºC/min to 320 ºC for 2 min and the injection port temperature tracked the oven temperature. The Mass Spectrophotometer conditions for Electron Impact (EI) ionization of MPE derivatives were as follows: ion energy, 70 eV; ion source temperature, 230 ºC; selected ions, m/z 91 (for MBzP) and 163 (for all other MPE derivatives). A typical chromatogram showing peaks for MEP, MBuP, MEHP, MnOP, and MBzP are shown in Figure 12. MPEs were quantified using calibration curves constructed by spiking autoclaved sediment and water with known amounts of MPE, adding the internal standard, and taking the sample through the extraction and derivatization procedure. Calibration curves were linear ($r^2 = 0.92 – 0.99$) for all MPEs. A typical calibration curve for MEHP is shown in Figure 13.

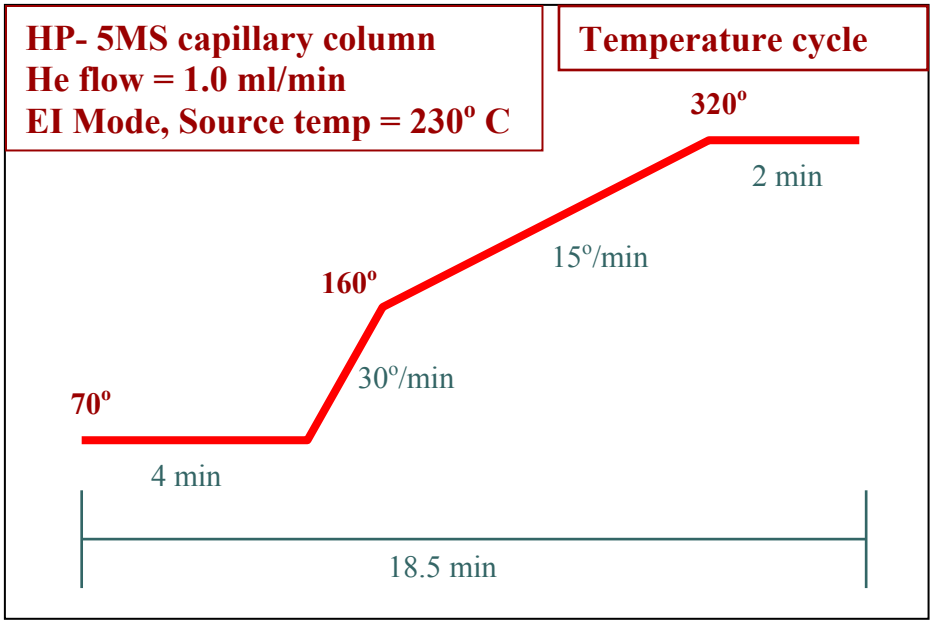


Figure 11: Temperature cycle program in GC for MPE derivative analysis.

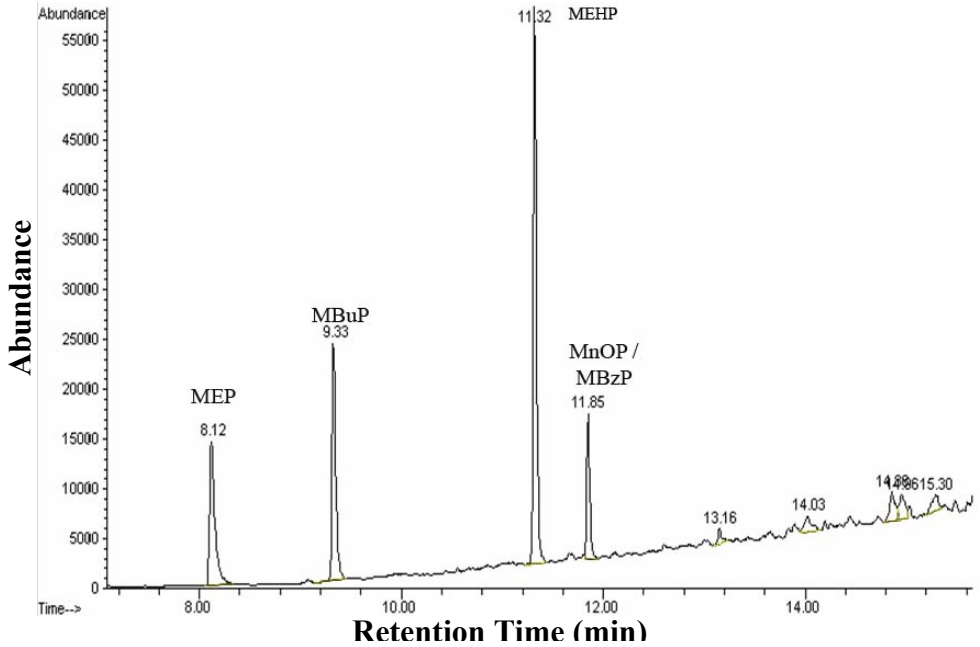


Figure 12: Chromatogram showing peaks, retention time, and abundance for MEP, MBuP, MEHP, MnOP and MBzP.

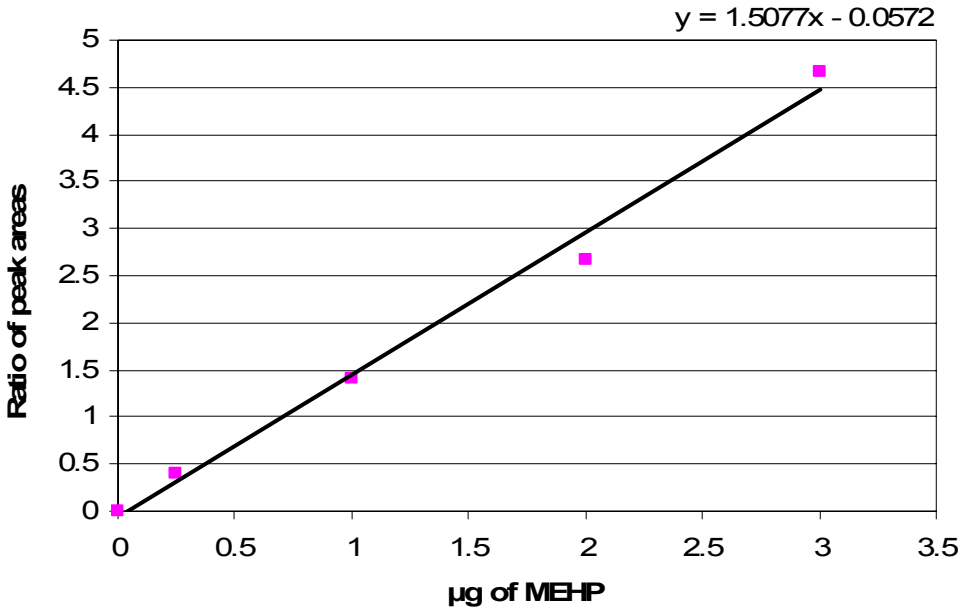


Figure 13: Calibration curve for MEHP with MnOP as internal standard. Solid line represents regression line (y=1.5077x - 0.0572). R^2 = 0.99.

2.2.5.2 Data analysis and statistics

Quantitation was achieved by measuring the relative response factor (RRF) for each analyte. The RRF relates the peak areas for two compounds, "i" and "j" (e.g., the test analyte and the internal standard).

$$RRF_{(i/j)} = \frac{Peak\ \ Area_{(i)}}{Peak\ \ Area_{(j)}} \qquad \textbf{Equation 12}$$

where $RRF_{(i/j)}$ is the relative response factor, Peak $Area_{(i)}$ and Peak $Area_{(j)}$ are the peak areas for the test chemical and internal standard, respectively, obtained from the GC chromatograms.

The RRFs calculated from the calibration standards were plotted against concentrations to obtain calibration curves. The regression equation obtained from these calibrations curves were used to obtain concentrations of samples.

MPEs C6, C7, C9 and C10 are mixtures of isomers, thus resulting in a group of peaks of varying abundance. To simply quantification, the most abundant peak in each congener was chosen as the representative peak.

Chemical mass balance for each experiment was quantified comparing the total concentration of MPE in water and sediment to the amount spiked initially to account for losses of chemical. The Mass balance was quantified as follows:

$$Mass\ \ balance\ \ (\%) = \frac{(W + S) * 100}{(MPE_{(t=0)})} \qquad \textbf{Equation 13}$$

where W is the total mass of MPE in the water phase, S is the total mass of MPE in the sediment phase and $MPE_{(t=0)}$ is the amount of MPE spiked into water at the start of incubation.

The sediment and water concentrations are expressed as the mean concentration along with two standard deviations. Sediment water partition coefficient (K_{SW}) and organic carbon normalized partition coefficient (K_{OC}) were calculated using the sediment (C_S) and water (C_W) concentrations at equilibrium.

Statistical analysis software, SAS version 9.1 was used to perform non-linear regression tests on MC7, MC9 and MC10 data sets. Non-linear regression equations are described in section 3.4.2.

2.2.6 Quality assurance and control of data (QA/QC)

Blanks (both water and sediment) were run for each chemical and samples concentrations were corrected for background contamination, if any.

2.3 Methods for Environmental Concentrations of MPEs in Field Samples

For MPEs concentration in the field (False Creek), the sediment and water samples were collected (as described in sections 2.1.3 and 2.1.4) and analyzed by Joel Blair, IOS, Sidney, B.C., Canada, following the methodology described in Blair *et al.,* (In preparation).

3 RESULTS AND DISCUSSION

3.1 Total Organic Carbon (TOC) Content

The TOC content of the False Creek sediment (unautoclaved) was found to be 2.89 ± 0.43 % (n=12, 2sd), while the processed sediment (autoclaved) was found to be 2.92 ± 0.56 % (n=3, 2sd), comparable to False Creek sediment collected by Mackintosh *et al*., (2003), 2.80 ± 0.31 %. The Buntzen Lake sediment had a higher TOC of 10.81 ± 2.38 % (n=3, 2sd) compared to the False Creek sediment.

3.2 Moisture Content (MC)

The moisture content of the processed marine sediment was found to be 5.48 ± 0.4 % (n=3, 2sd) while the processed fresh water sediment had moisture content of 4.06 ± 0.6 % (n=3, 2sd).

3.3 Culturable Micro-organisms from Autoclaved Sediment

The Easicult plates incubated with the sterilized sediment showed no growth of microbial colonies indicating that treated sediments were sterile (Figure 14).

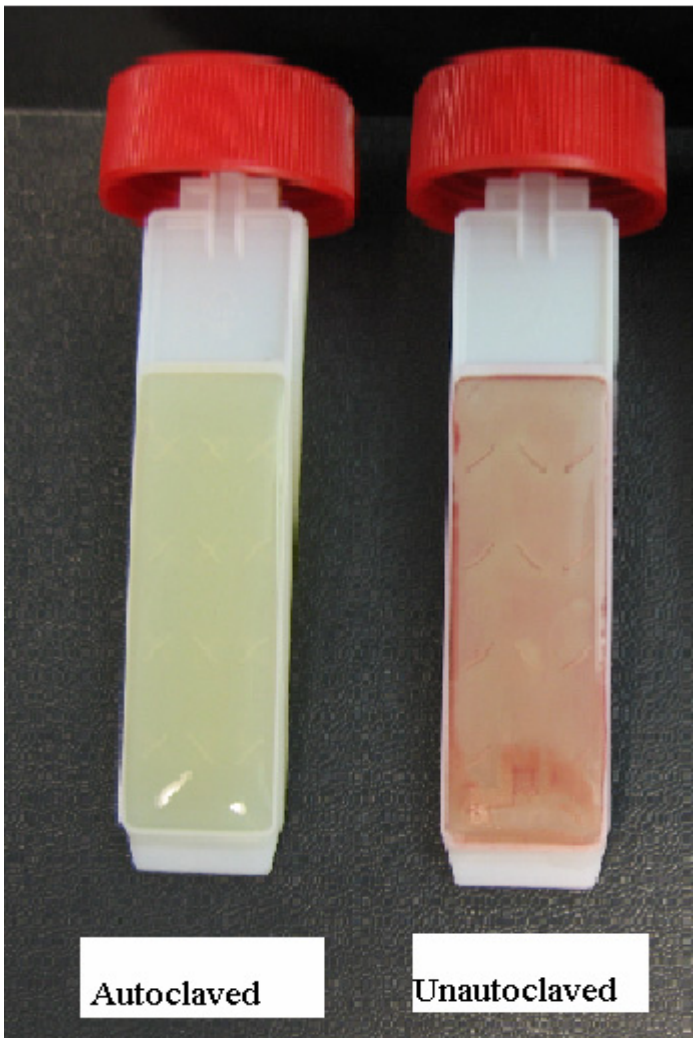


Figure 14: Easicult plates (autoclaved (left) and unautoclaved (right) sediment inoculations).

3.4 Sorption

3.4.1 Effect of sterilization technique on sorption coefficients – autoclaving vs chemical treatment ($HgCl_2$)

Similar sorption coefficients (K_{OC}) for MEHP were obtained from autoclaved sediment and chemically treated sediment ($HgCl_2$): 52 ± 8 (t=5day, n=3, 2sd) and 65 ± 9 (t=5day, n=3, 2d), respectively (Figure 15). Log K_{OC} values were 1.72 and 1.81 from autoclaved sediment and chemically treated sediment, respectively. There was no statistically significant difference (p=0.35 at 95%, t-test) between the sorption

coefficients of monoesters in sediment sterilized by autoclaving vs. mercuric chloride. Lotrario *et al*., (1995) investigated the effects of sterilization methods (autoclaving, gamma radiation) on soil and its implications on sorption studies. As with the present study, the authors found that there was no significant difference in the sorption coefficients obtained by either method. They also investigated the effect of autoclaving on the organic carbon content and found that the organic carbon content showed little to no change in its composition as a result of sterilization by autoclaving. A potential disadvantage of the chemical treatment is competitive sorption between the biological inhibitor (mercuric chloride) and the contaminant (MPEs). Another potential environmental concern is the formation of toxic methyl mercury upon usage and disposal. Therefore, we performed all subsequent sorption studies with autoclaved sediment.

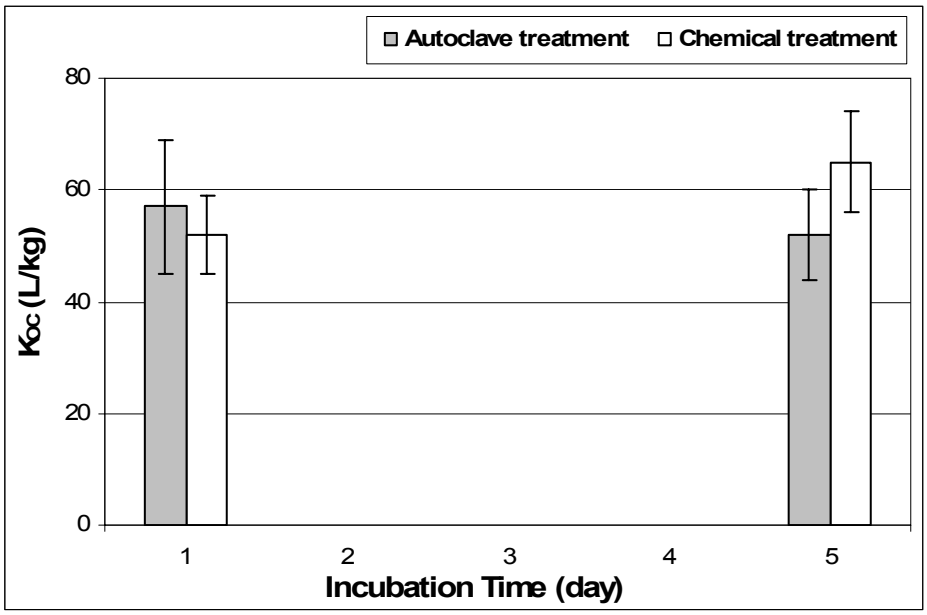


Figure 15: A comparison comparison of MEHP sorption coefficients (K_{OC}) on marine sediments sterilized by two methods – autoclaving and chemical treatment (mercuric chloride treatment). For K_{OC} values n=3 and error bars are 2 standard deviations. No statistical difference (p=0.35 at 95%, t-test) between K_{OC} values.

3.4.2 Sorption kinetics measured in laboratory studies

The concentration time plots for MEP, MBuP, MBzP, MC6, MEHP and MnOP (Figure 16 to Figure 24) showed that the uptake of MPEs from water phase into marine sediment phase was rapid. The sediment and water concentrations reached maximum values with in 24 hours. After reaching equilibrium, the dissolved and sorbed concentrations remained relatively constant (p=0.25 for MC6 to p=0.77 for MEHP, one-way ANOVA, α=0.05) until the end of the incubation period. Though equilibrium was reached within 24 hours, longer periods (2-6 days) of incubation were adopted to assure complete sorption of MPEs onto marine sediments. The equilibrium concentrations of sediment and water are shown in Table 4.

A similar rapid uptake of MPEs into fresh water sediment was observed (Figure 25 to Figure 30). The concentrations of MPEs in sediment remained relatively constant (p=0.27 for MBzP to p=0.55 for MEHP, one-way ANOVA, α=0.05).

Three congeners, MC7, MC9 and MC10 appeared to be reaching equilibrium towards the end of the 5 day incubation period both in marine and fresh water sediments (Figures 19, 20, 21, 31, 32 and 33). However, a little longer incubation period (i.e., 6 to 9 days) would have been preferable to ensure that equilibrium was achieved. Non-linear regression analysis (equation 12) was used to analyze data obtained from sorption experiments of both marine and fresh water sediments on MC7, MC9 and MC10. Rate constants (Table 6 and Table 7) were determined and partitioning at time infinity were calculated. However, the 5 day results also provide a very close approximation of the sorption coefficient.

$$K_{SW} = \frac{k_{ws}}{k_{sw}}\left[1 - e^{-k_{sw}t}\right] \qquad \textbf{Equation 14}$$

$$K_{SW}\left(t = \infty\right) = \frac{k_{ws}}{k_{sw}} \qquad \textbf{Equation 15}$$

The lower K_{OW} contaminants should reach equilibrium quickly in the laboratory while higher K_{OW} contaminants take longer periods (Gobas and MacLean, 2003). All the nine MPE congeners tested (except MC7, MC9 and MC10) reached equilibrium quickly within 24 hours.

3.4.3 Experimentally determined sorption coefficients of MPEs

The equilibrium water and sediment concentrations and sorption coefficients for various MPEs in both marine and fresh water sediments are presented in Tables 4 – 7.

The sediment water partition coefficients (K_{SW}) were normalized to organic carbon for both the marine and fresh water sediments.

Chemical mass balances for all the chemicals were calculated for both marine sediment (Table 4) and fresh water (Table 5). The mass balance values presented as average with 2 standard deviations represent the mass balance for each MPE during the period of incubation. It was found that the total spiked amount of MPE was mostly recovered from the water and sediment phases. For MC7, MC9 and MC10 congeners, both in marine and fresh water sediments, the mass balances were reported as 100 % as the water concentrations for these congeners were obtained by indirect method as described in the earlier section.

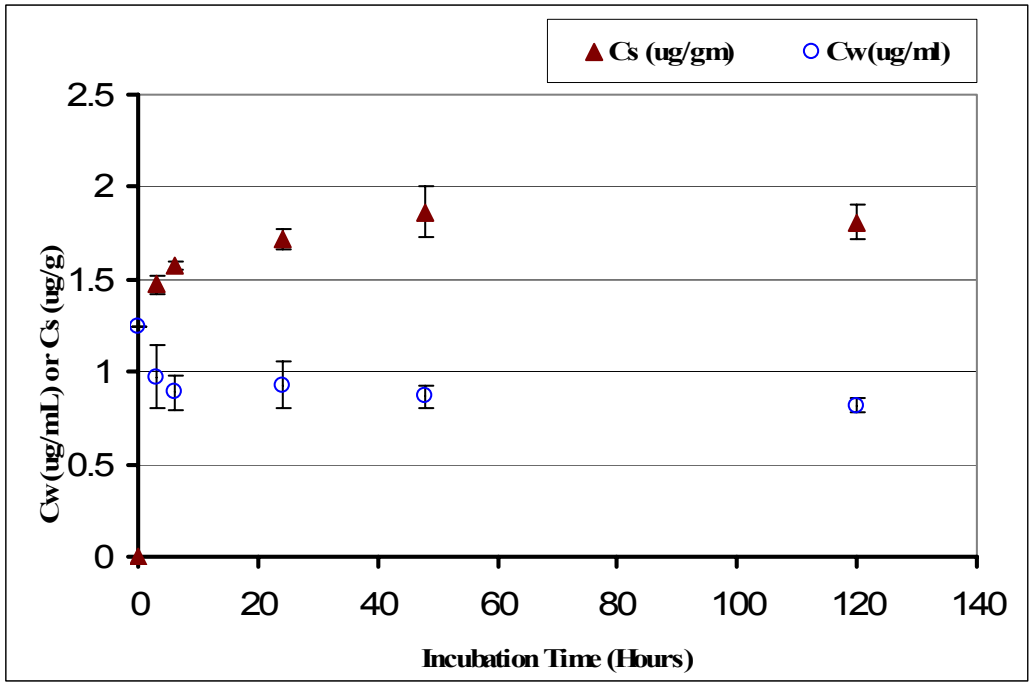


Figure 16: Sorption curve for MEHP on marine water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached an equilibrium within 24 h and there is no significant difference (p=0.33 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

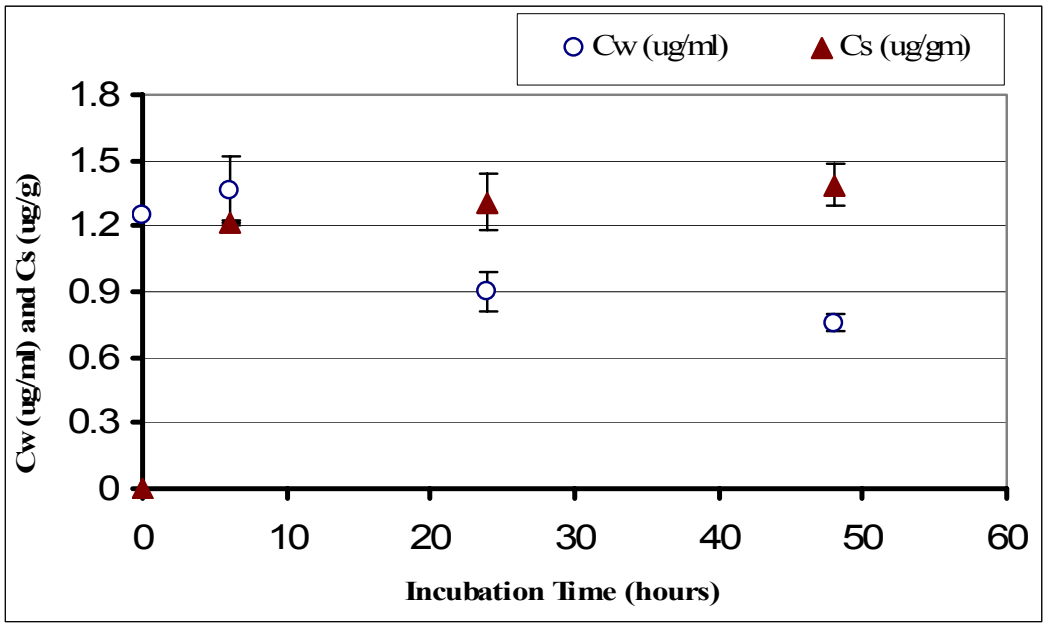


Figure 17: Sorption curve for MBuP on marine water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.25 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

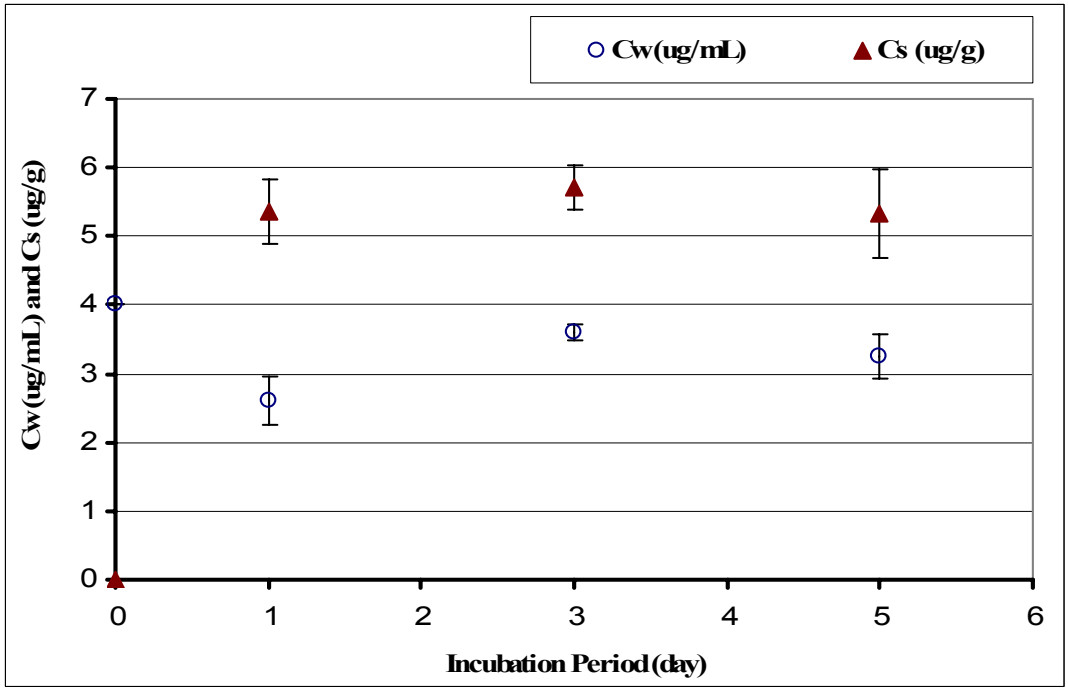


Figure 18: Sorption curve for MC6 on marine water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.20 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

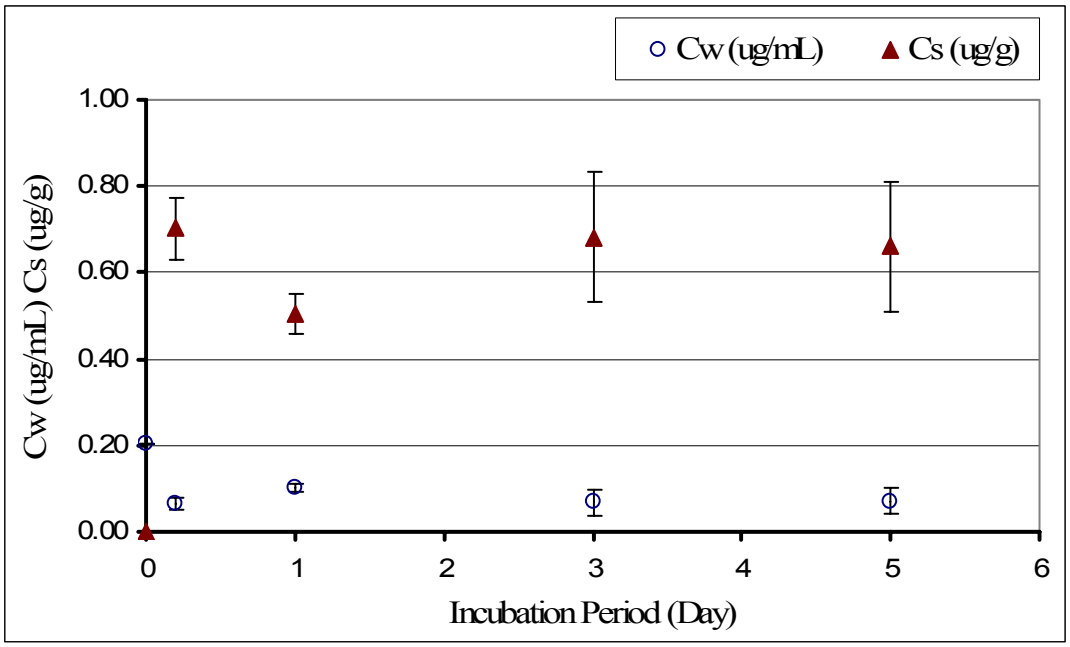


Figure 19: Sorption curve for MC7 on marine water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment uptake rate constant (k_{SW}) and water elimination rate constant (k_{WS}) are estimated to be 6.05 and 0.45 respectively (non-linear regression using equation 14).

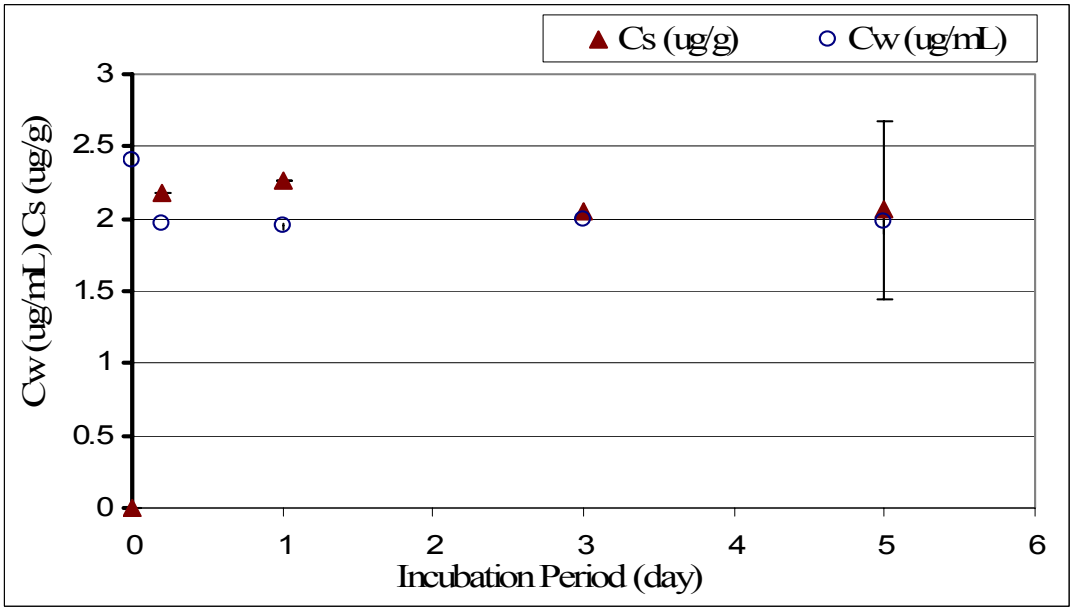


Figure 20: Sorption curve for MC9 on marine water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment uptake rate constant (k_{SW}) and water elimination rate constant (k_{WS}) are estimated to be 19.2 and 17.1 respectively (non-linear regression using equation 14).

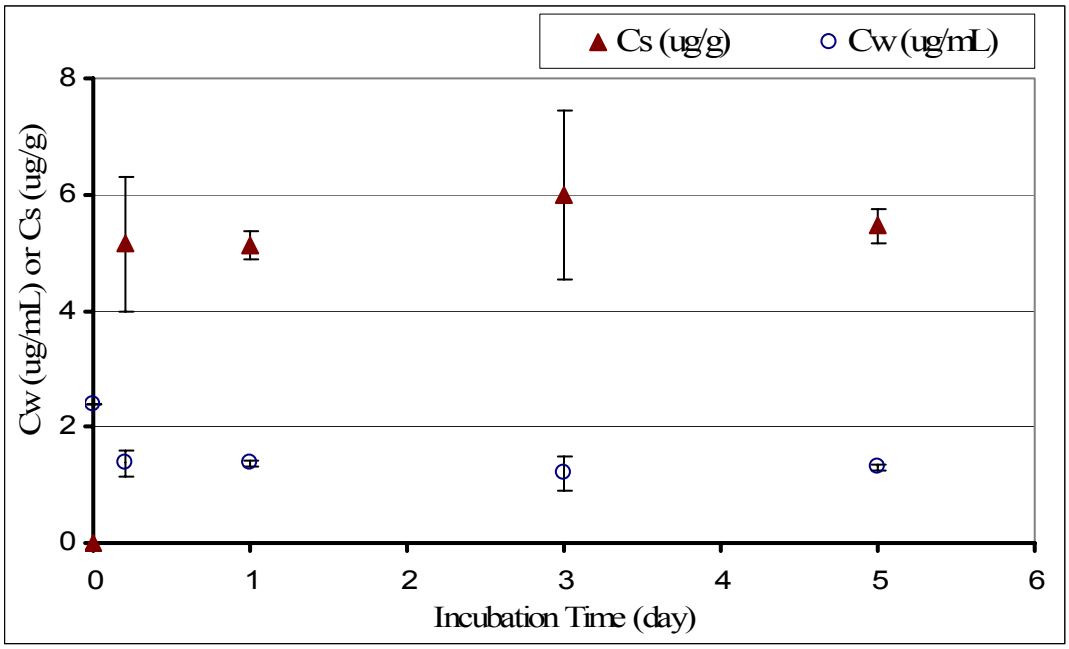


Figure 21: Sorption curve for MC10 on marine water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment uptake rate constant (k_{SW}) and water elimination rate constant (k_{WS}) are estimated to be 86.6 and 18.6 respectively (non-linear regression using equation 14).

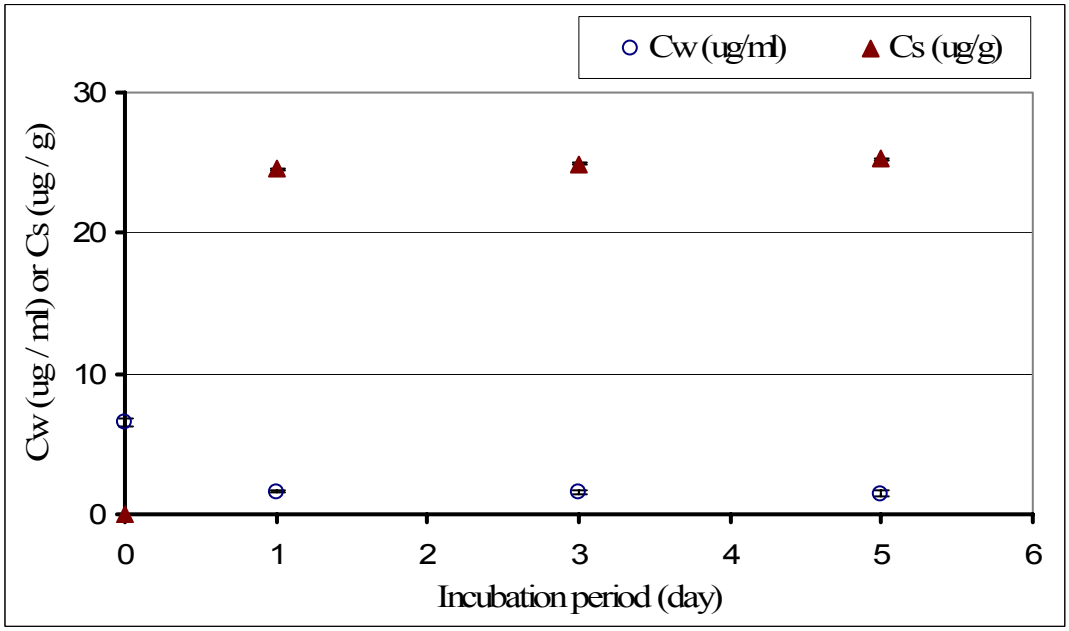


Figure 22: Sorption curve for MEP on marine water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.57 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

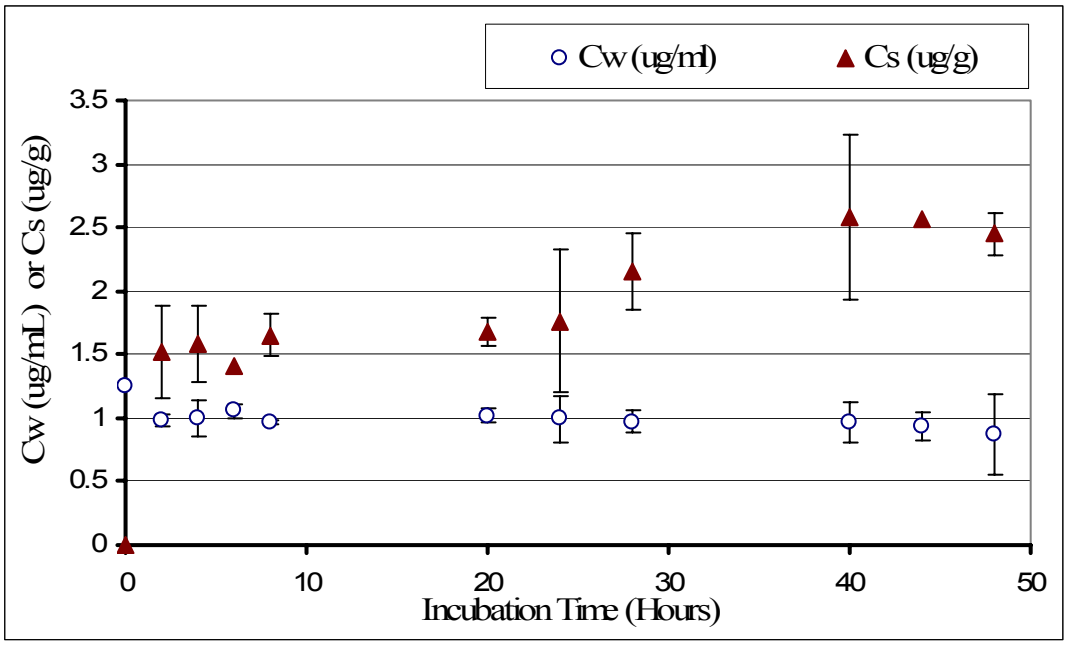


Figure 23: Sorption curve for MnOP on marine water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.38 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

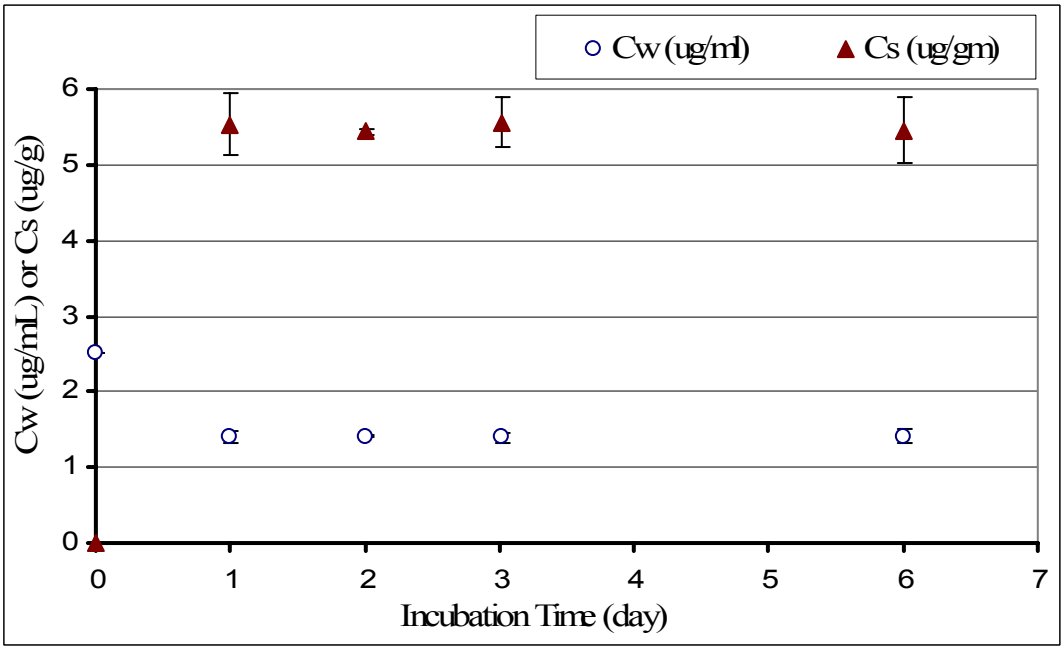


Figure 24: Sorption curve for MBzP on marine water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.76 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

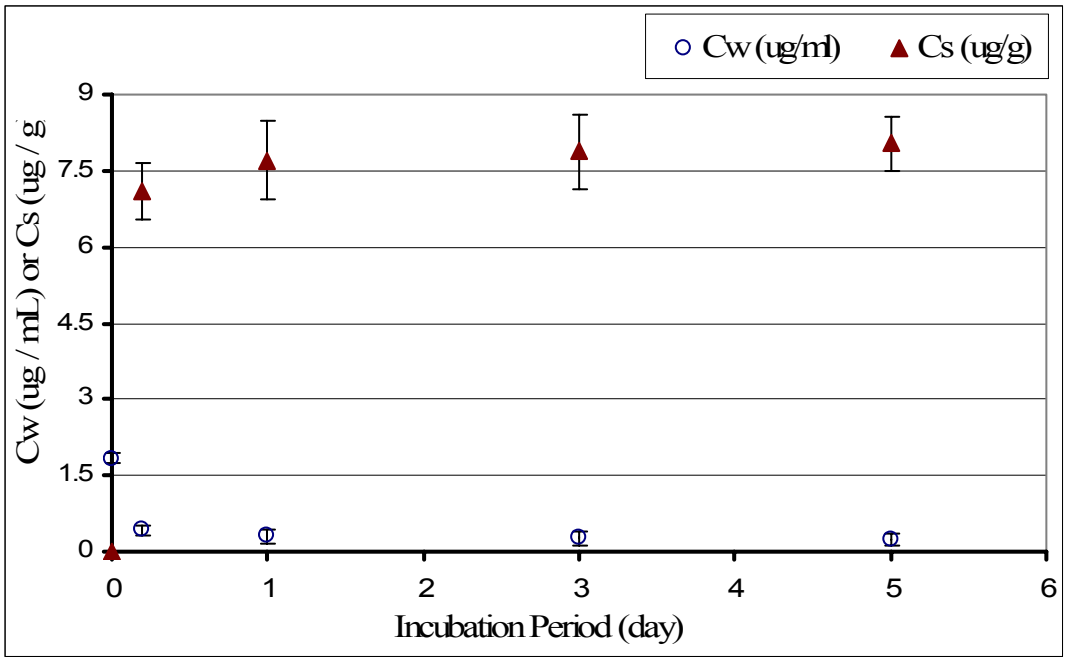


Figure 25: Sorption curve for MEHP on fresh water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.55 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

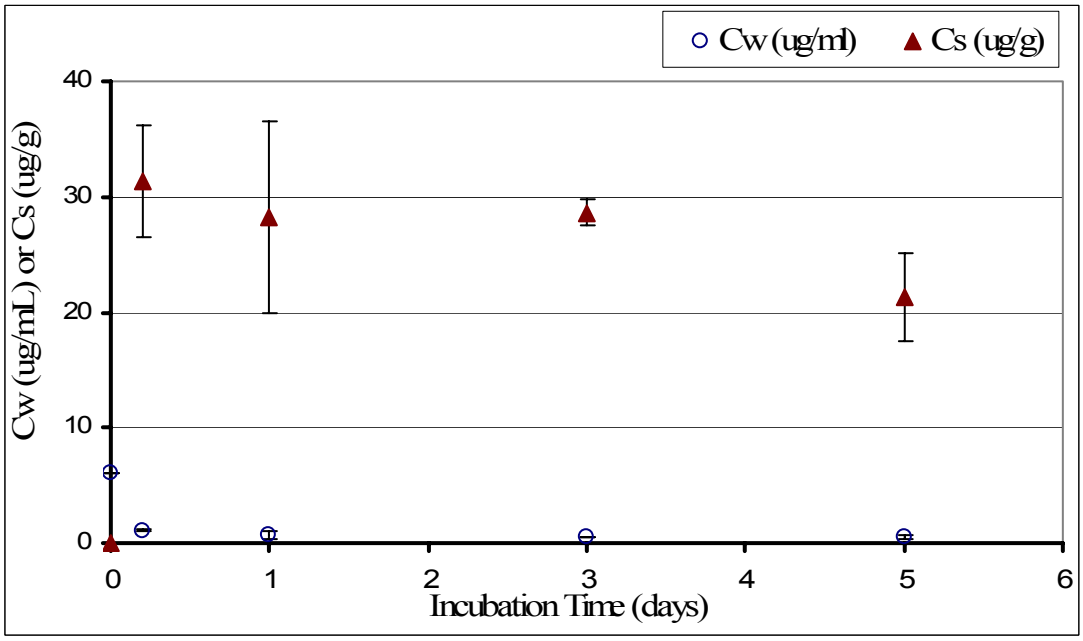


Figure 26: Sorption curve for MEP on fresh water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.35 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

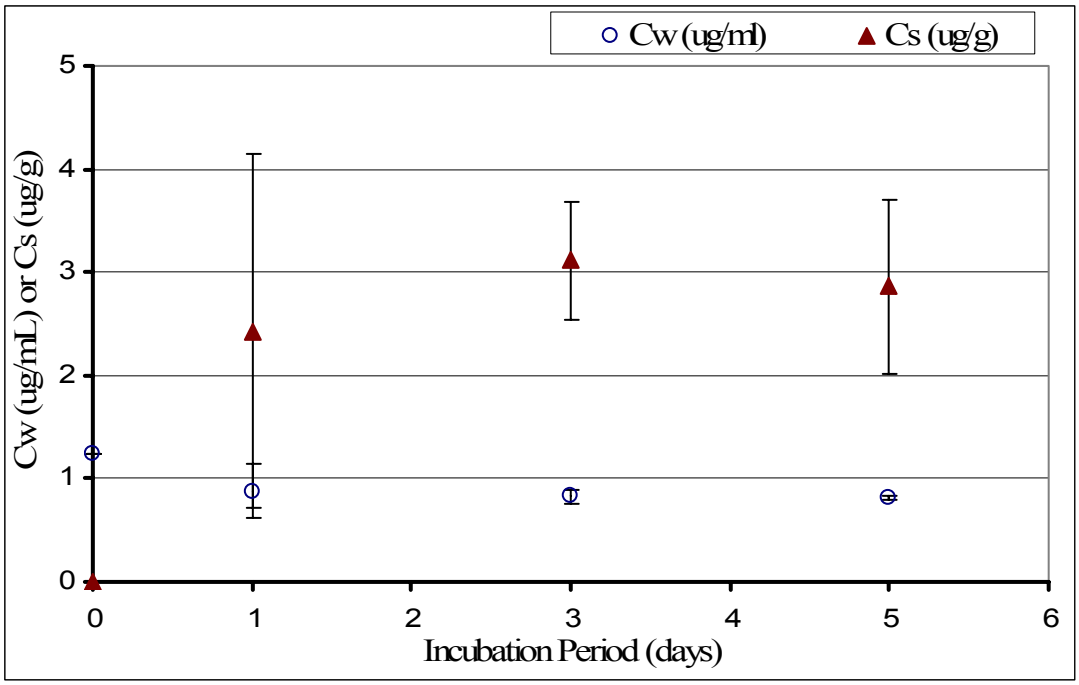


Figure 27: Sorption curve for MBuP on fresh water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.40 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

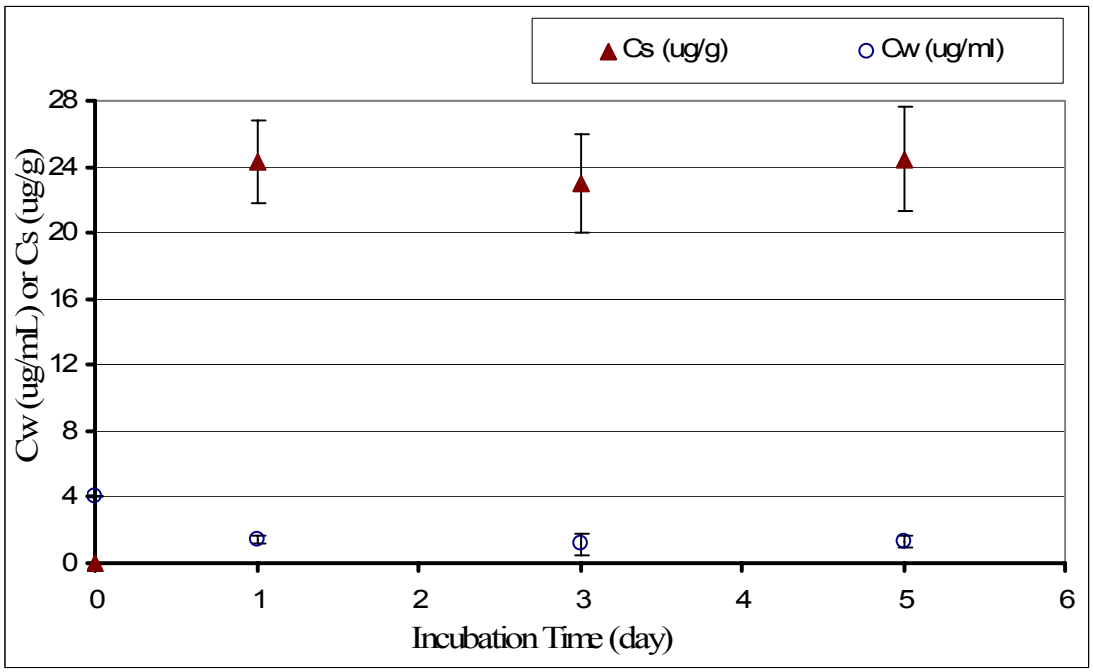


Figure 28: Sorption curve for MC6 on fresh water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.45 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

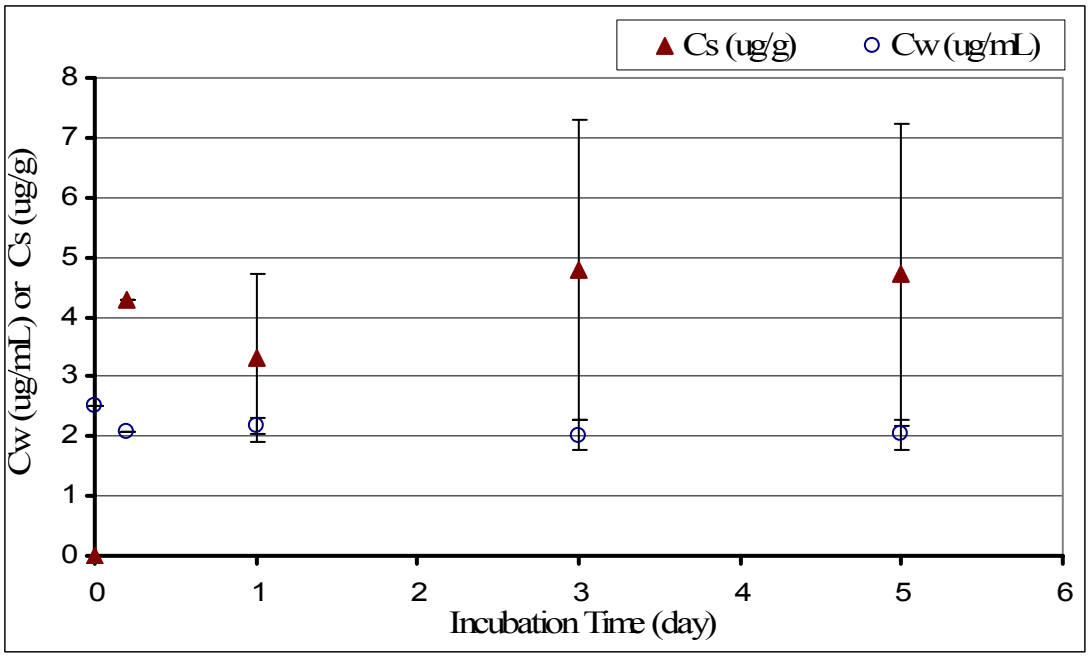


Figure 29: Sorption curve for MBzP on fresh water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.27 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

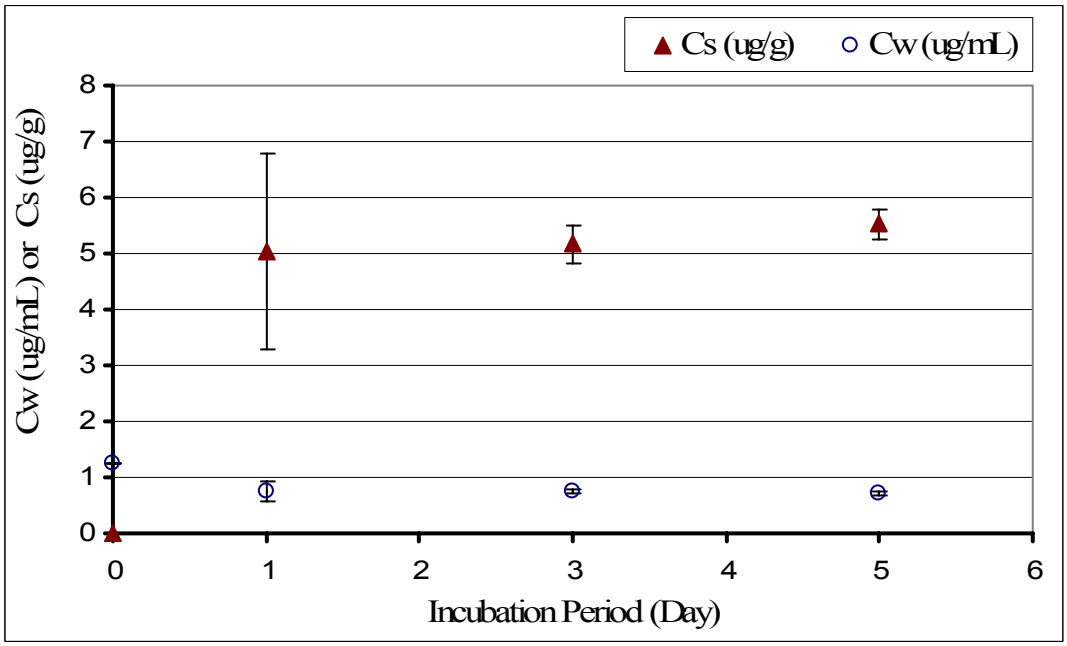


Figure 30: Sorption curve for MnOP on fresh water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment reached equilibrium within 24 h and there is no significant difference (p=0.45 at 95%, one-way ANOVA) in sediment concentration from day 1 to till the end of the incubation period.

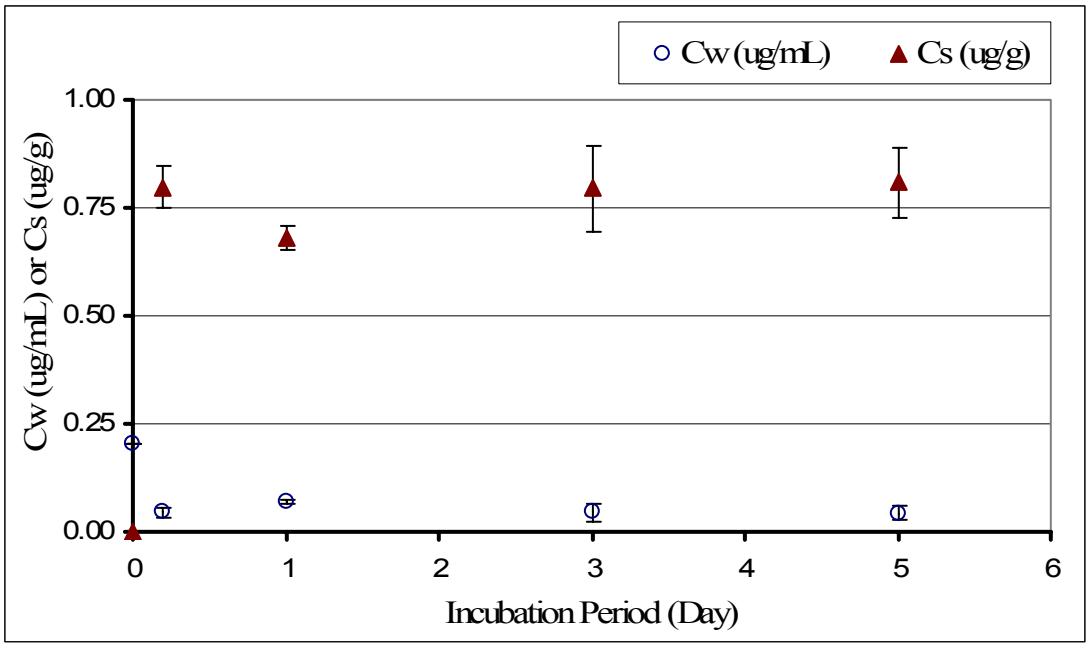


Figure 31: Sorption curve for MC7 on fresh water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment uptake rate constant (k_{SW}) and water elimination rate constant (k_{WS}) are estimated to be 20.9 and 1.0 respectively (non-linear regression using equation 14).

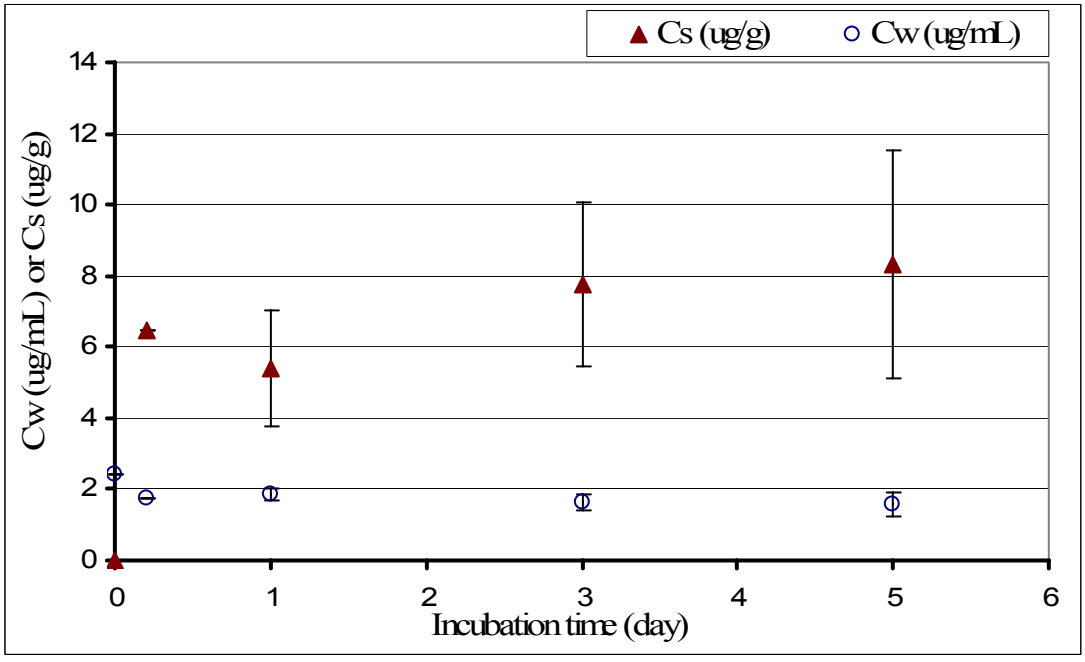


Figure 32: Sorption curve for MC9 on fresh water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment uptake rate constant (k_{SW}) and water elimination rate constant (k_{WS}) are estimated to be 5.98 and 1.39 respectively (non-linear regression using equation 14).

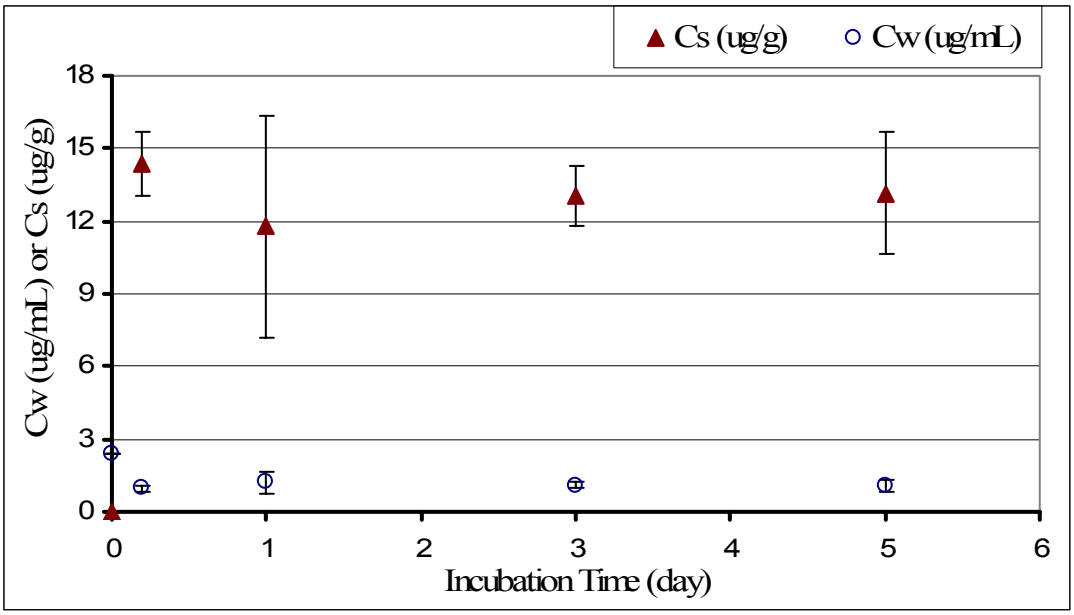


Figure 33: Sorption curve for MC10 on fresh water sediment. Water and sediment concentrations for each time point are mean of 3 values (n=3), error bars are 2 standard deviations. Sediment uptake rate constant (k_{SW}) and water elimination rate constant (k_{WS}) are estimated to be 17.8 and 1.47 respectively (non-linear regression using equation 14).

Table 4: Marine water sediment (False Creek): Sediment sorption results showing water and sediment concentrations at equilibrium, sediment water ratio, organic carbon content, pH of the sediment-water system during incubations, p-values indicating that sediment reached equilibrium concentration within 24h and there is no significant change from day 1 till the end of the incubation period (one-way ANOVA), partition coefficients (K_{SW} and K_{OC}), mass of chemical spiked into water at t=0, amount of chemical sorbed to sediment and chemical mass balance of sorption experiments for various MPE congeners. ([1] equilibrium concentrations at t=2 day; [2] equilibrium concentrations at t=6 day; [3] Sediment was sterilized by mercuric chloride).

MPE Congener	C_w (t=5 day) (µg/mL)		C_S (t=5 day) (µg/g)		Sediment / water ratio	% OC (Marine Sediment)	pH	p value	K_{SW} (L/kg)		K_{OC} (L/kg)		Spiked mass of MPE (µg)	Sorption to sediment (%)	Mass Balance (%)	
	Mean	2sd	Mean	2sd					Mean	2sd	Mean	2sd			Mean	2sd
MEP	1.45	0.2	25.5	0.1	1:5	2.92	6	0.57	17.6	2.79	603	150	65.0	78	100	-
MBuP	0.76[1]	0.1	1.39[1]	0.1	1:5	2.92	6	0.25	1.83	0.16	62.6	13.2	12.5	22	100	37
MBzP	1.41[2]	0.1	5.45[2]	0.4	1:5	2.92	6	0.76	3.86	0.42	132	29.1	25.0	87	100	-
MC6	3.25	0.3	5.33	0.6	1:5	2.92	6	0.20	1.64	0.43	56.0	16.7	40.0	25	106	24
MEHP	0.82	0.1	1.81	0.1	1:5	2.92	6	0.33	2.21	0.12	75.6	15.1	12.5	29	99	6.0
MEHP	0.68	0.1	2.43	0.3	1:5	2.92	4	0.77	3.59	0.53	123	29.8	9.70	50	122	7.0
MEHP	0.79[3]	0.2	1.49[3]	0.1	1:5	2.89	6	0.59	1.89	0.25	65.3	8.76	14.8	20	80	16
MnOP	0.90[1]	0.3	2.45[1]	0.2	1:10	2.92	6	0.38	2.70	0.93	93.2	36.6	12.5	20	94	5.0

Table 5: Fresh water sediment (Buntzen Lake): Sediment sorption results showing water and sediment concentrations at equilibrium, sediment water ratio, organic carbon content, pH of the sediment-water system during incubations, p-values indicating that sediment reached equilibrium concentration within 24h and there is no significant change from day 1 till the end of the incubation period (one-way ANOVA), partition coefficients (K_{SW} and K_{OC}), mass of chemical spiked into water at t=0, amount of chemical sorbed to sediment and average chemical mass balance during incubation period of sorption experiments for various MPE congeners.

MPE Congener	C_w (t=5 day) (µg/mL)		C_S (t=5 day) (µg/g)		Sediment / water ratio	% OC (Fresh water sediment)	pH	p value	K_{SW} (L/kg)		K_{OC} (L/kg)		Spiked mass of MPE (µg)	Sorption to sediment (%)	Mass Balance (%)	
	Mean	2sd	Mean	2sd					Mean	2sd	Mean	2sd			Mean	2sd
MEP	0.52	0.2	21.2	3.8	1:5	10.81	6	0.35	41	18	377	192	59.9	71	102	33
MBuP	0.81	0.1	2.86	0.9	1:10	10.81	6	0.40	3.5	1.0	32.5	12.0	12.5	23	90	3.0
MBzP	2.03	0.3	4.7	2.5	1:10	10.81	6	0.27	2.3	1.3	21.4	12.6	25.0	19	100	-
MC6	1.30	0.3	24.5	3.2	1:10	10.81	6	0.45	19	3.6	174	41.7	40.0	61	92	13
MEHP	0.24	0.1	8.03	0.6	1:5	10.81	6	0.55	34	14	309	147	18.4	87	100	2.0
MnOP	0.71	0.1	5.52	0.3	1:10	10.81	6	0.45	7.8	0.5	72.0	16.0	12.6	44	100	-

Table 6: Results for marine water sediment (False Creek): Sediment sorption experiments for MC7, MC9 and MC10 showing water and sediment concentrations at equilibrium, sediment water ratio, organic carbon content, pH of the sediment-water system during incubations, partition coefficients (K_{SW} and K_{OC}), mass of chemical spiked into water at t=0, amount of chemical sorbed to sediment and average chemical mass balance during incubation period of sorption experiments for various MPE congeners. (C_w was determined by subtracting the C_s value from the total amount spiked).

MPE cong-ener	C_w (t=5 day) (µg/mL)		C_S (t=5 day) (µg/g)		Sedi-ment / water ratio	% OC (Marine sedi-ment)	pH	Rate constants		K_{SW} (L/kg)	K_{OC} (L/kg)	Spiked mass of MPE (µg)	Sorption to sediment (%)	Mass Balance (%)
	Mean	2sd	Mean	2sd				k_{sw}	k_{ws}					
MC7	0.07	0.1	0.66	0.2	1:5	2.92	6	6.05	0.45	13.6	466	2.04	65.0	100
MC9	1.99	0.3	2.10	0.1	1:5	2.92	6	19.2	17.1	1.12	38.4	24.0	17.5	100
MC10	1.31	0.1	5.46	0.3	1:5	2.92	6	86.6	18.6	4.66	159	24.0	45.5	100

Table 7: Results for fresh water sediment (Buntzen Lake): Sorption experiments showing water and sediment concentrations at equilibrium, sediment water ratio, organic carbon content, pH of the sediment-water system during incubations, partition coefficients (K_{SW} and K_{OC}), mass of chemical spiked into water at t=0, amount of chemical sorbed to sediment and average chemical mass balance during incubation period of sorption experiments for various MPE congeners. (C_w was determined by subtracting the C_s value from the total amount spiked).

MPE congener	C_w (t=5 day) (µg/mL)		C_S (t=5 day) (µg/g)		Sediment / water ratio	% OC (Fresh water sediment)	pH	Rate constants		K_{SW} (L/kg)	K_{OC} (L/kg)	Spiked mass of MPE (µg)	Sorption to sediment (%)	Mass Balance (%)
	Mean	2sd	Mean	2sd				k_{ws}	k_{sw}					
MC7	0.04	0.03	0.81	0.15	1:5	10.81	6	20.9	1.0	20.9	193	2.04	79	100
MC9	1.57	0.31	8.30	3.21	1:10	10.81	6	5.98	1.39	4.30	39.8	24	35	100
MC10	1.1	0.25	13.2	3.21	1:10	10.81	6	17.8	1.47	12.1	112	24	55	100

3.5 Effect of pH

Sorption experiments were carried out at 2 different pH levels (pH 4 and pH 6) to investigate the effect of ionization on sorption coefficients. The K_{OC} values for both pHs for MEHP are tabulated (Table 4).

Sorption experiments for MEHP at pH 4 revealed log K_{OC} to be 2.09 compared to a lower value of 1.88 at pH 6. Dissociation constants, pKa for MPEs are estimated by ASTER to be 4.2 all MPEs (Peterson and Parkerton, 1999). Thus, the fraction of non-ionized MEHP present at pH 4 and 6 are 61 % and 2 %, respectively. However, the increase in log K_{OC} value was not proportional to the increase in the non-ionized fraction. Thus, it can be speculated that the pKa values of these MPEs may be lower than estimated. Measured pKa values for two congeners, MMP and MEP were found to be 3.18 and 3.26 respectively (Chantooni and Kolthoff, 1975) which are lower than the estimated values, 3.57 and 4.2 respectively (Peterson and Parkerton, 1999).

Esteves da Silva and Marques (2007) found that ionic substances showed an increased solubility in water in lower ionic strengths while the solubility decreased in the presence of higher ionic strengths relative to their solubility in pure water. These two opposite variations in the aqueous solubility of ionic substances may result from the effect of 'salting in' and 'salting out' as a function of ionic strength.

The apparent solubility of MPEs may be higher than their solubility in pure water at neutral pH. The increase in solubility of the MPEs can be explained due to three factors: (i) solubility in water; (ii) ionization; (iii) interaction with dissolved organic matter; all these factors contribute the higher concentrations in water driving the sorption

coefficients lower than expected from the prediction models. The fraction of non-ionized PMEs that may be present the sorption experimental setup at pH 6 given that pKa is 4.2 can be estimated to be approximately 1.6 %. Thus 1.6 % of PMEs spiked is available for partitioning to sediment. Even in the natural environment and the prevalent pH conditions (False Creek water has a pH of 6), weakly acidic chemicals are predominantly present as ions with negative charge (Bintein and Devillers, 1994).

The main components of organic matter (typically containing 58% of organic carbon) in sediments are contains humic acids, fulvic acids and humin. Fulvic acids have a lower molecular weight and a higher percentage of carboxyl groups than humic acids (Wershaw, 1986). These carboxyl groups may ionize and increase the solubility of fulvic acids in the water component than the humic acids. Fulvic acids are soluble at low pH values (pH 2) where humic acids precipitate. The colloidal surfaces of most natural soils are negatively charged and therefore have an affinity for positively charged molecules, but not much affinity for negatively charged molecules (Green, 1974).

Humic acids of bottom sediments may have lower affinity to binding ionic substances due to the hydrophilic character of the weak organic acids (De Paolis and Kukkonen, 1997) like MPEs. However, the non-ionized form of the chemicals may bind to the organic carbon, thus the fraction of non-ionized chemical in turn the pKa of PMEs are one of the important factors controlling the sorption to organic carbon.

3.5.1 Field concentrations

Field concentrations were obtained in years 2004 and 2006. In samples collected in 2004, MBP MEHP and MiNP congeners were detected in sediment and MBP and

MEHP were detected in water. For samples collected in 2004, concentrations of detected MPEs in sediment and water were reported (Figure 34 and Figure 36). Sorption coefficients were not calculated. In samples collected in 2006 10 MPE congeners, MMP, MEP, MBP, MEHP, MBzP, MC6, MC7, MnOP, MiNP and MC10 were detected in sediment (Figure 35) while in water 8 congeners except MnOP and MC10 were detected (Figure 37).

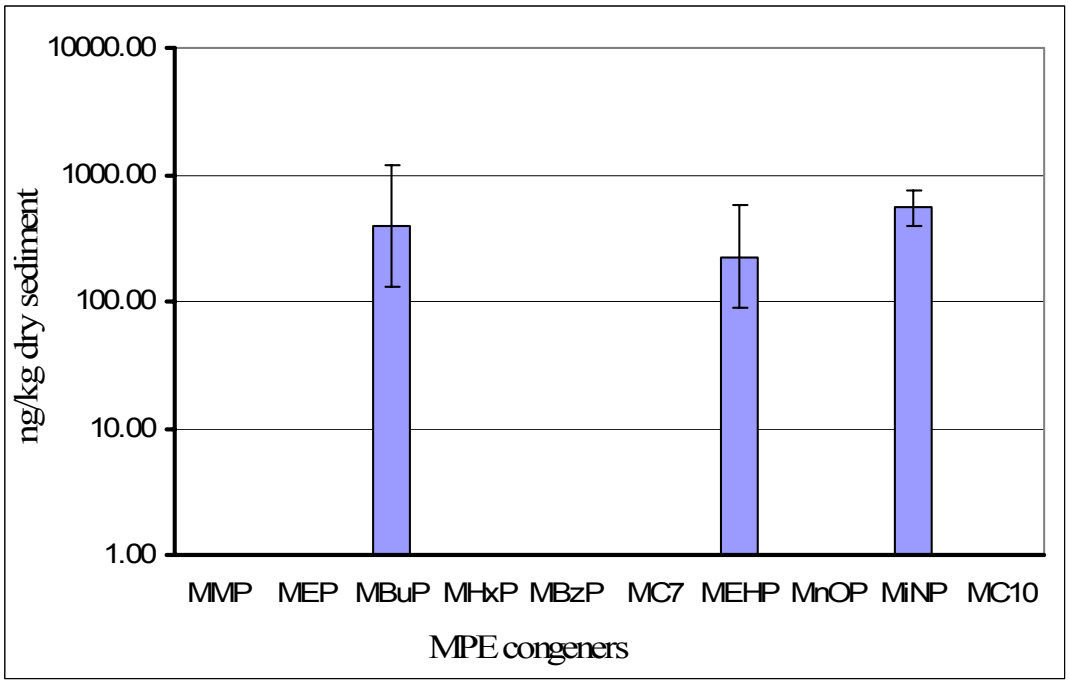


Figure 34: Concentrations (geometric mean ± 1SD) (ng/kg dry sediment) of MPE congeners in the False Creek sediment collected in the year 2004.

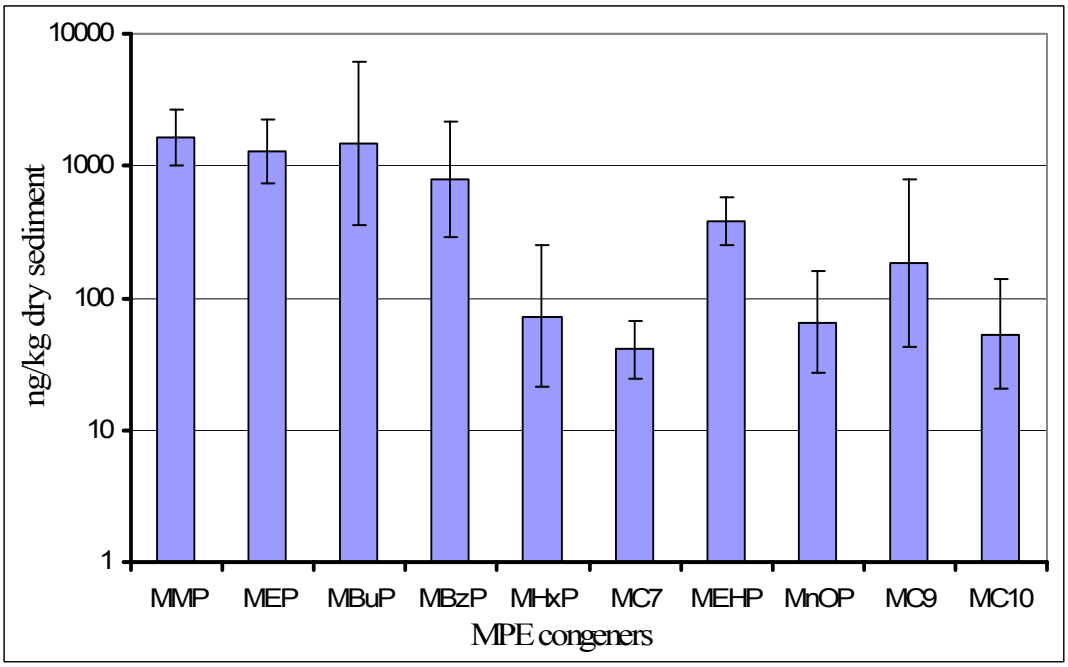


Figure 35: Concentrations (geometric mean ± 1 SD) (ng/kg dry sediment) of MPE congeners in the False Creek sediment collected in year 2006.

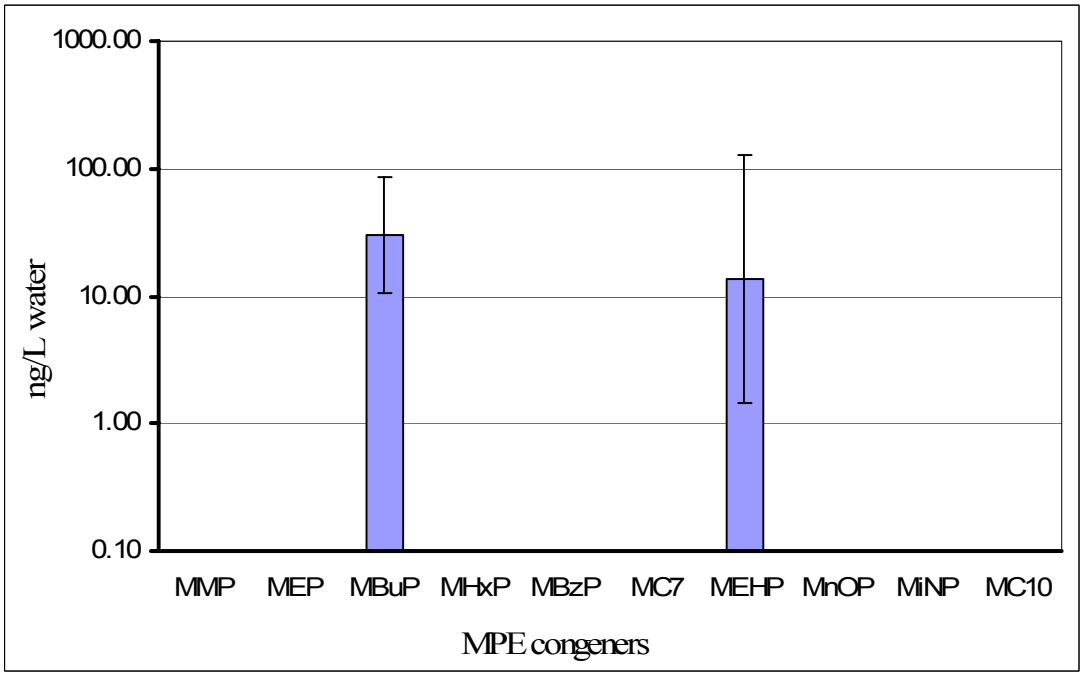


Figure 36: Concentrations (geometric mean ± 1SD) (ng/L water) of MPE congeners in False Creek water collected during the year 2004.

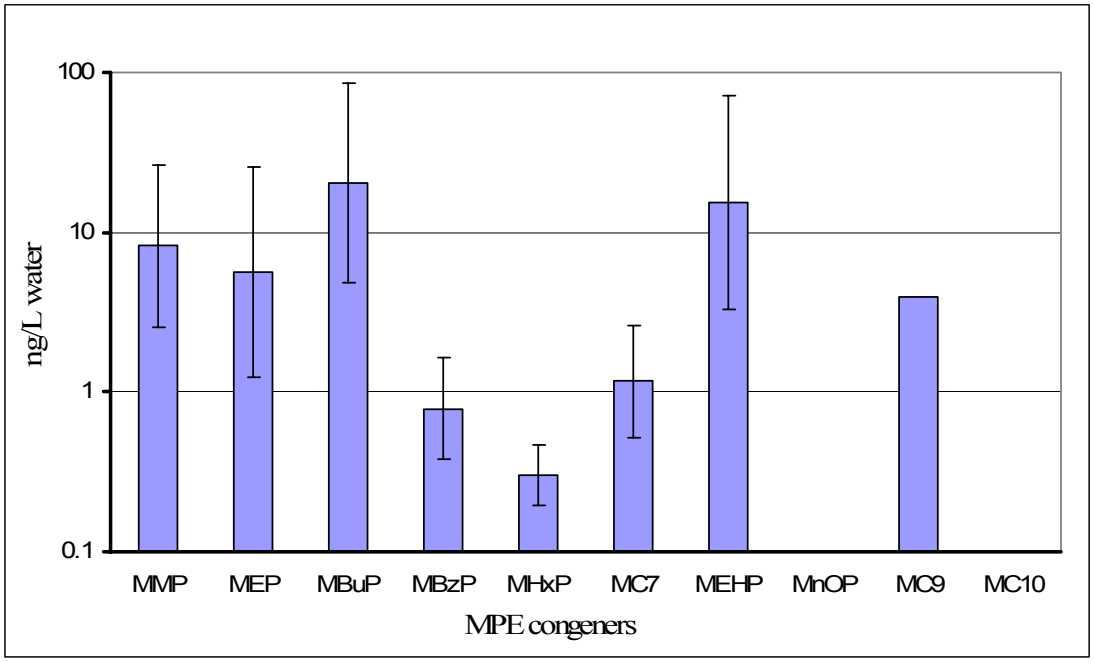


Figure 37: Concentrations (geometric mean ± 1SD) (ng/L water) of MPE congeners in False Creek water collected during 2006.

3.6 Field Observations

Water and sediment samples were collected from 4 random sampling sites. However no effort was made to look for variations between sampling sites as no variation was observed in the previous study (Mackintosh *et al.*, 2006).

It has been observed in the previous studies that the field estimated sorption coefficients are larger than the laboratory developed sorption coefficients (Gobas and MacLean, 2003). Sorption behaviour is generally determined by their aqueous solubility especially for the neutral organic compounds (Zhou and Liu, 2000; Karickhoff, 1979). Salinity of marine water is also important environmental factor that may affect sorption behaviour of organic contaminants on marine sediments. In marine water, there are various inorganic ions such as sodium ions, potassium ions, chloride ions and sulphate ions, which can effect the solubility of the PMEs (Jurner and Rawling, 2001). The aqueous solubility of these PMEs will correspondingly decrease with increasing salinity

(Zhao et al., 2004). As a result, the amount of these PMEs partitioning into the sediment as a result of salting will increase. The salinity, in turn salting of electrolytes will effect the release of organic matter into the water from sediment increasing the amount of organic carbon in the sediment thus the sorptive capacity resulting in higher K_{OC} than the laboratory based K_{OC}.

It is observed that lower K_{OW} PMEs (MMP, MEP, MBuP, MBzP and MC6) exhibit sorption coefficients that are order of magnitude greater than their corresponding estimated values (Table 8), while the differences between the observed and estimated values drops for higher K_{OW}s (MEHP, MC7 and MC9). This confirmed the results of Gobas and MacLean (2003) who stated that "*Substances with low K_{OW} exhibit distribution coefficients that are orders of magnitude greater than their corresponding equilibrium partition coefficients. The discrepancy between the actual distribution coefficients and the equilibrium partition coefficients drops with increasing K_{OW}. Only chemicals with the highest K_{OW} values exhibit distribution coefficients that are comparable to the equilibrium partition coefficients"* (Gobas and MacLean, 2003).

3.7 Predicted vs Laboratory vs Field Measurements of Sorption Coefficients

The sorption coefficients (K_{SW}) derived from field data were found to be higher than those determined in lab experiments by approximately an order of magnitude. No correlation was found between K_{SW} and molecular weight or the K_{OW} (Figure 38). There was no significant relationship between the K_{OC} (both field and lab) with the predicted K_{OW} (Figure 38). K_{OW} may not a good predictor of sorption coefficients for ionizable chemicals. Log D, a new predictor, is the ratio of the equilibrium concentrations of both

the unionized and ionized species of a molecule in octanol to same species in the water phase. It differs from the log K_{OW} in that ionized species are considered as well as the neutral form of the molecule. Log D values for MPE congeners at pH 6 (Table 8) were calculated using JChem software suite. The calculated Log D values for MPE congeners were lower than Log K_{OW}, ranged from -1.14 (MMP) to 2.45 (MC10). The Log D values for MC9 and MC10 were 1.99 and 2.45, respectively, which correspond to log K_{OW} 2, approximately. The field and laboratory derived log K_{OC} values for these two congeners (Table 8) showed an increase with the increase in log K_{OW} values as predicted by EPIWIN (Figure 39). However, the lower Log D congeners (which correspond to less than Log K_{OW} 2) does not show any trend in the log K_{OC} values. This may suggest that the MPE congeners with lower log K_{OW} (< 2) may not partition only based on the hydrophobicity. The higher field sorption coefficient values compared to laboratory sorption coefficients, may be possible due to the following reasons.

Effect of salinity: The False Creek marine water has salinity of 30 % while the water in the lab experiments did not contain salts. The high salinity decreases the water solubility of negatively charged species (Zhao *et al.*, 2004), and hence increases the sorption coefficients (Chiou *et al.*, 1979; Site, 2001). In marine water, there are numerous solutes (both organic and inorganic) and that can effect the aqueous solubility (Means, 1995; Turner and Rawling, 2001) of the organic compounds like MPEs. 'Salting out' is the phenomenon where the aqueous solubility of organic compounds is reduced as a result of increased solute / ion concentration (Means, 1995; Turner and Rawling, 2001; Zhao *et al.*, 2004). However, one study by Abernathy and Davidson as reported in (Means, 1995), found that the increased salinity increased sorption of Prometryne, a

neutral triazine compound, while it decreased the sorption of the polar herbicide Flumeturon. Karickhoff (1984) found a 15 % increase in sorption coefficient of pyrene and methoxychlor in response to increased salinity. In addition to the reduction in aqueous solubility, salinity was also found to have effect on the sediment resuspension of organic matter. The ability of sediment to resuspend into the water phase was also decreased due to salting out. Thus as a consequence of salting out, the combination of effects both in the water and sediment are altering the sorption coefficients of MPEs from field to the lab.

Table 8: A collection of log Koc values predicted (from empirical equations) and experimental data from this study. 1:ASTER (Peterson and Parkerton, 1999); 2:EPIWIN (Peterson and Parkerton, 1999); 3:Binstin and Devillers (Bintein and Devillers, 1994); 4:Frank Gobas (personal communication); 5:Experimental (Marine Sediment); 6:Column 5 values corrected for ionization at pH 6; 7:Experimental (Field); 8:Experimental (Fresh Sediment); Log D values were calculated for MPE congeners at pH 6 using Marvin Sketch (JChem) model. ND represents non-detects.

Congener	Log K_{OW}	Log D	Predicted values				Experimental values			
			Log K_{OC} (1)	Log K_{OC} (2)	Log K_{OC} (3)	Log K_{OC} (4)	Log K_{OC} (5)	Log K_{OC} (6)	Log K_{OC} (7)	Log K_{OC} (8)
MMP	1.37	-1.14	2.03	1.00	0.81	0.94	-	-	3.75	-
MEP	1.86	-0.79	2.32	1.11	1.26	1.40	2.78	2.97	3.67	2.58
MBuP	2.84	0.07	2.9	1.64	2.17	2.31	1.80	1.99	3.56	1.52
MBzP	3.07	0.64	2.99	2.45	2.39	2.53	2.12	2.31	4.66	1.33
MC6	3.85	0.86	3.47	2.17	3.11	3.26	1.75	1.95	4.16	2.24
MEHP	4.73	1.66	3.98	2.67	3.93	4.07	1.88	2.07	2.31	2.49
MnOP	5.22	1.66	4.26	2.91	4.39	4.53	1.97	2.16	ND	1.86
MC7	5.57	1.26	4.36	3.05	4.71	4.86	2.67	2.14	3.02	2.29
MC9	5.30	1.99	4.33	2.97	4.46	4.61	1.56	1.75	3.64	1.69
MC10	5.79	2.45	4.62	3.23	4.92	5.06	2.15	2.34	ND	2.05

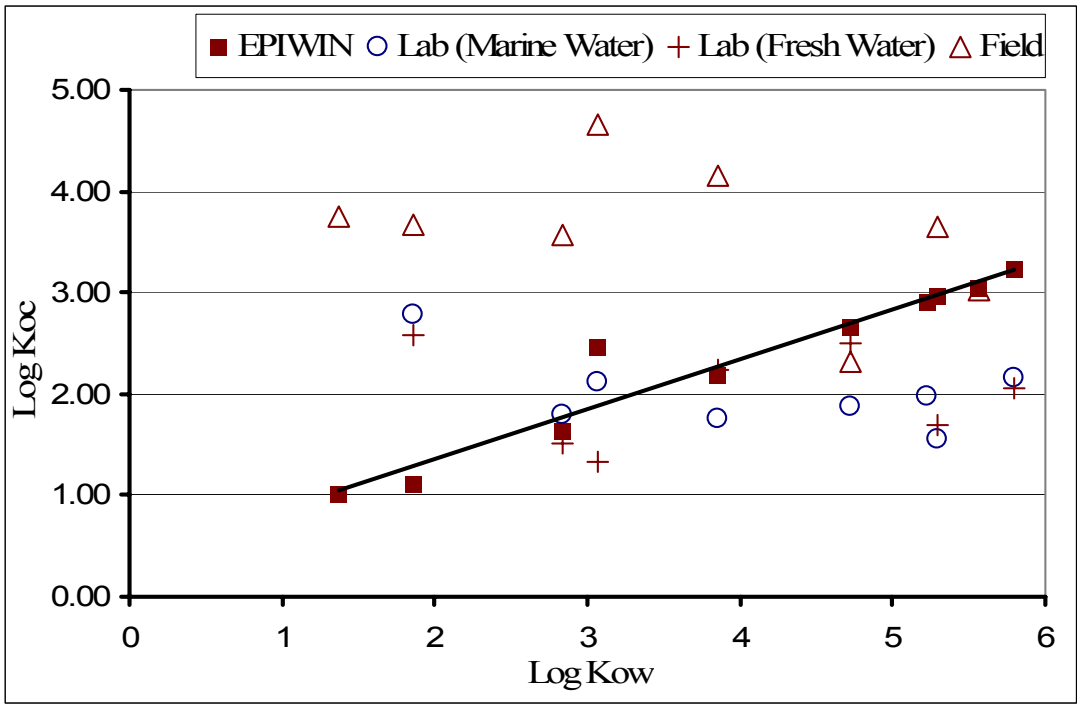


Figure 38: Graph showing Log K_{OC} (EPIWIN calculated Log K_{OC}, Experimental Log K_{OC} (Marine sediment and Fresh water sediment) and Field derived Log K_{OC} values vs Log K_{OW}. Soild line represents regression fit for the predicted values by EPIWIN (r^2=0.94).

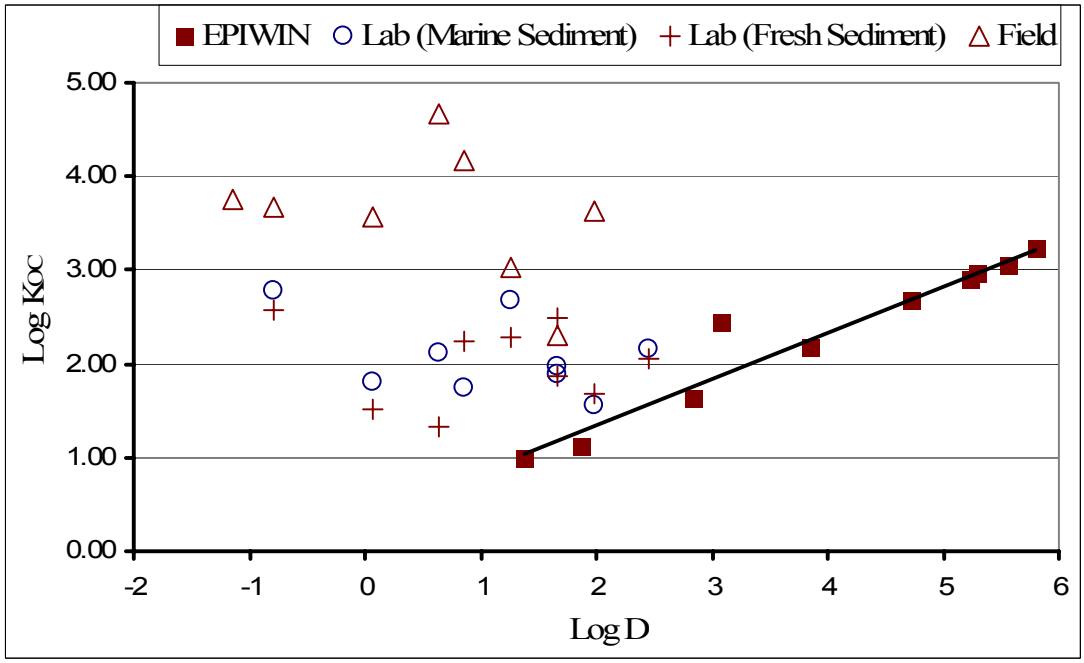


Figure 39: Graph showing Log K_{OC} (EPIWIN calculated Log K_{OC}, Experimental Log K_{OC} (Marine water sediment and Fresh water sediment), and Field derived Log K_{OC} values vs Log D. Solid line represents regression fit for the predicted (EPIWIN) Log K_{OC} values (r^2 =0.94).

3.8 Conclusion

The sorption of nine different monoesters congeners onto both marine and fresh water sediments reached equilibrium within 24 hours in the laboratory experiments. The organic carbon content of the sediment played an important role in the extent of sorption of monoesters. Fresh water sediment (high organic carbon content) showed higher sediment-water partition coefficients than marine water sediments. Organic carbon-normalized sorption coefficients for 9 different monoester congeners in the laboratory experiments ranged between 38 and 600 L/kg for marine sediment and between 21 and 380 L/kg for fresh water sediment. Sorption coefficients measured in the field sediments were greater, and ranged between 200 and 46,000 L/kg. Organic carbon-normalized sorption coefficients for MEHP at pH 6 and pH 4 were 75 and 123 respectively. The sorption coefficient of MEHP increased with decreasing pH due to the weak acidic nature of MEHP. The sorption coefficients of MPEs measured in the laboratory studies were substantially lower than the predicted values based on the K_{OW} of the undissociated form. Log K_{OW} based models may not be the good predictors of K_{OC} for the ionizable chemicals like MPEs, as these models does not account for the ionization. The partition of MPEs may not be just of the non-ionic form but also the ionic form, which is more predominant at pH 6. Log D, which accounts for the ionic form, may be more appropriate for predicting K_{OC}. These results demonstrate that the dissociation of MPE at environmental pH levels needs to be considered when assessing the environmental fate of these substances in aquatic environments.

The strength of association of pollutants to the matrix (sediment or soil), will determine the the extent to which those interactions can be disrupted, allowing the

contaminant to become more bioavailable. Sorption coefficients are one of those properties which measures the interaction of the pollutants to the matrices.

APPENDIX

APPENDIX A

Table 9: Numerical data showing field concentrations of MPE congeners in False Creek water and sediment matrices collected during years 2004 and 2006. Concentrations are reported as geometric means with upper and lower standard deviation. ND represents non-detects.

MPE Cong-ener	2004						2006					
	MPE Cong-ener			2004			2006			Sediment Concentrations (ng/kg)		
	Geometric mean	Upper SD	Lower SD	Geometric mean	Upper SD	Lower SD	Geometric mean	Upper SD	Lower SD	Geometric mean	Upper SD	Lower SD
MMP	ND	-	-	ND	-	-	8.200	17.94	5.629	1656	1046	641.5
MEP	ND	-	-	ND	-	-	5.625	19.83	4.381	1288	983.7	557.9
MBuP	30.31	56.83	19.77	390.2	777.9	259.8	20.27	65.14	15.46	1475	4732	1124
MBzP	ND	-	-	ND	-	-	0.789	0.854	0.410	789.3	1371	501.0
MHxP	ND	-	-	ND	-	-	0.300	0.166	0.107	73.07	174.1	51.47
MEHP	13.77	116.1	12.31	225.8	341.3	135.9	15.42	56.08	12.09	381.1	199.0	130.7
MoC7	ND	-	-	ND	-	-	1.165	1.443	0.645	40.64	26.01	15.86
MnOP	ND	-	-	545.0	208.1	150.6	ND	-	-	65.87	95.80	39.03
MoC9	ND	-	-	ND	-	-	3.888	-	-	186.0	611.6	142.6
MoC10	ND	-	-	ND	-	-	ND	-	-	52.79	84.14	32.44

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