BIODEGRADATION OF PHTHALATE ESTERS IN FALSE CREEK SEDIMENTS

by

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B.Sc. University of Alberta, 1999

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Abstract

Phthalate diesters (DPEs) are a family of industrial chemicals used in a vast array of consumer products. Because of their broad commercial applications, many DPEs have become ubiquitously distributed in the environment. An in vitro test was conducted to measure the biodegradation rate of seven DPEs in temperate marine sediments. Low molecular weight congeners degraded rapidly with sediment half-lives ranging from 3.0 and 8.0 days, while high molecular weight congeners exhibited slow (2-ethyl-hexyl phthalate sediment half-life of 340 days) or no significant biodegradation. While previous studies show that the high molecular weight congeners are inherently biodegradable, the current study showed that they were recalcitrant in natural sediments. A mechanistic biodegradation model was developed based on the premise that all phthalates are inherently biodegradable, but only the freely dissolved fraction of the chemical concentration in sediments is bioavailable for microbial degradation. The model showed good agreement with empirical observations.

Keywords: Phthalate ester, Biodegradation, Bioavailability, Sediment, Environmental persistence

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1: Introduction

Phthalate diesters (DPEs) or phthalates, are a class of chemicals known as plasticizers that are widely used to impart flexibility to plastic and polyvinyl products (Staples et al., 1997).

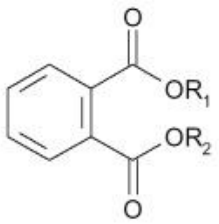


Figure 1. Generalized chemical structure for phthalate diesters. Variation of side chain (R1, R2) length and branching pattern differentiates between congeners.

They are produced primarily in commercial applications for vinyl manufacturing, but are also found in consumer items including personal care products (nail polish, lotions and perfumes), paints, insect repellents, children's toys and food packaging material (Mortensen et al., 2005, Lovekamp-Swan & Davis, 2003). There are many different congeners of DPEs; those commonly used in commerce have side chain lengths of 1 to 13 carbon atoms (Stanley et al., 2002). Because of their usefulness and broad functionality in commercial applications, many phthalates have been classified as high production volume chemicals (i.e., > 1000 tonnes produced per year) (OECD, 2004);

consequently they have become widely distributed in the environment to the point where they are considered to be ubiquitous (Staples et al., 1997).

Table 1. Suite of DPEs and MPEs included in biodegradation study and associated environmental partitioning parameters, as reported in Staples et.al., (1997), and Otton et al, 2008).

Phthalate Ester	Abbrev.	CAS No.	AQ Solubility (mg/L)	Log K_{ow}
DPEs				
Dimethyl phthalate	DMP	131-11-3	4200	1.61
Diethyl phthalate	DEP	84-66-2	1100	2.38
Di-n-Butyl phthalate	DnBP	84-74-2	11.2	4.45
Butylbenzyl phthalate	BBP	85-68-7	2.7	4.59
Di -2-Ethylhexyl phthalate	DEHP	117-81-7	0.003	7.50
Di-n-Octyl phthalate	DnOP	117-84-0	0.0005	8.06
Diisononyl phthalate	DINP	28553-12-0; 68515-48-0	<0.001	9.40
MPEs				
Monomethyl phthalate	MMP	4376-18-5	3738	1.37
Monoethyl phthalate	MEP	2306-33-4	1212	1.86
Mono-n-butyl phthalate	MnBP	131-70-4	126	2.84
Monobenzyl phthalate	MBzP	2528-16-7	52	3.07
Mono-2-ethylhexyl phthalate	MEHP	4376-20-9	1.49	4.73
Mono-n-octyl phthalate	MnOP	5393-19-1	NR	5.22
Monononyl phthalate	MC9P	(mixture)	0.408	5.30

NR indicates value not reported

DPEs are not covalently bonded to their associated commercial products; hence, the primary source of phthalates in the environment is slow release from plastics and vinyl products due to weathering (Stanley et al., 2002). In recent decades, concerns over DPEs have increased for two main reasons:

- The vast global production of phthalates (estimated at 5.2 million tons per year) has resulted in ubiquitous human exposure (Parkerton and Konkel, 2000) and,

- The potential for estrogenic or teratogenic effects as a result of long term exposure, or exposure during sensitive life stages (e.g., fetal development) to certain phthalates and their primary metabolites (Harris et al., 1997; Lovekamp-Swan and Davis, 2003, Lehmann et al., 2004; Howdeshell et al., 2008).

For these reasons, in recent decades, there have been significant efforts to characterize the environmental fate of phthalates (Staples et al., 1997; Peterson and Staples, 2002; Liang et al., 2008). Abiotic and biotic degradation of phthalates (Peterson and Staples, 2002) as well as the environmental partitioning behaviour of phthalate esters (Cousins et al., 2002, Mackintosh et al., 2006) have been evaluated. The outcome of these studies indicate that phthalates will tend to accumulate in sediments of aquatic systems, and that biodegradation by microorganisms is the dominant mode of decomposition (Staples et al., 1997; Cousins et al., 2002).

1.1 Phthalate Toxicity

In order to provide context for this DPE biodegradation study, the following brief overview of phthalate toxicity is provided.

Due to the concerns listed above, significant effort has been expended determining the potential effects and toxic modes of action phthalates may induce in mammalian and aquatic receptors.

1.1.1 Mammalian Toxicity

Mammalian data suggest that a variety of toxic effects are possible due phthalate exposure. These include endocrine disruption, developmental toxicity and carcinogenesis (Hoppin et al., 2002). Studies have shown that DnBP and DEHP are capable of affecting male fertility, particularly when exposure occurs in utero during sexual differentiation (Saillenfait et al., 2009). Male rats exposed to DnBP, and DEHP (in utero) display effects in reproductive tissue that indicate suppression of fetal testicular testosterone and insulin-like 3 hormone production (Howdeshell et al., 2008). A study by Duty et al. (2005) suggested that at current exposure rates, some phthalates may be associated with alterations in reproductive hormone levels (FSH and inhibin B) in adult men.

DPEs have been suggested as some of the potential causes of human testicular disgenesis syndrome (TDS) due to the similarity of TDS symptoms to reproductive effects in male rates following DPE exposure (Howdeshell et al., 2008). Lehmann et al. (2004) observed that fetal testicular testosterone was significantly reduced when female rats were exposed to DBP by gavage at concentrations greater than 50 mg/kg/day.

Experimental evidence has indicated that the compounds responsible for the observed toxic effects are not DPEs but their primary metabolites (MPEs) (Barr et al., 2003). For example, Yagi et al. (1980) observed the same toxic effects in mice exposed to DEHP and smaller doses of MEHP, suggesting increased toxicity of MEHP. In addition, several studies have found that MEHP can activate the expression of several target genes associated with peroxisome proliferation and hepatic carcinogenesis in rats (Hurst and Waxman 2003, 2004).

Despite the rapid metabolism and elimination of DPEs (particularly in primates) (David and Gans, 2002), measurable concentrations in adult humans (in saliva, urine, serum, amniotic fluid) indicate that an equilibrium is reached between the rates of elimination and the exposure to phthalates from dietary sources and personal care products (Duty et al., 2003; Silva et al., 2004). The level of dosage used in animal studies is generally three to four orders of magnitude greater than the estimated daily exposure of humans (Martino-Andrade and Chahoud, 2010). For example, the USEPA Integrated Risk Information System (IRIS) has posted a lowest observed adverse effect level (LOAEL) in humans for DnBP and DEHP at doses of 600 mg/kg bw/d, and 14 mg/kg bw/d, respectively (USEPA, 2010). These values are well above the estimated levels of human exposure by Wittassek et al., (2008) who predicted median exposure rates to the general German population of 0.0021 (DnBP) and 0.0027 (DEHP) mg/kg bw/day.

1.1.2 Ecotoxicity

Due to the propensity of DPEs to accumulate in aquatic systems, many ecotoxicological studies have been conducted using aquatic species.

Of the seven DPEs included in this study, only four have been shown to be acutely or chronically toxic to aquatic species (Staples et al., 1997b). Bradlee and Thomas (2002) reported that the acute toxicity for algae (measured by inhibition of cell growth) and invertebrates and fish (measured by survival) of the four DPEs occurred at the following concentration ranges:

- DMP 29.0 – 377 mg/L,
- DEP 10.3 – 131 mg/L,

- DBP 0.35 – 6.29 mg/L, and;
- BBP 0.21 – 5.30 mg/L.

The other three DPEs (DEHP, DnOP and DiNP) do not show aquatic toxicity at their associated limits of aqueous solubility. A possible explanation for the lack of observed toxicity for the other three DPEs is their low solubilities. Table 1 shows there is a 1000 fold decrease in solubility between the low molecular weight (LMW) DPEs (those with side chains with 1-6 carbon atoms) and high molecular weight (HMW) DPEs (side chain length >6 C). Due to this difference in solubility, it is possible that insufficient quantities of the HMW DPEs partition into the aqueous phase to exert an acute or chronic toxic effect.

1.2 Chemical Regulation in Canada

In Canada, regulation of chemical substances is controlled by the Canadian Environmental Protection Act (CEPA, 1999). Evaluation of the relative potential harm for chemicals in Canada is comprised of three factors: environmental persistence (P), bioaccumulative potential (B) and inherent toxicity (T); together, referred to as PBT. Criteria for determining whether a chemical is defined as persistent under CEPA are defined in the Persistence and Bioaccumulation Regulation (1999) as follows: a substance is categorized as persistent if:

- its half-life is equal to or greater than 2 days in air, or it is subject to atmospheric transport from its source to a remote area;
- its half-life is equal to or greater than 182 days in water;
- its half-life is equal to or greater than 365 days in sediments;

- its half-life is equal to or greater than 182 days in soil.

There are two common endpoints for biodegradation testing, i.e., primary degradation and mineralization. Primary degradation is defined as any single structural alteration of a chemical, and is determined by measuring the disappearance of the parent substance from a test system over time. Mineralization is the complete biodegradation of an organic compound to carbon dioxide (under both aerobic and anaerobic conditions), methane (under anaerobic conditions) and water (OECD, 2003).

The CEPA persistence criteria do not specify whether the measured half-life endpoint is associated with primary degradation of mineralization; but primary degradation is likely intended, as it is expected to reduce the persistence, bioaccumulation and/or toxicity (PBT) of most organic compounds (Aronson and Howard, 1999). However, for some chemicals, primary degradation may not result in the loss of PBT qualities and in certain cases, they may even increase (Ioannides and Lewis, 2004). For example, several phthalate diesters have monophthalate metabolites that are reportedly capable of causing estrogenic effects (Howdeshell et al., 2008).

The inherent toxicity and bioaccumulative potential of phthalates have been evaluated elsewhere (Staples et al., 1997; Bradlee & Thomas, 2002; Mackintosh, 2002). However, the environmental persistence of DPEs has been less well characterized because a large percentage of the biodegradation literature has been generated using test methodologies that do not utilize natural test media or environmentally realistic test conditions. When considering biodegradation data, it is imperative to understand the test conditions under which the data were generated, as they can have a significant effect on results (Boethling et al., 2009).

1.3 Biodegradation Testing

Biodegradation can be defined as the biologically mediated alteration of a chemical (Aronson and Howard, 1999), and is assessed though a variety of standardized and non-standardized testing methods. These methods can be classified into two broad categories: screening tests, and simulation tests. The biodegradation endpoint for screening tests is mineralization whereas the biodegradation endpoint for simulation tests is primary biodegradation.

For organic chemicals, using biodegradation studies to predict the rate of removal and half-life in the environment presents a challenging problem. This is because the rate of degradation depends on both the intrinsic properties of the chemical and also on environmental conditions (Mackay and Webster, 2006). Generally, in biodegradation studies using organic chemicals (including phthalates), up to three distinct degradation phases can be observed. The first is a "lag" phase where the degradation rate is slow or does not occur at all, the second is the degradation phase where the bulk of the chemical of interest is degraded, and the third is the stationary phase where degradation is negligible (Figure 2). Further discussion regarding the three distinct phases of biodegradation is presented in section 1.4.

1.3.1 Screening Tests

Screening tests are used to test the biodegradability of organic chemicals and are generally conducted using sewage inocula as the degrading microbial community. The Organisation for Economic Cooperation and Development (OECD) has developed methodologies for a series of 28-day screening tests (e.g., OECD 301, 302, 304A) designed to assess the ready or inherent biodegradability of commercial chemicals.

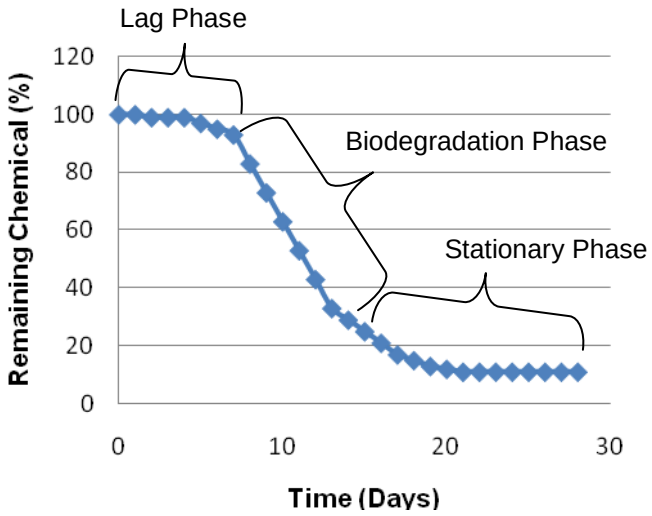


Figure 2. Illustrative relationship between the fraction of the original chemical concentration in the sediment throughout the incubation period of a microbial biodegradation experiment showing lag, degradation and stationary phases.

A substance can be defined as readily biodegradable if more than 60% of the original chemical concentration has degraded over the 28-day test, and if the time between the onset of biodegradation and the stationary phase is less than 10 days. Inherent biodegradability is measured similarly but a higher concentration of degrading inoculum is used and the test is carried out over a longer period (up to 14 days) (Pagga, 1997). Generally, in screening tests, the concentration of degrading organisms, and the concentration of compound of interest in test chambers are higher than those found under natural conditions. Furthermore, indirect detection methods are often used to quantify the degree of ultimate degradation of chemicals of interest (e.g., measurement of biological and chemical oxygen demand (BOD/COD) or total CO_2 evolution) (Beek et al., 2001). If the assessment of biodegradation is carried out by monitoring the accumulation of ultimate metabolites, to ensure that the ultimate metabolites were derived from the compound of interest, screening studies often use radiolabeled parent compounds. Environmental half-lives derived from ultimate degradation are generally longer than

those derived from primary degradation studies. For example, Gledhill et al. (1980) published half-lives for primary and ultimate biodegradation of butylbenzyl phthalate of <4 and 28 days, respectively.

Screening tests are limited in terms of their ecological realism as they use artificially active substrates (sewage inocula), and they often have detection limits for test substrate that are above concentrations found in the environment (Wesnigk et al. 2001). For these reasons, screening tests are generally used to classify chemicals into three categories (Wesnigk et al., 2001):

- chemicals with potential to be easily and rapidly biodegraded;
- chemicals with potential for degradation under specific environmental circumstances; and
- chemicals with no biodegradation potential.

Screening tests have been carried out to quantify the inherent and ready biodegradability of phthalates (Sugatt et al., 1984; Scholtz et al., 1997) and see Table 2. These have indicated that both LMW and HMW phthalates are readily biodegradable.

1.3.2 Simulation Tests

Unlike screening tests, simulation tests are generally conducted without a standardized method but are designed to approximate natural conditions and so utilize natural water, soil or sediment and their associated bacterial communities as the testing medium for biodegradability. Microcosm bench-top tests are the most commonly used to derive the degradation kinetics of a chemical in an environmental medium. These tests are generally accomplished by directly measuring the disappearance of parent compound

after prescribed time intervals by gas chromatography (GC) or high performance liquid chromatography (HPLC) (OECD, 2003). Simulation tests, while generally more environmentally realistic than screening tests must be undertaken with consideration of the many factors that can influence the rate of biodegradation (e.g., oxygen availability, temperature, concentration of parent compound, length of incubation etc.) (Aronson and Howard, 1999). These factors are discussed further in following sections. In addition to test conditions, chemical properties (solubility, $K_{ow,}$ K_{oc}) can affect rates of biodegradation in test systems (Zhang et al., 1998). It is therefore not surprising that there is disagreement in the scientific literature regarding the biodegradation of some HMW phthalates in simulation tests. Several authors have shown that DEHP, DnOP, DINP do not readily degrade in sediment simulation tests (Johnson and Lulves, 1975; Rubin et al., 1982; Johnson et al., 1984; Painter and Jones, 1990) whereas others have reported degradation (Yuan et al., 2002; Chang et al., 2005).

1.4 Phthalate Biodegradation

Primary biodegradation of phthalates involves hydrolysis of one of the DPE's ester side chains, resulting in the formation of a monoester metabolite and the corresponding alcohol (Figure 3). Each metabolite is further degraded by various biochemical pathways depending on which microorganisms are present, as well as oxygen availability (Staples et al., 1997). Diagrams of primary and ultimate phthalate biodegradation are presented in Figures 3 and 4.

Screening and simulation studies for phthalates have been completed under both aerobic and anaerobic conditions, and in various environmental media. A summary of recent biodegradation studies is provided in Table 2.

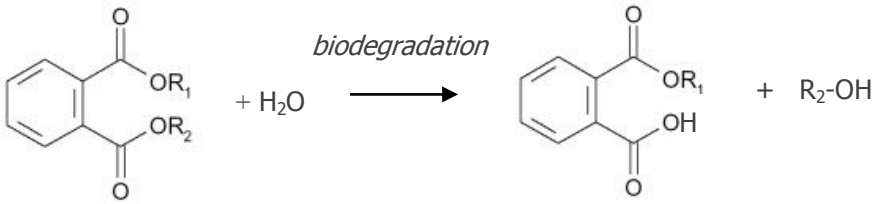


Figure 3. Generalized primary biodegradation reaction for phthalate diesters and primary metabolites (monoester and associated alcohol). Variation of side chain (R_1, R_2) length and branching pattern differentiate between congeners.

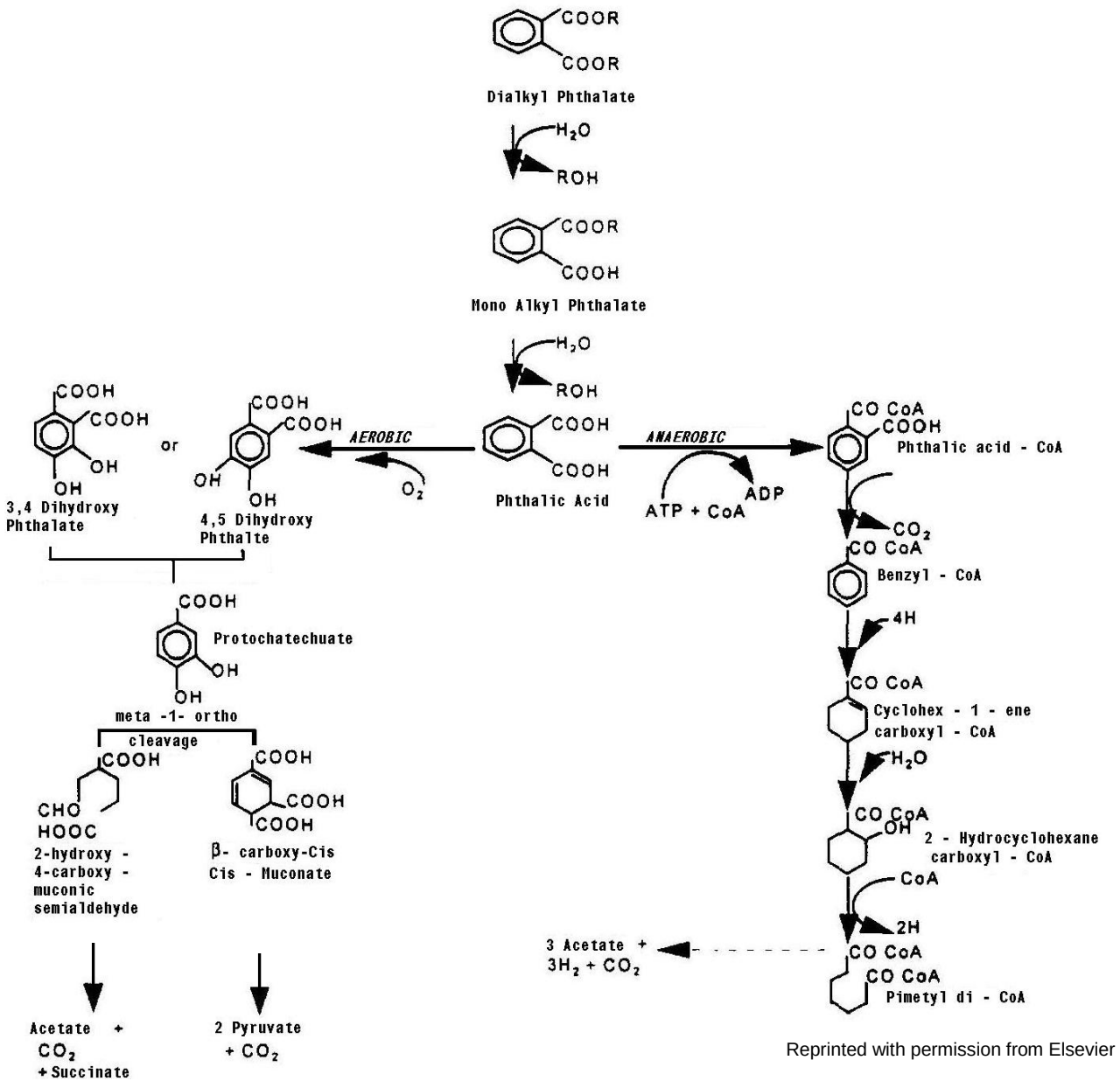


Figure 4. General biodegradation pathway for DPEs (from Staples et al., 1997)

Table 2. Summary of DPE biodegradation literature (2002 – 2010)

Medium[1]	Phthalate	Test		Result[3]	Reference
		Type[2]	Conditions		
AS, SM	DnOP	Sim	Aero / 30°C	$t_{1/2}$ ≈ 16 h	Wu et al., 2010
Se, SM	DnBP	Screen	Aero / 30°C	$t_{1/2}$ ≈ 1d	Zhou et al. 2009
SM	DMP	Screen	Aero / 30°C	85% removal in 30h	Gu et al., 2009
Se, SM	DMT	Screen	Aero / 25°C	96% removal in 12d	Luo et al., 2009
S, C	DnBP	Screen	Aero / 30°C	$t_{1/2}$ ≈ 2.3 d	Chang et al., 2009
S, C	DEHP	Screen	Aero / 30°C	$t_{1/2}$ ≈ 6.8 d	Chang et al., 2009
AS, SL, SM	DMP	Screen	Anaero / 30°C	$t_{1/2}$ ≈ 2.4 d	Wu et al., 2008
S	DnBP	Sim	Aero / 20-30°C	$t_{1/2}$ ≈ 8.0 d	Xu et al., 2008
S	DEHP	Sim	Aero / 20-30°C	$t_{1/2}$ ≈ 28 d	Xu et al., 2008
Se, SM	DnBP	Sim	Anaero / 16°C	$t_{1/2}$ ≈ 1.5-2.2 d	Lertsirisopon et al., 2006
Se, SM	BBP	Sim	Anaero / 16°C	$t_{1/2}$ ≈ 1.2-1.6 d	Lertsirisopon et al., 2006
Se, SM	DEHP	Sim	Anaero / 16°C	$t_{1/2}$ ≈ 207-280 d	Lertsirisopon et al., 2006
Se, SM	DiNP	Sim	Anaero / 16°C	$t_{1/2}$ ≈ 347-660 d	Lertsirisopon et al., 2006
Se	DnBP	Sim	Anaero / 30°C	$t_{1/2}$ ≈ 75 d	Kao et al., 2005
Se	DEHP	Sim	Anaero / 30°C	$t_{1/2}$ ≈ 147d	Kao et al., 2005
Se, SM	DEP	Sim	Anaero / 30°C	$t_{1/2}$ ≈ 15 d	Chang et al., 2005
Se, SM	DnBP	Sim	Anaero / 30°C	$t_{1/2}$ ≈ 9.0 d	Chang et al., 2005
Se, SM	DEHP	Sim	Anaero / 30°C	$t_{1/2}$ ≈ 26 d	Chang et al., 2005
S, AS	DMP	Sim	Aero / 25°C	$t_{1/2}$ ≈ 2.3 d	Jianlong et al., 2004
S, AS	DEP	Sim	Aero / 25°C	$t_{1/2}$ ≈ 3.7 d	Jianlong et al., 2004
S, AS	DnBP	Sim	Aero / 25°C	$t_{1/2}$ ≈ 8.5 d	Jianlong et al., 2004
S, AS	DnOP	Sim	Aero / 25°C	$t_{1/2}$ ≈ 28 d	Jianlong et al., 2004
Se, SM	DEP	Sim	Anaero / 30°C	$t_{1/2}$ ≈ 34 d	Yuan et al., 2002
Se, SM	DnBP	Sim	Anaero / 30°C	$t_{1/2}$ ≈ 14 d	Yuan et al., 2002
Se, SM	BBP	Sim	Anaero / 30°C	$t_{1/2}$ ≈ 19 d	Yuan et al., 2002
Se, SM	DEHP	Sim	Anaero / 30°C	$t_{1/2}$ ≈ 35 d	Yuan et al., 2002
Se, SM	DEP	Sim	Aero / 30°C	$t_{1/2}$ ≈ 2.5 d	Yuan et al., 2002
Se, SM	DnBP	Sim	Aero / 30°C	$t_{1/2}$ ≈ 2.9 d	Yuan et al., 2002
Se, SM	BBP	Sim	Aero / 30°C	$t_{1/2}$ ≈ 3.1 d	Yuan et al., 2002
Se, SM	DEHP	Sim	Aero / 30°C	$t_{1/2}$ ≈ 15 d	Yuan et al., 2002

[1] Types of test media

Se=Sediment SL = Sludge

SM= Synthetic medium C=Compost

S=Soil AS = Activated Sludge

[2] Test type (simulation or screening)

[3] If not presented in the original publication, half lives were calculated where possible

The results presented in Table 2 indicate that most recent biodegradation studies incorporate synthetic growth media, and that biodegradation results can vary widely. For example two of seven half-life measurements for DEHP were > 140 days, with all other studies reporting half-life times of < 35 days. For a specific example, studies by Chang et al. (2005) and Lertsirisopon et al. (2006) both conducted biodegradation experiments in freshwater sediments amended with synthetic media. Their reported half-life times for DEHP differed by approximately an order of magnitude (26 d and >200 d, respectively). There are several possible explanations for the wide variation in results, including differences in incubation temperature and different proportions of sediment to synthetic medium. Only two of the studies listed in Table 2 used natural media without synthetic medium amendments: Xu et al. (2008) determined soil half-lives for DnBP and DEHP of 8.0 and 28 days, respectively, and Kao et al. (2005) determined sediment half-lives for DnBP and DEHP of 75 and 147 days, respectively. While both studies utilized a natural consortium of degrading organisms, the tests are difficult to compare directly as they were conducted in different media (soil vs. sediment) and under different conditions (e.g., oxygen demand, temperature). Careful consideration of the individual test conditions is required before extrapolating test results to predictions of biodegradation in the environment as the incubation conditions may be very different than those expected in natural soils or sediments.

In 2008, Liang et al. published a review of DPE biodegradation that focused on tests employing microorganisms isolated from select environmental media, and cultured in synthetic media. Their review of the literature indicated that a broad range of DPEs can be degraded rapidly by bacteria isolated from many different freshwater and marine

sediments, soils, and sludges. While these results are meaningful with regards to determining the inherent biodegradability of DPEs, caution should be employed when extrapolating results to natural media, as consideration must be given to differences between synthetic media and the natural media, and how these differences may affect the reported rates of biodegradation.

The review by Staples et al. (1997) identified 12 studies (up to 1997) of five individual phthalates (DMP, DnBP, BBP, DEHP, DiNP) which included sediments as the degrading medium. Within the identified studies (presented in Table 3), a wide range of phthalate degradation results were reported under a variety of conditions (temperature, concentration etc.) and over various incubation periods. These studies indicate that in general, when tested in natural sediment, a greater degree of biodegradation is observed for lower molecular weight phthalates than for higher molecular weight phthalates.

1.4.1 Lag Phases in Biodegradation Studies

The length and occurrence of the lag phase is unpredictable and can vary with many of the same factors that affect biodegradation rates (i.e. location, chemical concentration, microbial community, temperature and others) (Aronson and Howard, 1999). If the lag period is sufficiently long, it may be environmentally significant as the chemical may persist long enough to become widely distributed, and affect

Table 3. Summary of phthalate biodegradation literature in sediments (1975 – 1995) (modified from Staples et al., 1997)

DPE	Acclimation / Nutrients added?	Oxygen Demand / Endpoint	Temp. (°C)	Initial Test Conc. (ppm)	Test Duration (Days)	Degra-dation (%)	Reference
DMP	N/N	Anaerobic - Primary	35	81	56-96	18-40	Madsen et al. 1995
DMP	N/N	Anaerobic - Primary	35	50	56-96	18-40	Madsen et al. 1995
DnBP	N/N	Aerobic - Primary	28	<3.8	7	32-49	Steen et al. 1980
DnBP	N/N	Aerobic - Primary	25	0.4-0.55	7-9	100	Walker et al. 1984
DnBP	N/N	Aerobic - Primary	28.5	10-1000	42-56	59-96	Tagatz et al. 1986
DnBP	N/N	Aerobic - Ultimate	22	0.082-8.2	14	71-85	Johnson et al, 1984
DnBP	N/N	Aerobic - Ultimate	12	nr	14	80	Johnson et al, 1984
DnBP	N/N	Aerobic - Ultimate	28	nr	14	95	Johnson et al, 1984
DnBP	N/N	Aerobic - Ultimate	5	nr	14	50	Johnson et al, 1984
BBP	N/N	Aerobic - Primary	nr	1	7	95	Gledhill et al. 1980
BBP	N/N	Aerobic - Primary	20	0.01-0.1	2	47-60	Adams et al. 1989
BBP	N/Y	Anaerobic - Primary	30	62	42-100	100	Painter and Jones, 1990
BBP	N/N	Anaerobic - Primary	nr	1	2	50	Adams and Saeger, 1993
BBP	N/N	Aerobic - Ultimate	nr	1	28	50	Adams and Saeger, 1993
BBP	N/N	Aerobic - Ultimate	nr	1	28	51-65	Gledhill et al. 1980
BBP	N/N	Aerobic - Ultimate	20	0.01-0.1	30	10.4	Adams et al. 1989
BBP	N/N	Anaerobic - Ultimate	nr	1	28	45	Adams and Saeger, 1993
DEHP	N/Y	Anaerobic - Primary	30	7.8-78	365	0-14	Painter and Jones, 1990
DEHP	N/N	Anaerobic - Primary	nr	nr	14	0	Schwartz et al. 1979
DEHP	N/N	Aerobic - Ultimate	10	1.4	27	38	Sodergren, 1982
DEHP	N/N	Aerobic - Ultimate	5	0.018	28	1	Johnson et al, 1984
DEHP	N/N	Aerobic - Ultimate	12	0.018	28	3	Johnson et al, 1984
DEHP	N/N	Aerobic - Ultimate	22	0.018-10	28	6-24	Johnson et al, 1984
DEHP	N/N	Aerobic - Ultimate	28	0.018	28	10	Johnson et al, 1984
DEHP	N/N	Aerobic - Ultimate	20	nr	28	20	Fish et al. 1977
DEHP	N/N	Anaerobic - Ultimate	22	1	300	0	Johnson and Lulves, 1975
DiNP	N/N	Aerobic - Ultimate	22	0.02-10	28	<1-8	Johnson et al, 1984

nr indicates value not reported

susceptible species before it is degraded (Wiggins et al., 1987). There are several possible mechanisms that may account for lag phases in biodegradation testing; these include:

- time needed for preferential growth of degraders to a level at which degradation of test compound is detectable (Chen and Alexander, 1989);
- time required for the induction of enzyme(s) in a reaction pathway (Aronson and Howard, 1999);
- preferential use by micro-organisms of other organic compounds before the test chemical is utilized as a carbon source (Ingerslev, 2000);
- degrader predation by heterotrophic protozoa (Wiggins et al., 1987).

While induction of certain necessary enzymes in bacteria has been proposed as a factor contributing to the lag phase, the time needed for such induction is normally considered short (minutes or hours) (Wiggins et al., 1987). Ingerslev et al. (2000) investigated the importance of test volume on lag phase in biodegradation of *p*-nitrophenol and observed longer / more variable lag phases, or a lack of degradation in samples of river water less than 50ml. The authors postulated that one possible explanation for this observation is that at small test volumes, the total number of degraders was lacking or reduced to a level where they were unable to proliferate. Other possible explanations include that the microbial community was not completely present; so that the degrading species were either not present, or metabolically active (as they may depend of partners). Also, it is possible that the physiochemical environment of the test's

incubation changed the proportion of degraders in the community, which affected the rate of *p*-nitrophenol degradation (Ingerslev et al., 2000).

1.4.2 Biodegradation Phase

Once biodegradation proceeds, the kinetics of this process can be mathematically described. While there are several rate equations that can describe the rate of biodegradation (Scow et al., 1986; Roslev et al., 1998) a first order kinetic model is commonly assumed (Aronson and Howard, 1999). First order rate constants are most applicable when the concentration of microbial substrate (parent compound in biodegradation tests) is low, resulting in little or no change in bacterial biomass. First order kinetics is mathematically described by the following equations:

$$\frac{dX_t}{d_t} = -k \cdot X_t \qquad \text{or,} \qquad (1)$$

$$X_t = X_{t0} \cdot exp^{(-kt)} \qquad (2)$$

where X_{t0} (g) represents the initial mass of chemical, X_t (g) is the mass of chemical in a test at time interval t, t (day) represents time and k (day^{-1}) is the observed (or measured) first order rate constant of degradation.

The first order rate constant can be used to determine the half-life of a chemical in various environmental media using the following equation:

$$t_{1/2} = \frac{\ln 2}{k} \qquad (3)$$

where $t_{1/2}$ (day) represents the half-life time in sediments, and k (day^{-1}) is the first order biodegradation rate constant. Rate constants and half-lives determined in simulation tests can then be used in multi-media environmental fate models which take into account

partitioning behaviour, advective, and reactive processes present in situ for each medium to determine an overall environmental half-life (Webster et al., 1998).

1.4.3 Stationary Phase

In many biodegradation studies, including those involving DPEs, (Sholtz et al., 1997; Ingerslev et al., 2000; Hu and Wan, 2006), chemicals are not completely degraded. There are several possible explanations for this phenomenon. Ruben et al. (1982) suggested that different microorganisms are responsible for chemical degradation at high and low concentrations. Specifically, specialized microorganisms (eutrophs) degrade chemicals quickly at high concentrations but they cannot sustain themselves at low concentrations of parent compound. Other microorganisms (oligotrophs) can degrade chemicals at low concentrations (Ruben et al., 1982). Oligotrophs are less specific, and may prefer to degrade other carbon sources and chemicals in the sample. Another possible explanation is that at very low substrate concentrations, insufficient chemical is present to induce the biochemical pathway in degrading microorganisms (Aronson and Howard, 1999). A third explanation is that the remaining low concentrations of chemical observed are not bioavailable to the degrading bacterial community. This fraction of chemical may be strongly bonded to sediment particles, or sample containers (Peterson and Staples, 2002). The latter scenario can be investigated by exploring the chemical properties of the compound in question (e.g., solubility, log K_{ow}, log K_{oc}). A final possibility is that required co-factors such as carbon, nitrogen or other nutrients have been depleted and therefore metabolism stops (Chi et al., 2007).

2: Theory

2.1 Development of a Theoretical model to describe DPE biodegradation rates

It is generally understood that chemicals that are adsorbed in sediments must first desorb into the aqueous phase before becoming bioavailable to microorganisms for metabolism (Beckles et al., 2007). The process by which organic chemicals are biodegraded in natural systems can be hypothetically separated into several sequential steps: desorption of chemical into the aqueous phase, mass transfer of chemical to biologically accessible regions (e.g., out of particle micro-pores), and biological uptake and transformation (Zhang et al., 1998). Therefore, it is possible for desorption to be an important rate limiting step during the biodegradation process, and therefore can influence the environmental fate, and ecotoxicity of sediment bound contaminants (Cornelissen et al., 2005). Using first order kinetics and equilibrium partitioning equations, a simple mathematical model was developed to explore the relationship between a chemical's biodegradation rate constant (k_t) and K_{ow}.

Model Terms

X_t = total mass of chemical in incubation jar (µg)
X_w = mass of chemical dissolved in water (µg)
X_s = mass of chemical sorbed to sediments (µg)
C_s = concentration of chemical sorbed to sediments (µg/g)
C_w = concentration of chemical dissolved in water (µg/l)
V_s = sediment volume in incubation jar (g)
V_w = water volume in incubation jar (l)
k_t = observed or apparent first order biodegradation rate constant (day^{-1})
k_i = inherent biodegradation rate constant (day^{-1})
k_{sw} = sediment/water partition coefficient (ml/g)

k_{ow} = octanol/water partition coefficient (unitless)
ϕ = fraction of freely dissolved chemical in incubation jar (unitless)
$\propto_{oc}$ = proportionality constant for K_{oc} prediction from K_{ow} (unitless)
ϕ_{oc} = fraction of organic carbon in False Creek sediment (unitless)

Working under the assumption that only freely dissolved compound is bioavailable to sediment microorganisms for metabolism, equation 1 can be modified as follows:

$$\frac{dX_t}{d_t} = -k_i \cdot \phi \cdot X_t \qquad (4)$$

where k_i represents an inherent biodegradation rate constant, ϕ is the fraction of the total mass of chemical that is dissolved, and X_t is the total mass of chemical in an incubation chamber. ϕ can be defined as follows:

$$\phi = \frac{X_w}{X_t} = \frac{X_w}{X_w + X_s} \qquad (5)$$

where X_w is the amount of chemical dissolved in water, and X_s is the amount of chemical associated with sediments.

With the following equations:

$$X_i = C_i \cdot V_i \quad (6), \quad \text{and} \quad K_{sw} = \frac{C_s}{C_w} \qquad (7)$$

where X_i is mass of chemical in medium *i*, C is concentration of chemical in medium *i*, and V is volume medium *i*; and K_{sw} is the sediment water partition coefficient; equation 5 can be re-written as follows:

$$\phi = \frac{1}{1+K_{sw} \cdot \frac{V_s}{V_w}} \qquad (8)$$

Therefore, equation 4 can be re-written as:

$$\frac{dX_t}{d_t} = -\frac{k_i}{1+K_{sw}\cdot\frac{V_S}{V_w}} \cdot X_t \qquad (9)$$

When comparing equations 1 and 9, it becomes apparent that

$$k_t = \frac{k_i}{1+K_{sw}\cdot\frac{V_S}{V_w}} \qquad (10)$$

Where k_t is the measured biodegradation rate constant. Eq. 10 illustrates that the apparent (actual) rate constant of biodegradation is a function of the inherent degradation rate constant k_i, but also of the degree of sorption of the chemical to the sediment. If the inherent biodegradability of all DPEs is the same, the biodegradation rates can vary among DPEs of different molecular weights due to differences in sorption coefficients to the sediment. This is illustrated by taking the logarithm of equation 10 after which we have the following arrangement:

$$\log k_t = \log k_i - \log\left(1 + K_{sw}\cdot\frac{V_s}{V_w}\right)$$

If $K_{sw}\cdot\frac{Vs}{V_w} \gg 1$ (the case for hydrophobic compounds), then

$$\log k_t = \log k_i - \log\left(K_{sw}\cdot\frac{V_s}{V_w}\right)$$

$$\log k_t = \log k_i - \log\frac{V_s}{V_w} - \log K_{sw}$$

$$\log k_t = \log k_i - \log\frac{V_s}{V_w} - \log \propto_{oc} \phi_{oc} K_{ow}$$

In this arrangement, $\propto_{oc}$ is a proportionality constant for K_{oc} conversion to K_{ow} (after Seth et al., 1999), and ϕ_{oc} is the fraction of organic carbon in sediments.

$$\log k_t = \log\left(\frac{k_i \cdot v_w}{V_s \cdot \propto_{oc} \cdot \phi_{oc}}\right) - \log K_{ow} \qquad (11)$$

Equation 11 indicates that the biodegradation rate constant (k_t) may vary proportionately to log K_{ow}, and fall with increasing K_{ow} of the chemical. This hypothesis can be tested using linear regression, by plotting the observed experimental log k_t vs. log K_{ow} (Figure 16) which is discussed further in section 4.3.

2.2 Study Objectives

The objectives of this study are to:

1) Conduct a simulation test to determine the biodegradation rates of a range of DPEs in natural sediments obtained from False Creek, British Columbia,

2) To confirm the biodegradation of DPEs by measuring the formation of MPE, one of the major metabolites of DPEs,

3) To test our mathematical model (equation 11) describing the relationship between the rate of phthalate biodegradation, and associated K_{ow},

4) To determine if any of the tested DPE congeners meet the criteria for persistence under the Canadian Environmental Protection Act (1999).

3: Methods

Methods are presented in three sections describing 1) the field sampling methodology, 2) experimental design, and 3) laboratory methods for sample extraction and analysis. Seven widely-used DPEs and their primary monoester metabolites were evaluated in the study, and are listed along with relevant chemical characteristics in Table 1, and their chemical structures are presented below in Figure 5.

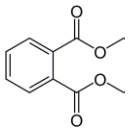
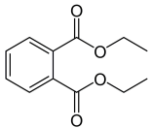
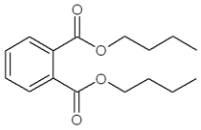
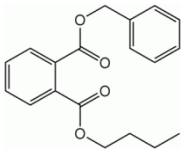
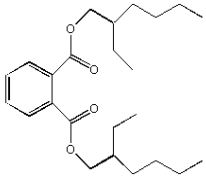
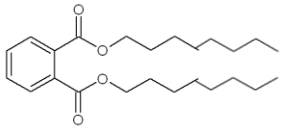
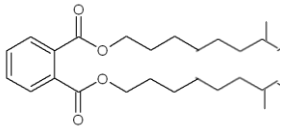
 Dimethyl phthalate (DMP)	 Diethyl phthalate (DEP)
 Di-n-butyl phthalate (DnBP)	 Butylbenzyl phthalate (BBP)
 Bis-2 di-ethylhexyl phthalate (DEHP)	 Di-n-octyl phthalate (DnOP)
 Diisononyl phthalate (DiNP)	

Figure 5. The structures of phthalate diesters used in this biodegradation study

3.1 Field Sampling Method

3.1.1 Study Site

False Creek is a shallow (mean depth ≈20m) urban embayment of the Strait of Georgia approximately 2.5 km in length located in downtown Vancouver, British Columbia (49°16'09"N 123°07'15"W) (Mackintosh, 2002). According to historic monitoring data, bottom temperatures range from 5 – 16^{o}C, pH ranges from 7.6 – 8.2 and dissolved oxygen from 6.2 to 10.4 mg/L (BCMOE, 2009). The fine silty sediments in False Creek contained approximately 2.8% total organic carbon, and 70% moisture content. Generally in marine sediments only a thin film of surface sediment contains molecular oxygen allowing aerobic respiration, below this zone the sediment is anoxic. These different geochemical conditions produce a stratified community structure of sediment microorganisms, with different assemblages in the aerobic and anaerobic zones. (Bertics and Ziebis 2009). There are many inputs of non point-source urban contamination into False Creek; according to a 2006 report from the British Columbia Ministry of Environment (BCMOE, 2006), there are 10 storm-water outfalls, and 5 combined sewer (sanitary overflow) outfalls discharging into the region (Figure 6).

3.1.2 Sampling Equipment

Due to the ubiquitous nature of phthalate esters, much effort was spent to minimize the potential for contamination of samples during collection and analysis. Sampling equipment and collection jars were cleaned according to a protocol developed at the Institute of Ocean Sciences (IOS); equipment was rinsed in acetone, toluene and dichloromethane (DCM) followed by baking for 10h at 400^{o}C. After baking, each item was then rinsed twice with iso-octane, once with DCM, twice with methanol and then

once again with DCM. Following this process, the collection jars and sampling equipment were covered with aluminium foil that had already been rinsed with hexane and DCM and baked at 400^{o}C overnight.

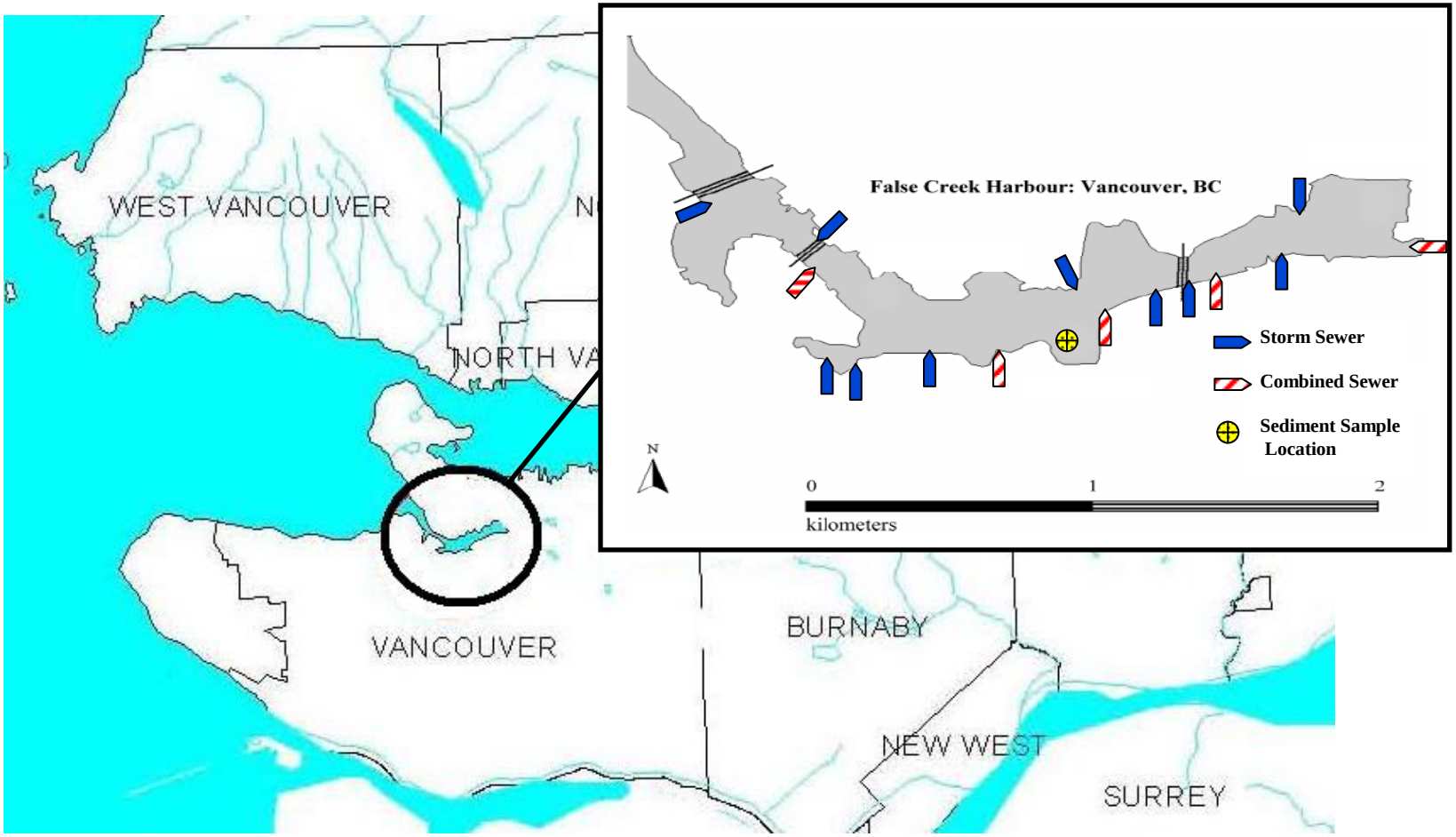


Figure 6. Location of sediment sampling, and approximate locations of combined sewer overflow and stormwater discharges in False Creek (modified from Mackintosh, 2002; BCMOE, 2006).

3.1.3 Sediment Sample Collection

Sediments were collected using a 6" petite Ponar ® sediment grab from a depth of 5m at the "Marina-South" location described in Mackintosh, 2002 (Figure 6). A single sample location in False Creek was deemed adequate for the biodegradation study, as two previous studies indicated that concentrations of DPEs do not vary significantly in surficial False Creek sediments (Mackintosh, 2002; McConnell, 2007). To collect sufficient sediment volume for biodegradation testing, multiple grabs were taken, and each was released into a shallow aluminium tray pre-cleaned according to the IOS protocol. The top 1 centimetre of sediment was collected and transferred to a large 2L jar and transported to the laboratory in an ice-filled cooler. False Creek water was collected in a 4L amber glass bottle from approximately 1 metre above the bottom using a 12-foot extendible stainless steel pole and equipment described in Mackintosh, 2002. After collection, the bottle was sealed with a foil-lined lid, placed on ice, and then transferred to a refrigerator.

3.2 Experimental Design

3.2.1 Materials

Individual phthalates DEP, DnBP, BBP and DEHP and internal standards (di-isobutyl phthalate (DIBP), di-n-octyl phthalate (DnOP) were purchased from Aldrich (Milwaukee, WI). Deuterated compounds: d_4-DMP and d_4-DnOP were purchased from Cambridge Isotope Laboratories (Andover, MA). Individual standard stock solutions were prepared at various concentrations in "distilled-in-glass" grade acetonitrile (ACN) (Caledon, ON, Canada) and combined into a spiking solution designed to produce

approximate nominal concentrations that were 100-fold greater than the background concentrations of DPEs measured in Mackintosh, 2002 and McConnell, 2007.

3.2.2 Glassware Preparation

Laboratory glassware was cleaned following the same method described above for the sampling equipment. Other materials such as caps of autosampler vials and filter paper which decompose at elevated temperatures, were rinsed twice with ACN.

3.2.3 Spiking Procedure

The DPE spiking solution was evaporated to near dryness in a 2L flask under a fume hood to minimize the effect of solvent on sediment micro-organisms. 500g of wet sediment was then added and stirred vigorously using a variable speed drill press for 12 hours at room temperature. An additional 1 kg of wet sediment was then added and stirred for an additional 2 hours. The objective of the spiking procedure was to provide a uniform concentration of DPEs in the incubation vessels (described below).

3.2.4 Incubation Procedure

The sediment incubation experiments were performed by transferring 30g of spiked sediments and 10ml of overlying False Creek water in 125ml pre-cleaned glass jars sealed with metal lids. Test jars were incubated at 12-14^{o}C in the dark for 144 days, while control jars were incubated for 96 days. To approximate natural conditions in False Creek, headspace gas exchange (i.e. lids were opened) was conducted twice per week to promote formation of an aerobic zone at the sediment surface and in overlying water. Lids of test jars were opened in a Microzone BM4-2a-49 Type II biosafety cabinet to prevent contamination of samples from microorganisms and DPEs sorbed to airborne dust.

Samples were then gently swirled at 120 rpm in a rotary shaker for 5 minutes. Dissolved oxygen (DO) in three dedicated DO control sample jars was monitored throughout the incubation using a YSI 58 laboratory DO meter. Temperature was monitored throughout the incubation period using an Extech 42275 data logger.

At pre-determined intervals (day 0, ½, 1, 2, 4, 8, 12, 24 48, 96 and 144), three jars representing triplicate samples were removed from the incubation chamber, amended with 300µl of a 1% $HgCl_2$ solution to stop metabolism, vortexed for 5 seconds and frozen at -20^{o}C to await extraction. Sediments were sampled with greater frequency early in the incubation in an attempt to capture the anticipated degradation of DPEs and formation of MPEs.

Controls consisted of triple autoclaved (24h between autoclaving) sediments amended with 300µl of a 1% $HgCl_2$ solution. Controls were incubated alongside experimental sediments to ensure that any observed disappearance of DPEs in the active test sediments could be attributed to the resident bacteria in the sediments. Easi-Cult ® TTC dip-slides (Orion Diagnostica) were used to demonstrate presence or absence of viable micro-organisms in sample and control sediments. These were inoculated by dipping into a 1:2000 dilution of sediment in sterile distilled water and incubated at room temperature for 48-hours.

3.3 Laboratory Methods

Sediments were extracted at SFU as described below, then shipped to the Institute of Ocean Sciences (IOS) for GC-MS quantitation of phthalate diesters, and LC/ESI-MS/MS analysis of phthalate monoesters.

3.3.1 Sediment Extraction

A diagram of the extraction process is provided in Figure 7. Wet sediment extraction was performed at room temperature using acetonitrile (ACN) as the extraction solvent with sonification by a Branson 5510 sonicator. Sediment extraction was initiated by weighing out 2.0 g of stirred wet sediments from each 125ml sample jar in a 20ml scintillation vial using a pre-cleaned spatula. 50µl of an internal standard solution containing 410 ng/µl DnOP, 35.3 ng/µl DIBP (in ACN) was then added along with an additional 10ml of ACN. Vials were then vortexed for 10 seconds, sonicated for 5 minutes and centrifuged for 5 minutes at 1300g. Overlying solvent was then removed and filtered into a 150ml round bottom flask using Whatman #1 filter paper. Sediments were extracted twice more (using 10ml ACN) and after the third filtering, the filter paper was rinsed with an additional 4ml of ACN. The pooled filtrate was then evaporated to near dryness using a rotary evaporator, and transferred to a 2ml autosampler vial and stored at -40^{o}C until shipped to the IOS laboratory for analysis.

Sediment extraction efficiencies were determined in a pilot study which compared the fraction of DPEs spiked in $HgCl_2$ amended sediments to the same amount of DPEs spiked in solvent. Extraction efficiencies for test phthalates are presented in Table 4.

3.3.2 GC/MS and LC/ESI-MS/MS Analysis of Sediment

Each sample was analyzed for DPEs and MPEs. A detailed description of the methods used for the analysis of DPEs and MPEs is provided in Mackintosh et al. (2004) and Blair et al. (2009) respectively. Briefly, DPE sample extracts were quantified using low-resolution gas chromatography mass spectrometry (GC/LR-MS). After GC/MS analysis,

sample extracts were analyzed for MPEs by LC/ESI using tandem mass spectrometry (MS/MS) for the quantification of the individual phthalate monoesters.

Table 4. Extraction efficiencies from DPE spiked sediment pilot study (standard deviation) (n=3).

Phthalate Ester	Abbrev.	Extraction Efficiency % (± SD)
DPEs		
Dimethyl phthalate	DMP	25 (2)
Diethyl phthalate	DEP	45 (5)
Di-n-Butyl phthalate	DnBP	86 (8)
Butylbenzyl phthalate	BBP	82 (6)
Di -2-Ethylhexyl phthalate	DEHP	82 (8)
Di-n-Octyl phthalate	DnOP	103 (4)
Diisononyl phthalate	DINP	81 (15)

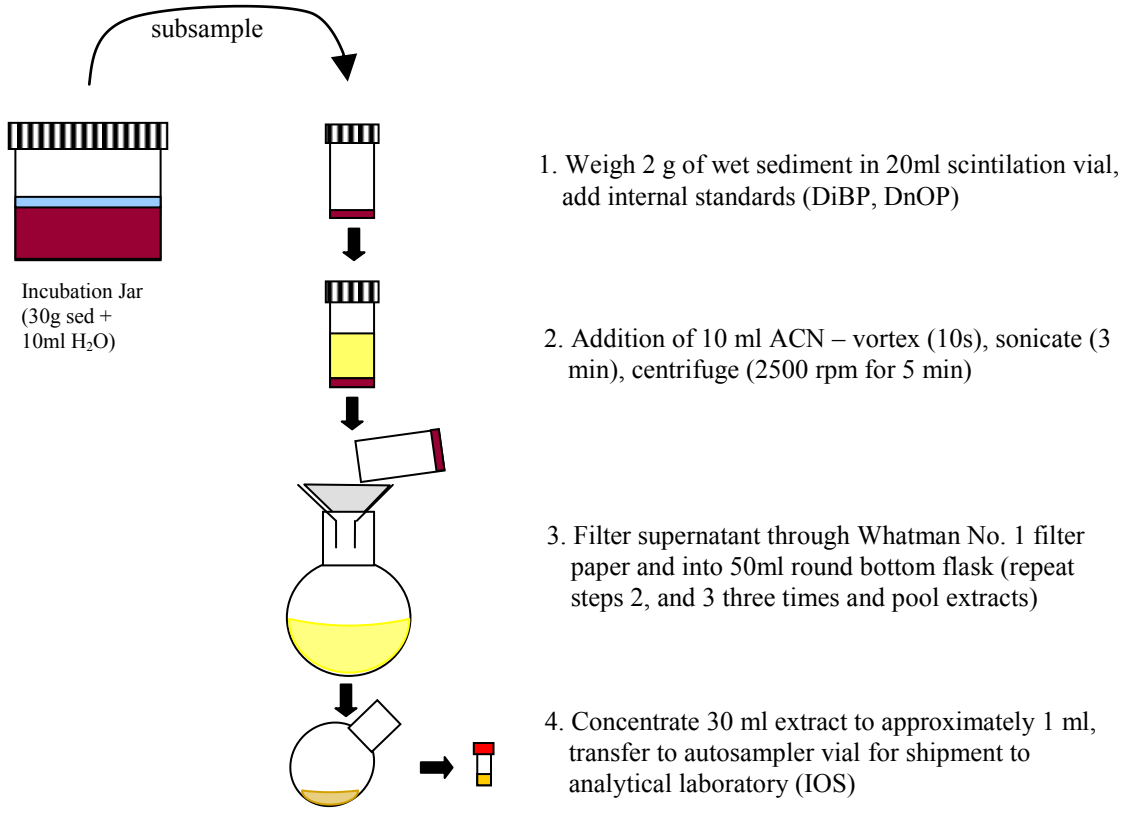


Figure 7. Schematic of the phthalate ester extraction methodology

3.3.3 Quality Assurance / Quality Control

Because of the ubiquitous nature of phthalates, three triplicate procedural blanks were prepared by performing the extraction process using solvents and glassware only (i.e., without test sediment). These samples were to provide a measurement of the background concentrations of phthalates introduced during the extraction process, and provide a basis for calculation of the method detection limit (MDL) for each detectable DPE. Measurable concentrations of only two DPE congeners (DnBP, and DEHP) were reported in procedural blanks; therefore, for these congeners, sample concentrations were corrected for the blank, and method detection limits (MDLs) were calculated as the mean + 3 standard deviations of the blanks. For the other DPE and MPE congeners, a limit of quantification was determined by taking the lowest detectable calibration standard (ng/ml) multiplied by the final volume of extraction solvent (ml), divided by the original amount of extracted sediment (g). Sterile (negative) and dip-slides (positive) controls were also implemented as discussed above in Section 2.2.4.

3.3.4 Data Analysis and Statistics

The biodegradation kinetics for each DPE congener were determined assuming first order kinetics by performing linear regression of the slope of geometric mean dry weight concentration data over the degradation phase of the incubation period; or if no degradation phase was apparent, over the entire incubation period. If the linear regression was statistically significant ($p < 0.05$), half lives were calculated according to equation (3).

4: Results and Discussion

4.1 Test Conditions

Figure 8 illustrates the temperature and dissolved oxygen concentrations in the sediment test that were recorded over the incubation period. Temperature was maintained at 13 ± 1.5°C and dissolved oxygen (DO) concentrations (measured in overlying water) slowly rose from an initial concentration of approximately 1 to 2 mg/L in the early stages of incubation, to approximately 5 mg/L after 70 days.

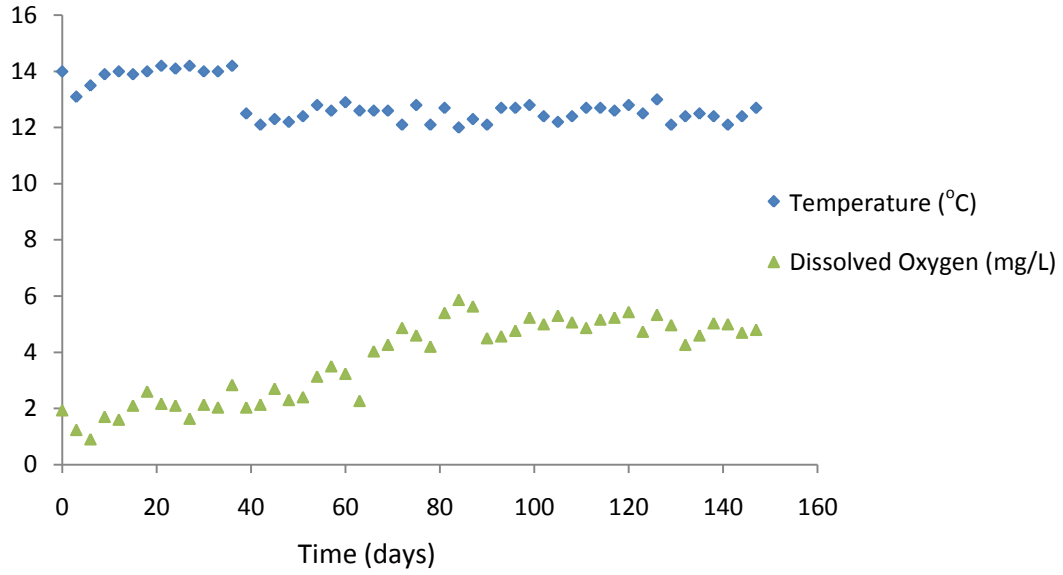


Figure 8. Temperature (°C) and dissolved oxygen (mg/L) over incubation period.

The incubation temperature was similar to the mid-upper range of False Creek, where bottom water temperature ranges from 5 to 16°C between winter and summer

(BCMOE, 2009). Dissolved oxygen in False Creek ranges from 6.2 to 10.4 mg/L (BCMOE, 2009), indicating that the initial DO concentrations observed in incubation jars were below the desired levels.

The initial low levels of DO in overlying water were of concern as one of the primary aims of the study was to incubate sediment under approximate natural conditions. Therefore while a more aggressive oxygenation method (sparging) was contemplated no action was taken as this would have introduced a higher risk of DPE contamination. In addition, aggressive oxygenation of sediments would have altered the conditions of the test so they were no longer consistent with False Creek sediments in situ. While oxygen is not a requirement for DPE biodegradation, Yuan et al. (2002) found that the rates of biodegradation for DEP, DnBP, BBP, DEHP are higher in aerobic sediments than for sediments incubated under anaerobic conditions. It is possible that the low initial levels of DO were a result of high metabolic activity of sediment microorganisms, utilizing readily available sources of carbon (DPEs and other natural substrates) and co-factors.

4.2 DPE Biodegradation and MPE formation

Results of the DPE simulation test are discussed below; Table 5 summarizes partitioning characteristics (log K_{oc}, log K_{ow}) and biodegradation rate constants, and associated sediment half-life times.

Table 5. log K_{ow}, log K_{oc}, degradation rate constants (k_{bio}) and half-life time ($t_{1/2}$) of DPEs in days.

Chemical	log K_{ow}	log K_{oc} [3]	k_t (day $^{-1}$) (*p-value*)	k_c (day $^{-1}$) (*p-value*)	Sediment Half-Life $t_{1/2}$ (days)
Dimethyl (DMP)	1.61	5.59	0.23 (0.0002)[1]	-0.0014 (0.33)	3.0
Diethyl (DEP)	2.38	3.77	0.15 (0.001)[1]	-0.0078 (0.20)	4.5
Di-n-Butyl (DnBP)	4.45	4.90	0.08 (0.09) [2]	-0.0087 (0.19)	8.0
Butylbenzyl (BBP)	4.59	6.21	0.24 (0.0001)[1]	-0.010 (0.17)	3.0
Di -2-Ethylhexyl (DEHP)	7.50	9.20	2.1E-03 (0.03)[1]	0.0056 (0.20)	340
Di-n-Octyl (DnOP)	8.06	8.91	4.1E-03 (0.14)	-0.0018 (0.31)	-
Di-isononyl (DINP)	9.40	9.99	5.8E-05 (0.99)	0.017 (0.01)[1]	-

[1] Indicates Statistically Significant ($p < 0.05$)

[2] Indicates Statistically Significant ($p < 0.10$)

[3] Observed log K_{oc} in False Creek Sediments from Mackintosh 2002.

- Half-life calculation not possible due to lack of statistically significant degradation (0.693/0.00)

Dip-slides taken on the 48th and 96th day of incubation showed microbial colony formation on dip-slides exposed to the test sediments, but none on dip-slides exposed to negative control sediments. Measured concentrations of DPEs and MPEs over the incubation period are presented in Table 6, and Table 7 respectively, normalized to sediment dry weights.

Detectable concentrations of MPEs were consistent with the disappearance of their parent diester, and their appearance coincided with the onset of rapid diester degradation. For higher molecular weight phthalates, slower degradation rates resulted in either low detectable concentrations of the monoester (e.g., MEHP) or non-detectable concentrations for the entire incubation (e.g., DiNP). Primary biodegradation of low and high MW MPEs has been determined to be very rapid in False Creek sediments (Otton et al., 2008); who found that after an 18 – 50 hour lag phase, both lower and higher molecular weight MPEs degraded rapidly with half lives ranging from 16 – 35 hours. Several MPEs (MnBP, MiBP, MiNP) have also been evaluated with OECD screening tests, and have been determined to be readily biodegradable (Scholtz, 2003).

Table 6. Summary of mean phthalate diester concentrations over time for experimental and negative control sediments (µg/g dw) (n=3) MDLs for DnBP and DEHP were determined to be 5.5 and 0.8 µg/g dw, respectively. Limit of Quantification (LOQ) for the remainder of DPEs was reported at 0.12 µg/g dw.

Day	d4 DMP		DEP		DnBP		BBP		DEHP		d4 DnOP		DNP	
	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev
Experimental Sediments														
0	19.2	5.78	61.3	18.3	50.4	28.7	329	94.4	834	75.8	36.7	9.94	16.1	0.71
0.5	11.9	6.48	40.0	19.5	46.1	32.6	202	55.3	879	7.08	21.9	5.25	15.8	1.56
1	14.9	10.0	49.0	24.1	18.3	9.84	188	20.7	949	57.0	22.1	13.0	14.3	0.89
2	11.6	5.98	51.1	9.29	94.6	129	146	22.3	966	246	21.4	6.29	11.2	5.15
4	3.57	0.89	19.6	4.40	125	28.1	57.3	29.2	977	22.4	16.9	6.66	12.7	0.24
8	2.29	1.10	13.3	6.18	182	110	65.7	44.9	983	163	23.5	13.3	14.6	2.54
12	1.00	0.18	9.80	2.18	238	137	21.7	21.7	963	8.32	24.1	5.02	14.9	0.17
24	0.32	0.09	10.6	7.74	15.5	5.17	<0.12	-	906	42.8	27.5	7.11	14.3	0.15
48	0.63	0.04	5.93	0.78	7.70	0.90	<0.12	-	900	74.0	31.8	13.7	13.5	1.62
96	0.39	0.20	4.89	3.17	6.46	4.42	<0.12	-	1034	311	9.71	6.92	14.8	5.67
144	0.17	0.17	3.98	1.54	8.21	4.01	<0.12	-	707	455	18.0	5.53	14.1	2.06
Control Sediments														
0	9.19	0.13	97.9	10.7	74.1	12.1	271	57.4	139	14.0	0.77	0.13	1.15	0.14
48	9.78	3.97	85.1	23.2	61.9	15.9	209	44.7	219	74.8	0.80	0.32	4.28	4.35
96	8.21	2.30	46.7	6.74	32.9	9.85	103	25.6	236	25.8	0.66	0.15	6.08	1.32

- indicates no calculation of standard deviation was possible due to a lack of detectable concentrations in test sediments

Table 7. Summary of mean phthalate monoester concentrations over time for experimental and negative control sediments (ng/g dw) (n=3). Limit of Quantification (LOQ) reported at 17.9 µg/g dw.

Day	MEP		MBP		MEHP		MoC9	
	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev
Experimental Sediments								
0	2.39	NA[a]	3455	167	48.31	2.68	3.80	0.08
0.5	<17	NA	3982	94.4	53.69	3.84	4.07	0.05
1	<17	NA	4424	1136	71.77	11.5	4.23	0.27
2	4256	17.27	6299	1091	81.39	14.6	6.34	1.54
4	2413	852	3535	1353	99.75	72.7	4.09	0.29
8	2020	1079	2877	1485	162.82	25.2	4.08	0.51
12	55.11	66.63	146	164	113.73	32.3	3.91	0.33
24	0.91	0.30	5.36	0.67	32.67	2.12	3.18	0.03
48	1.64	0.48	10.21	3.31	28.95	7.56	2.10	1.85
96	1.41	0.27	8.01	2.20	24.65	5.30	2.78	0.04
144	0.72	0.03	6.30	5.36	23.62	3.55	2.45	0.08
Control Sediments								
0	2.90	2.10	11.47	3.73	13.34	6.66	9.16	2.80
48	4.81	0.95	12.84	3.80	19.62	2.40	5.79	0.98
96	7.42	2.29	16.37	3.31	7.78	4.32	4.67	0.58

Shaded values indicate those that more than 3 times above the LOQ

[a] On day 0 MEP was detected in a single replicate, preventing calculation of standard deviation

4.2.1 Lower Molecular Weight Phthalates (1-7 carbon atoms in side chain)

d4 Dimethyl phthalate

The concentration of d4DMP exhibited an exponential decay pattern over time until stabilizing after approximately 24 days at approximately 0.3 µg/g (dry weight). This corresponded with a 98% depletion of the initial concentration (Table 6). Linear regression of the degradation period (day 0-12) indicated a sediment biodegradation rate constant (k_t ± 95% Confidence Interval (CI)) of -0.24 ± 0.07 d^{-1} (p=0.0002). The concentration of d4DMP in the inactivated sediments (negative control) did not decline significantly over 96 days, generating a rate constant (k_c ± 95% CI) of -0.0014 d^{-1}

(p=0.33), not statistically significant from zero. Due to the lack of statistical significance of control sediment results (slope of linear regression was not statistically different from 0) k_t was not corrected for abiotic degradation. A sediment half life time was calculated as 3 days (Table 5).

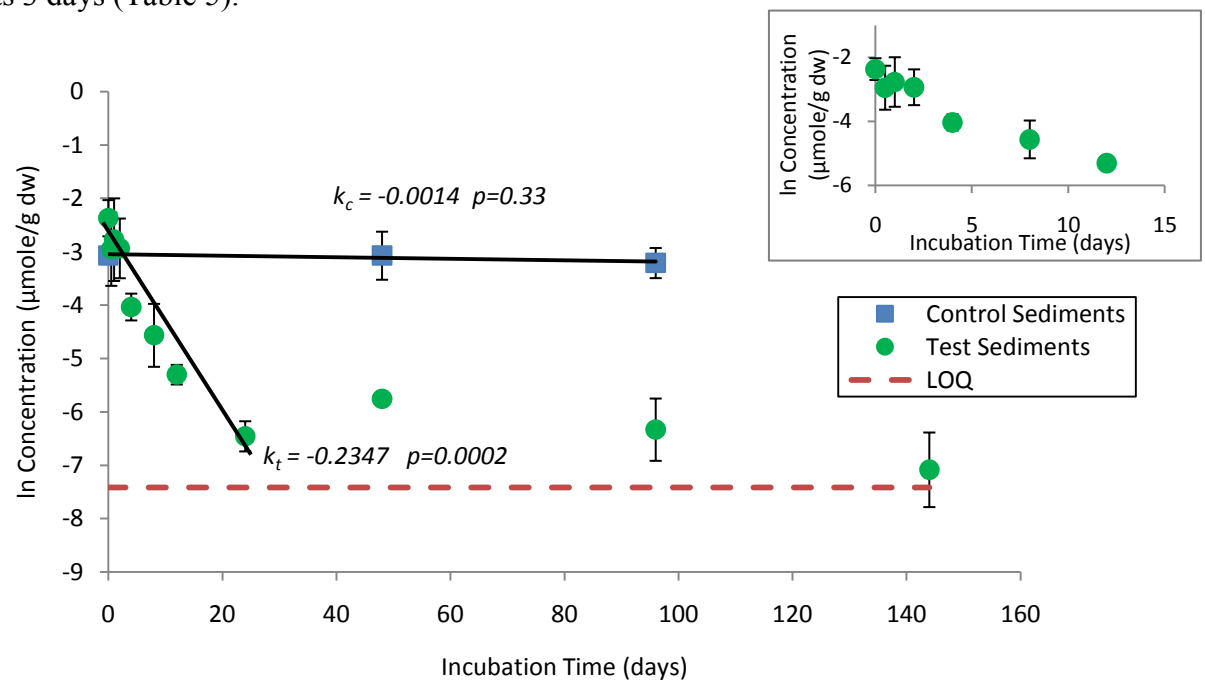


Figure 9. Mean ln concentration (µmol/g dw) of DMP in test and control sediments vs. incubation time. Insert shows concentration of d4DMP in sediment during the first 12 d of incubation. Biodegradation rate constants k_t and k_c were derived from the slope of the regression line. Error bars represent one standard deviation (n=3).

The rapid disappearance of d4DMP from test sediments is consistent with several tests using natural soils, for which biodegradation half-life times of 1.1 – 2.9 days (Suggat et al., 1984; Russell et al., 1985; Shanker et al, 1985) were reported. Biodegradation studies using synthetic media and bacteria derived from sediments (Li et al., 2005; Wang and Gu, 2006) reported biodegradation half lives of < 1 day. These very rapid degradation rates may have been influenced by the introduction of a nutrient

medium which may have: a) increased the dissolved fraction of chemical (enhancing bioavailability), or b) provided excess quantities of co-factors required in the biodegradation process.

d4Monomethyl phthalate

Due to an analytical error, d4MMP was not analyzed.

Diethyl phthalate

The concentration of DEP fell rapidly during the first 12 days of incubation and then stabilized at approximately 5 µg/g dw. This constituted a 92% decrease from original concentration) (Figure 10). Linear regression of DEP concentrations during the degradation period (day 0-12) yielded a sediment biodegradation rate constant (k_t ± 95% CI) of -0.15 ± 0.06 d^{-1} (p=0.002). DEP concentration in the negative control did not decrease significantly (k_c = -0.0078 p=0.20), so k_t was not corrected for abiotic degradation. The sediment half life time of DEP was calculated to be 5 days.

The rapid disappearance of DEP from test sediment agreed with observations by Yuan et al., (2002) who reported aerobic and anaerobic sediment half lives of 0.4-5.2 and 18.9-31.6 days, respectively, for tests using Taiwanese river sediments mixed with an artificial growth medium. In a similar study, Chang et al (2005) reported a sediment anaerobic biodegradation half life for DEP of 15.4 days at 30^{o}C. DEP has also been documented to degrade readily in freshwater (Ritsema et al., 1989; Furtmann, 1993).

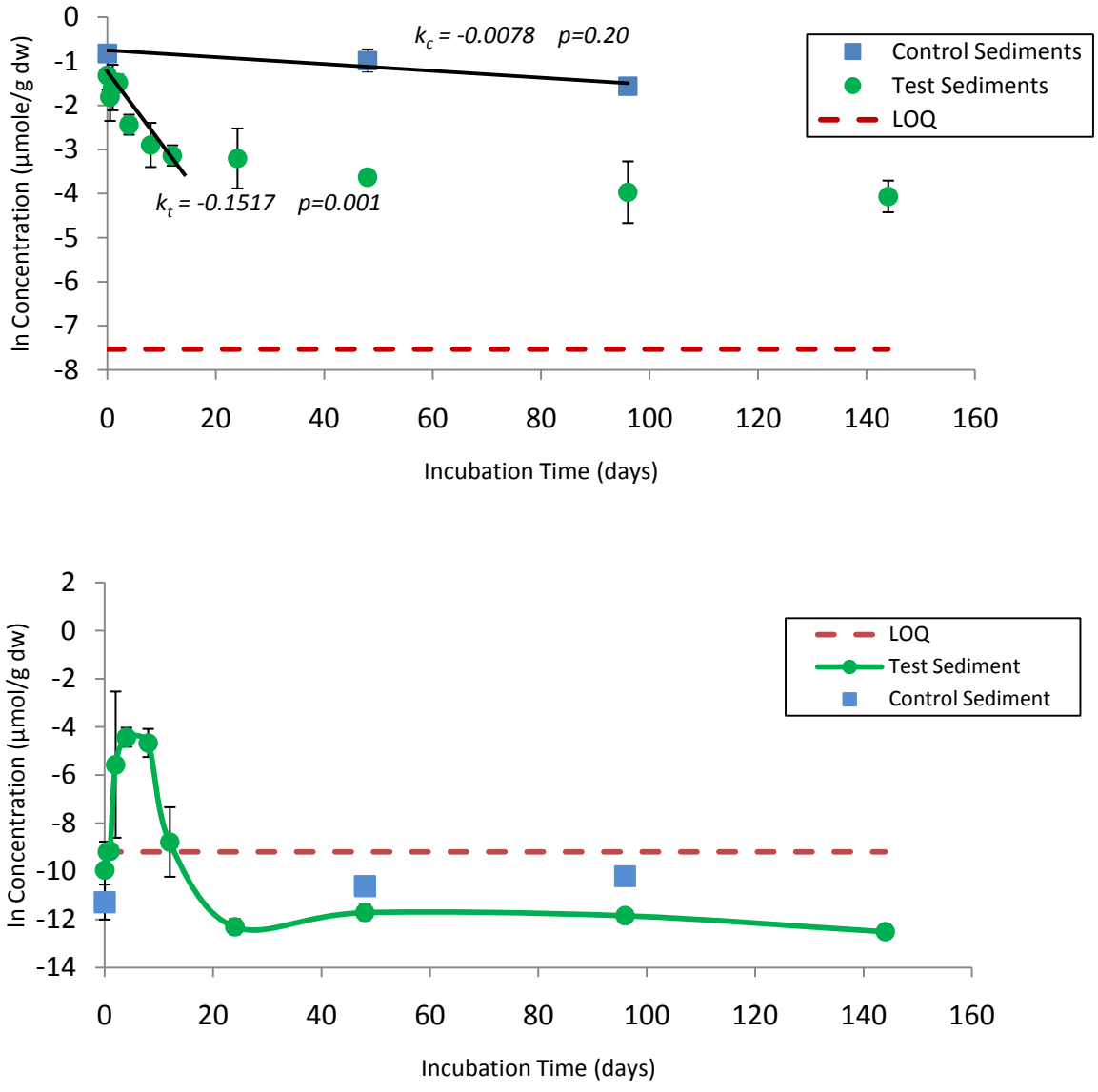


Figure 10. Mean ln concentration (µmol/g dw) of DEP (top) and MEP (bottom) in test and control sediments vs. incubation time. Biodegradation rate constants k_t and k_c were derived from the slope of the regression line. Error bars represent one standard deviation (n=3).

Monoethyl phthalate

Figure 10 illustrates that the rapid biodegradation of DEP was accompanied by an increase in the concentration of MEP, the expected main metabolite of DEP. Between days 1 and 2, MEP concentrations increased from the LOQ of 17ng/g to 4300 ng/g. MEP concentration then showed an initial gradual decrease (days 4-8), and then a rapid decline in MEP concentration until day 24, when concentrations of MEP stabalized below the LOQ. This temporal behavior of MPE corresponds with the biodegradation phase of DEP. The concentration of MEP fell from 2900 to 150 ng/g dw over the time period that coincided with the completion of DEP degradation (days 8–12), indicating that it degrades very quickly. This is consistent with observations from Otton et al, (2008); who reported a sediment half life for MEP of 1.5 ±0.42 days in False Creek sediments incubated at 22^{o}C.

Di-n-butyl phthalate

Dibutylphthalate did not follow the typical degradation pattern seen with d4DMP and DEP. Figure 11 shows that over the first day of incubation, DnBP concentration fell rapidly without a lag period; however, by day 2 the concentration of DnBP increased. Between days 1 and 12, the DnBP concentration in sediments increased more than 20-fold from 18 to 238 µg/g dw, after which time the concentration fell until stabilizing at approximately 7 µg/g on day 48 (86% reduction from the initial concentration). Analysis of the degradation period observed (day 8 – 48) yielded a statistically insignificant ($p = 0.09$) rate constant of $k_t = -0.0841$. No discernable contamination was observed in control sediments which showed no significant change in concentration of DnBP in sediments throughout the incubation period ($k_c = -0.0085$, $p=0.20$). The rise in concentrations of DnBP in test sediments indicates that despite the

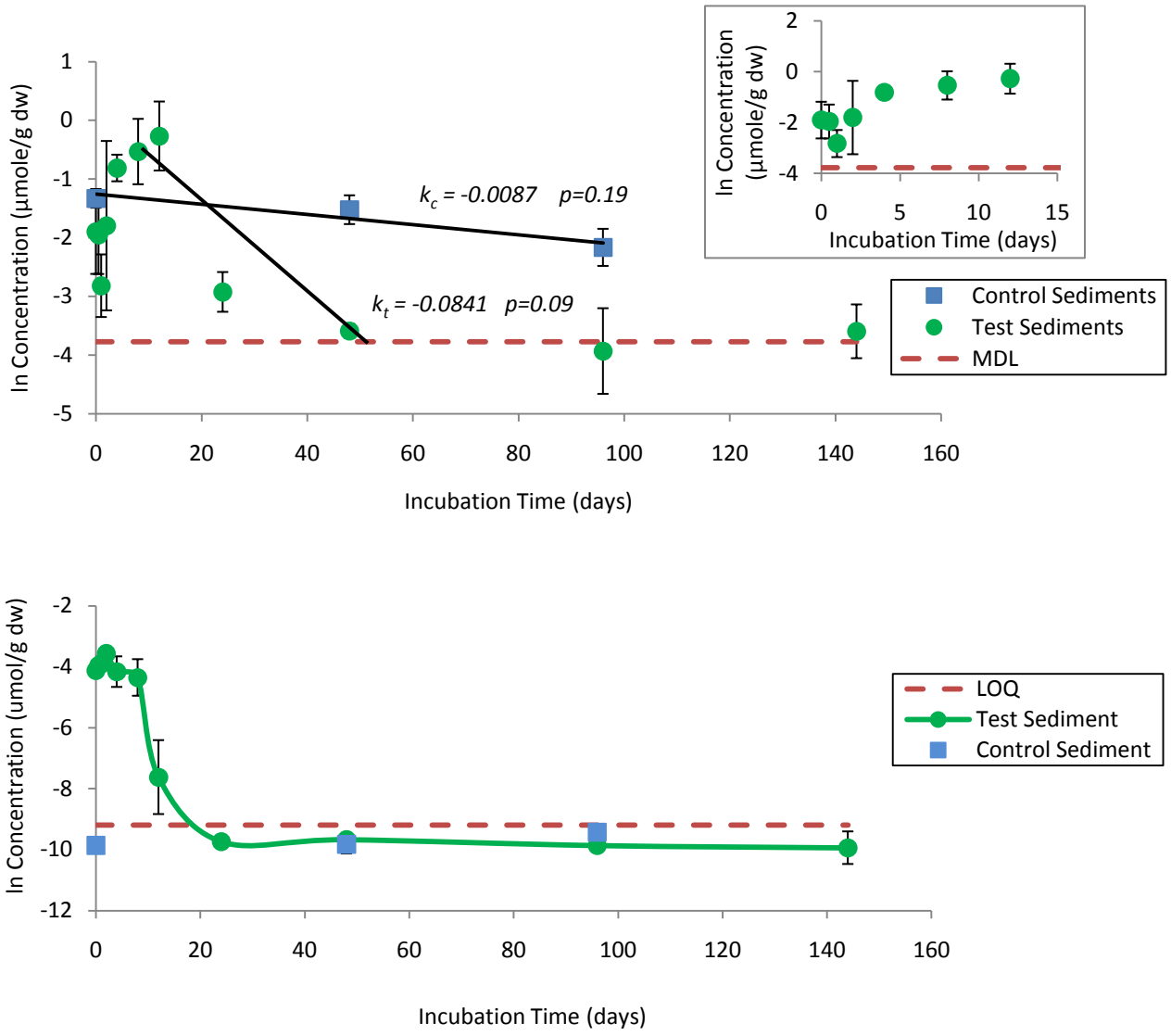


Figure 11. Mean ln concentration (µmol/g dw) of DnBP (top) and MnBP (bottom) in test and control sediments vs. incubation time. Top panel insert shows concentration of DnBP in sediment during the first 12 d of incubation. Biodegradation rate constants k_t and k_c were derived from the slope of the regression line. Error bars represent one standard deviation (n=3).

measures that were taken to discourage contamination of glassware and equipment, addition of DnBP to several samples occurred either during the incubation period, or during the extraction process. Several studies have revealed that DnBP and DEHP are commonly found laboratory contaminants (Frankhauser-Noti and Grob, 2007) and are also likely to be present in household dust (Rudel et al., 2003; Bornehag et al, 2005). It is unlikely that insufficient homogenization caused the observed increase in DnBP concentrations as all other DPEs in incubation jars were adequately mixed. Despite the fact that no statistically significant biodegradation rate was determined for DnBP, the data suggest that DnBP can biodegrade in False Creek sediments. The experimental design did include procedural blanks. The procedural blanks displayed no evidence of DnBP contamination. The apparent degradation in the first day of incubation suggests that DnBP biodegradation may occur similarly to the other LMW DPEs in this study (i.e., would have degraded rapidly in the absence of contamination), high initial concentrations of DnBP's primary metabolite (MnBP) support this hypothesis.

Our results are similar to the results of other studies on the sediment biodegradability of DnBP (Zhou et al., 2009; Lertsirisopon et al., 2006; Chang et al., 2005; Yuan et al., 2002) (Table 2). Two DnBP sediment biodegradation studies conducted in sediments above 5^{o}C and for longer than 7 days (Walker et al., 1984 and Johnson et al., 1984) reported DnBP sediment half life times of 7 and 5 days, respectively.

Mono-n-butyl phthalate

Initial MnBP sediment concentrations of 3000-6000 ng/g dw were detected and concentrations of MnBP remained high for the first 8 days of incubation. Concentrations

declined rapidly after 8 d until stabilizing below the LOQ after day 24 (Figure 11). MnBP was unique in this study as it is a primary metabolite of two of the tested diesters (DnBP and BBP), therefore observed concentrations of MnBP can be from either parent compound.

Butyl-benzyl phthalate

BBP underwent rapid biodegradation (with no initial lag phase) from the beginning of incubation (initial concentration of 328 µg/g) until day 24 when the concentration fell below the LOQ of 17 µg/g (Figure 12). Linear regression of the degradation period (day 0-12) returned a statistically significant sediment biodegradation rate constant (k_t ± 95% CI) of -0.24 ± 0.06 d^{-1} (p =0.0001). BBP did not degrade significantly in the negative control (k_c = -0.010 d^{-1} (p=0.16)). A sediment half life time of 3 days was calculated from k_t according to equation (3). This rate constant k_t is very similar to that reported for BBP in aerobic sediment by Yuan et al (2002) of 0.22 d^{-1}, and is in the same order of magnitude as that observed by Painter and Jones (1990), who reported a sediment biodegradation rate constant of -0.0949 d^{-1}.

Monobenzyl phthalate

Due to an analytical error, mono-benzyl phthalate (MBzP) was not analyzed. However, as discussed above (for MnBP), the other primary metabolite of butyl-benzyl phthalate (mono-n-butyl phthalate) was detected at high concentrations over the early stages of incubation (days 0-12). The high initial MnBP concentrations are likely (at least partially) the result of the high initial rate of BBP biodegradation. Both DnBP and BBP completed their biodegradation phases and entered their stationary phases at approximately the same (day 24), the time at which MnBP degradation reached

completion. The rapid clearance of MnBP from incubation jars subsequent to degradation of parent compounds is consistent with a previous reported sediment half life of 16 ±2 hours for MnBP in False Creek sediments, although incubated at 22°C (Otton et al., 2008).

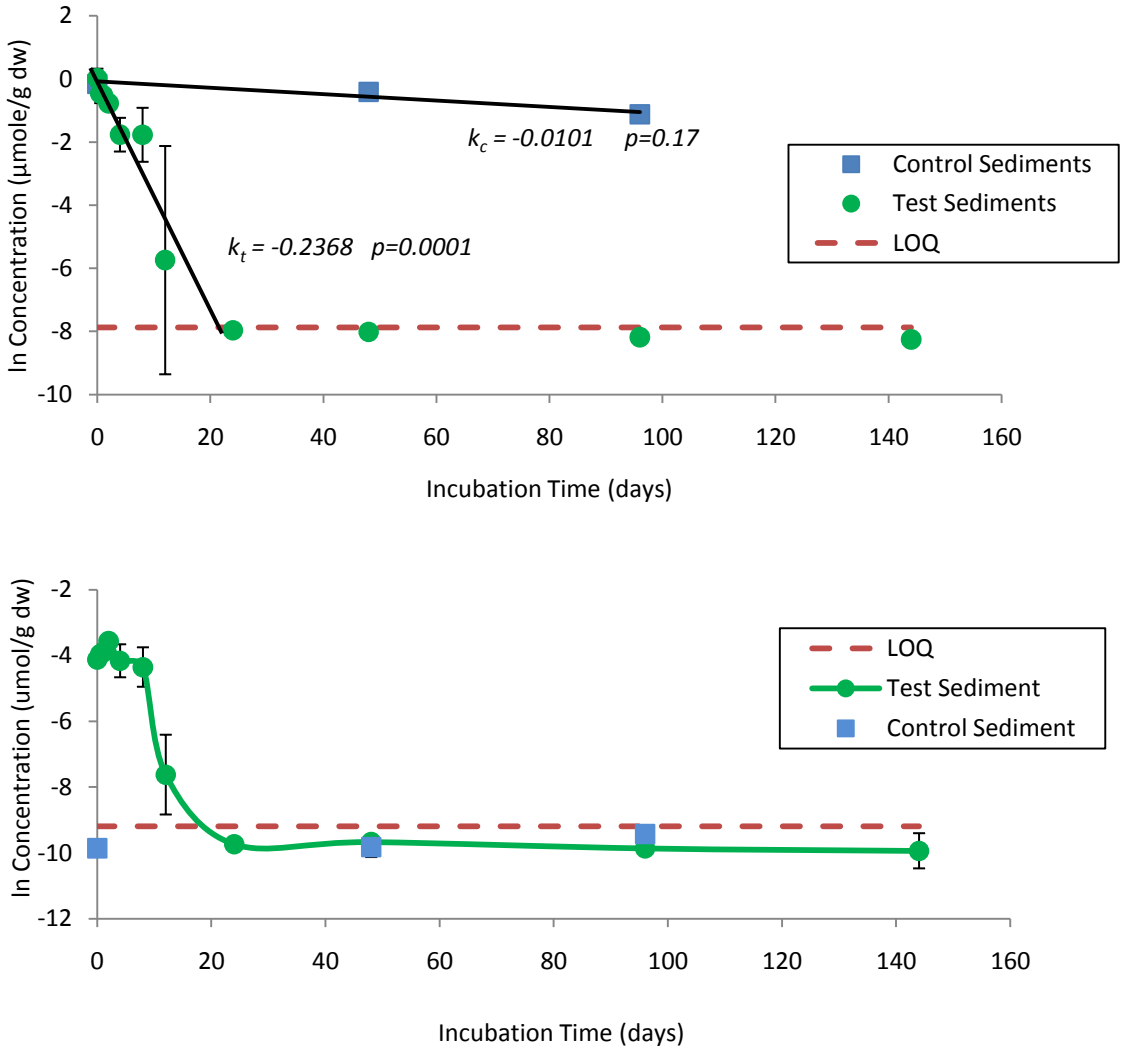


Figure 12. Mean ln concentration (µmol/g dw) of BBP (top) and MnBP (bottom) in test and control sediments vs. incubation time. Biodegradation rate constants k_t and k_c were derived from the slope of the regression line. Error bars represent one standard deviation (n=3).

4.2.2 Higher Molecular Weight Phthalates (>7 carbon atoms in side chain)

4.2.3 Bis-2 di-ethylhexyl phthalate

Concentrations of DEHP degraded slowly in test sediment over the incubation period. Approximately 85% of the original amount of chemical remained after 144 days (Figure 13).

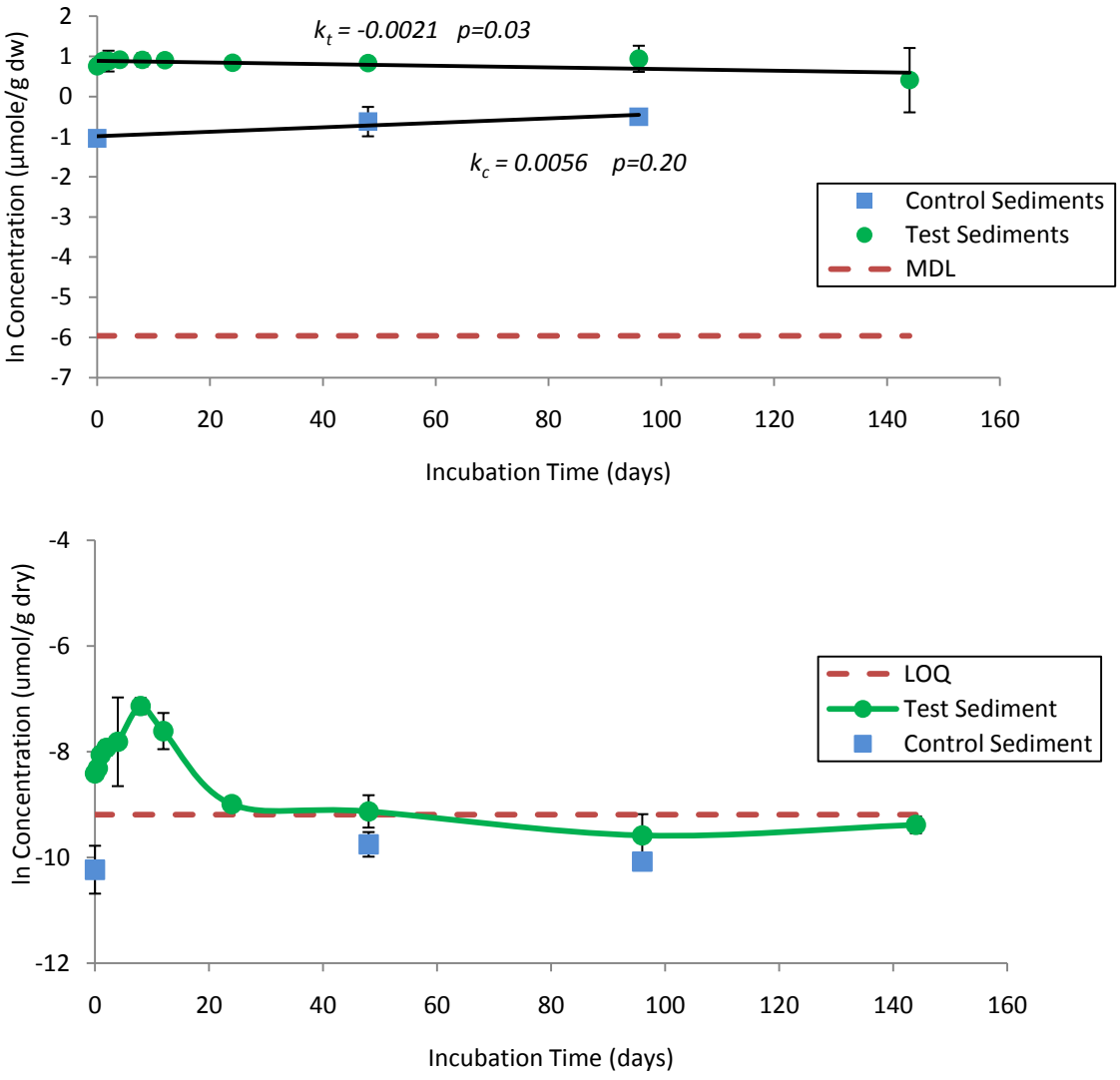


Figure 13. Mean ln concentration (µmol/g dw) of DEHP (top panel) and MEHP (bottom panel) in test and control sediments vs. incubation time. Biodegradation rate constants k_t and k_c were derived from the slope of the regression line. Error bars represent one standard deviation (n=3).

Linear regression of the logarithm of the DEHP concentration in the sediment versus time returned a statistically significant sediment biodegradation rate constant (k_t ± 95% CI) of -0.0021 ± 0.0017 d^{-1} (p=0.02), and a sediment half life of approximately 337 days. There was no statistically significant change in DEHP concentration in sterile control sediments during the incubation (k_c = 0.0056 ± 0.02 d^{-1} (p=0.20). While low molecular weight DPEs all showed extensive degradation over the first 12-24 days of incubation, this compound was relatively recalcitrant. This result is consistent with the findings from several sediment biodegradation studies. Lertsirisopon et al. (2006) Johnson et al. (1984); Painter and Jones (1990); and Johnson and Lulves (1977) each reported low rate constants for biodegradation (-0.003, -0.0008, -0.0006, no degradation respectively) at temperatures ranging from 10 to 30^{o}C (Table 3). Several studies have reported DEHP degradation in Taiwanese sediments (Yuan et al., 2002; Chang et al., 2005) and soils (Chang et al., 2009); however, these studies were all performed using 45ml of synthetic nutrient media seeded with 5g of sediment or soil. Use of a synthetic medium may have facilitated the observed biodegradation of DEHP in these studies due to the addition of supplemental nutrients and carbon into test sediments (Chang et al., 2009). Furthermore, the partitioning behaviour of DEHP may have been altered due to the inclusion of the synthetic medium (i.e., increasing the proportion of dissolved vs. sediment bound DEHP).

Mono-2-ethylhexyl phthalate

MEHP was detected above the limit of quantification, and it slowly increased from 48 to 162 ng/g dw over the first 12 days of incubation before stabilizing at 25-30 ng/g dw for the remainder of the incubation (Figure 13). Detection of MEHP despite the

lack of rapid biodegradation was likely due to the high concentration of DEHP in test sediments (approximately 850 µg/g dw), combined with the slow (but statistically significant) biodegradation rate. While degradation of MEHP has been shown to occur quickly ($t_{1/2}$ of 26 ± 9 hours at 22°C) (Otton et al., 2008), there may have been a continuous input from the slow degradation of the large pool of DEHP.

d4 Di-n-octyl phthalate

Concentrations of d4DnOP remained relatively stable in test sediment (compared with lower molecular weight DPEs) over the incubation period. Approximately 50% of the original amount of chemical remained after 144 days (Figure 14). Linear regression of test sediments indicated that the decrease in concentration was not statistically significant in either test (k_t = -0.0036 ± 0.0049 d^{-1} (p=0.12)), or control sediments (k_c = -0.0016 ± 0.017 d^{-1} (p=0.45)). As the slope of the linear regression was not significantly different from 0, no estimation of sediment half life was possible.

The low or non-existent rate of d4DnOP degradation is consistent with other published biodegradation values in the literature. Johnson et al. (1984) reported a first order biodegradation rate constant of -0.0008 d^{-1} for di-iso-octyl phthalate; and Shelton and Tiedje, 1981; Ziogou et al., 1989; Shelton et al., 1984 reported low amounts of degradation (0-44% over 70 days of incubation at 35-37°C). Furthermore, each test was performed using optimal temperature, and used sludge as the degrading media. These test conditions provide a high potential for degradation to occur, in comparison to the natural sediments and lower temperature used in the current study.

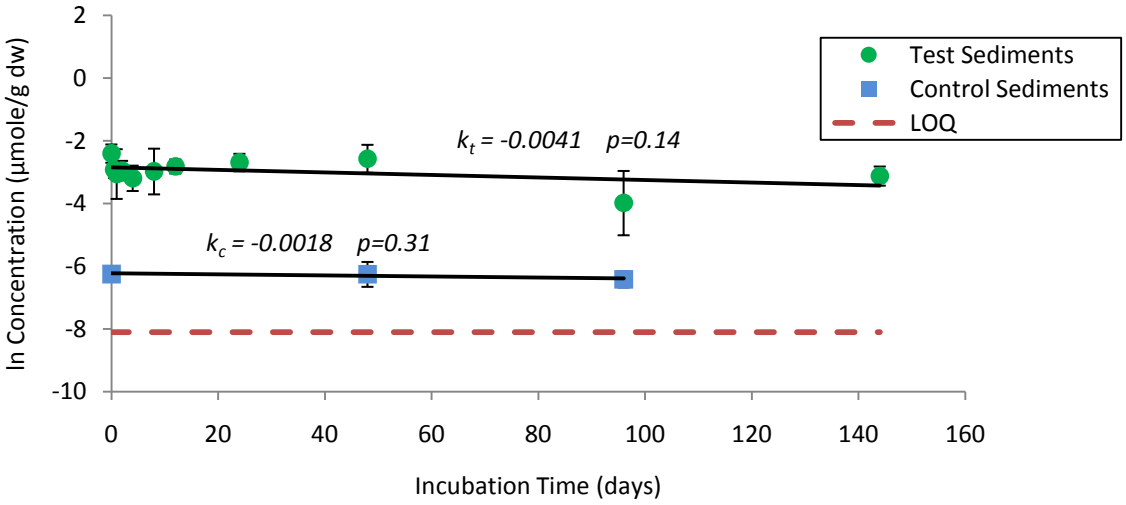


Figure 14. Mean ln concentration (µmol/g dw) of d4DnOP in test and control sediments vs. incubation time. Biodegradation rate constants kt and kc were derived from the slope of the regression line. Error bars represent one standard deviation (n=3).

d4Mono-n-octyl phthalate

Due to an analytical error, d4DnOP was not analyzed.

Di-isononyl phthalate

Like the two other higher molecular weight DPEs, concentrations of DiNP remained stable in test sediment (compared with LMW DPEs) over the incubation period (Figure 15). Approximately 87% of the original amount of chemical remained after 144 days. The slope of the linear regression of test sediment concentration over time was not statistically significantly different from 0 (k_t = -0.00005 ± 0.002 d^{-1}, p=0.99). Johnson et al. (1984) reported no significant DiNP degradation of 28 days at 12°C. DiNP concentration increased rapidly in control sediments, resulting in a significant positive rate constant of k_c = 0.0172 ± 0.013 d^{-1} (p=0.01) which corresponds with a doubling rate of approximately 40 days.

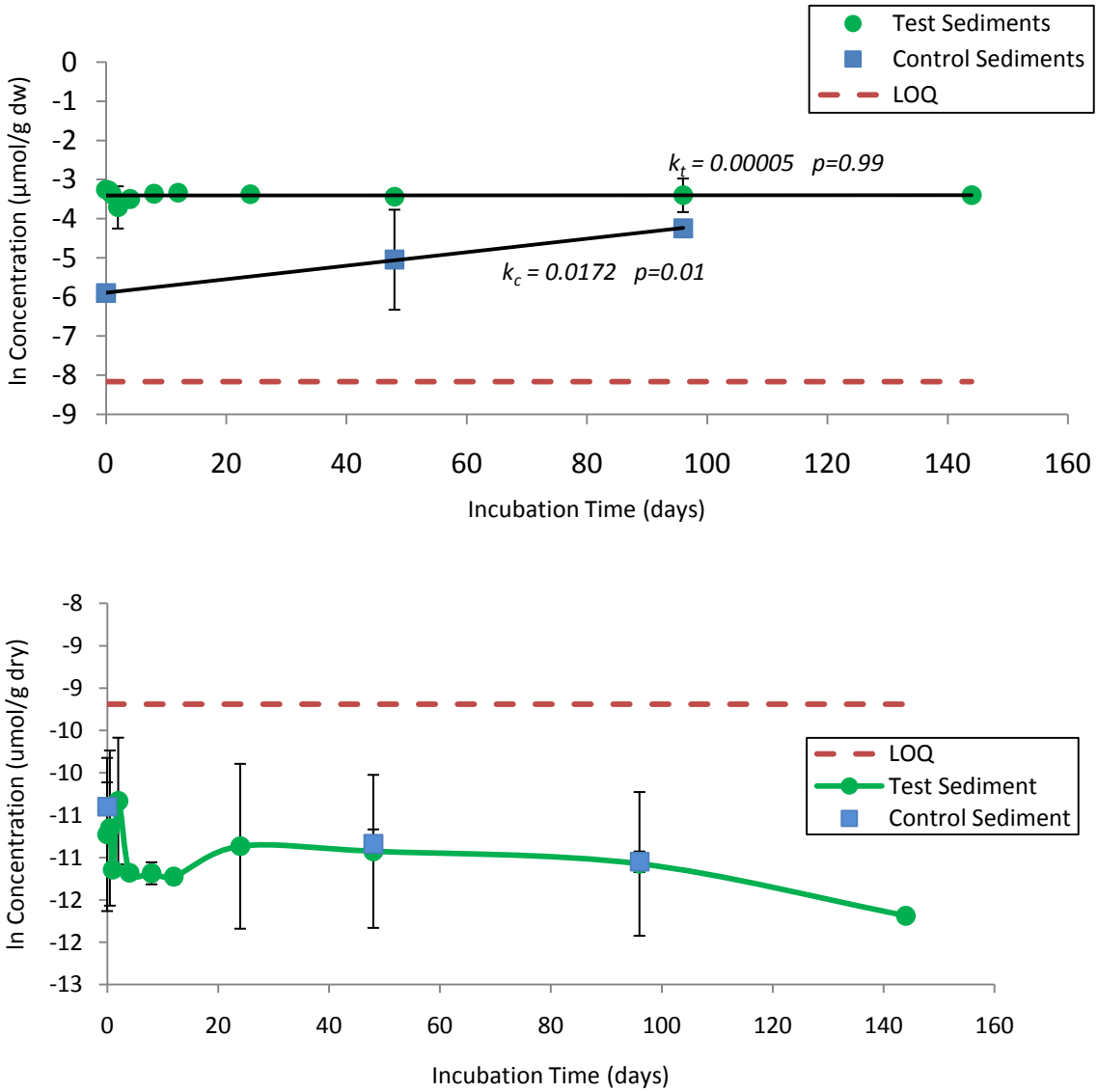


Figure 15. Mean ln concentration (µmol/g dw) of DiNP (top) and MoC9 (bottom) in test and control sediments vs. incubation time. Biodegradation rate constants k_t and k_c were derived from the slope of the regression line. Error bars represent one standard deviation (n=3).

Given that DiNP was not identified as a significant risk for contamination in piloting studies, and is less common in background household dust than many other DPEs (Bornehag et al, 2005) it seems unlikely that concentrations of DiNP rose by approximately 5 fold during the 96 day control incubation. It is possible that an error

occurred during the sample extraction or analysis of DiNP in sediments from incubation day 0.

Mono-isononyl phthalate

A mixture of C9 MPE isomers was used to quantify MiNP, and it was determined that no C9 MPEs were present above the LOQ in either test or control sediments (Figure 15). This result is consistent with DiNP biodegradation results which indicated no significant degradation of the diester.

In general, biodegradation of LMW DPEs (d4DMP, DEP, DnBP, BBP) occurred rapidly (with sediment half-lives <8 days), whereas HMW DPEs (DEHP, d4DnOP, DiNP) degraded either very slowly, or not at all over the 144 day incubation (Table 5). MPE metabolites appeared transiently during periods of rapid biodegradation of DEP, DnBP, and BBP, and during slow degradation of DEHP. When compared with test sediments, no significant degradation of diesters or formation of monoesters was observed in the negative control sediments indicating that biologically-mediated degradation was responsible for the observed decline in DPE concentrations and associated increases in MPE concentrations in test sediment. These data are consistent with very low rates of abiotic degradation of DPEs in aquatic media, (predicted to be between 3 and 2000 years) (Staples et al., 1997).

4.3 Phthalate Partitioning and Sorption

With the exception of DnBP, there were two distinct results of simulation testing:

1. rapid biodegradation of lower molecular weight (log K_{ow} < 5) DPEs with sediment half life times ranging from 2.9 to 4.6 days; and

2. very slow biodegradation (DEHP $t_{1/2}$ = 335 days) to no significant biodegradation of higher molecular weight (log K_{ow} > 5) DPEs.

In order to explore potential reasons for the observed differences in LMW and HMW DPE biodegradation results, I tested the model described in section 2.1.

As described in section 2.1.1, the mathematical model was tested using linear regression, to determine k_i (the y-intercept), by plotting the observed experimental log k_t vs. log K_{ow} (Figure 16). Performance of a linear regression between the $\log_{10} k_t$ over log K_{ow} showed a significant relationship between the two variables (p=0.004) and fit the data reasonably well with an r^2 value of 0.83. This indicates that the observed rate constant of biodegradation in the test decreases with increasing hydrophobicity and approaches 0.003 (corresponding half life of approximately 200 days) for compounds with a log K_{ow} greater than approximately 7.

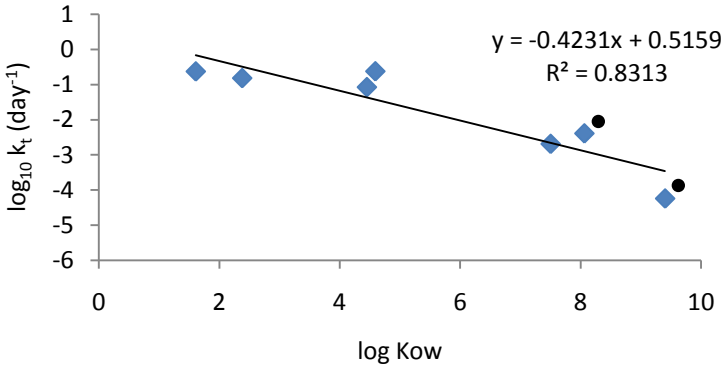


Figure 16. Observed log biodegradation rate (k_t) versus log K_{ow}. ● Denotes k_t values which are not statistically significantly different from 0 (DnOP and DiNP).

It is important to highlight the uncertainty in the log k_t values for the three HMW DPEs plotted in Figure 16. Rate constants for d4DnOP and DiNP (-2.4, and -4.2,

respectively) were not statistically different from 0. Also, the measured k_t for DEHP may be an over-estimation of biodegradation in natural sediment due to its very high spiking concentration (discussed further in section 3.4). Despite these uncertainties, the experimental evidence has indicated that LMW DPEs degrade much more quickly than HMW congeners, and it is also apparent that the observed biodegradation rate constants correlate well with the sorptive properties of each phthalate (i.e., K_{ow}).

Using equation 8, it is possible to estimate the dissolved fraction of each DPE in the test system using K_{sw}, which is estimated from K_{ow} by multiplying with α_{oc} (0.35 from Seth et al., 1999) and ϕ_{oc} (0.028 from Mackintosh, 2002). The model was also used to calculate biodegradation rate constants using equation 11, and the y-intercept of the regression line from Figure 9 (which represents k_i, the inherent rate of biodegradation). In essence, k_i can be represented most closely by k_t of the lowest molecular weight, and most soluble DPE (DMP), i.e., a compound that can desorb readily from the sediment phase into the aqueous phase. Modelled values for DPE dissolved fractions and biodegradation rate constants are presented in Table 8. According to the model, a significant proportion (10 to 99%) of LMW DPEs is predicted to be dissolved in the aqueous phase, (dissolved fraction decreasing with increasing K_{ow}). Conversely, only a small fraction of HMW DPEs (0.014 to 0.0002%) is predicted to be present in the dissolved form.

Predicted sediment half-life times ranged from 0.02 to 20 days for LMW DPEs, and from 41 to > 3200 years for HMW DPEs. For LMW DPEs, model predictions of k_t

Table 8. Model predictions for the dissolved (bioavailable) fraction of DPE in sediments, biodegradation rate constant and corresponding half-life.

DPE	Calculated ϕ[a]	Predicted log k_t (d^{-1})[b]	Predicted sediment half-life (d)[c]	Measured log k_t (d^{-1})	Measured sediment half-life (d)
DMP	0.99	4.0E+01	1.9E-02	2.3E-01	3.0
DEP	0.95	6.3E+00	1.1E-01	1.5E-01	4.6
DnBP	0.14	5.0E-02	1.3E+01	8.4E-02	8.2
BBP	0.10	4.0E-02	1.9E+01	2.4E-01	2.9
DEHP	1.4E-04	5.0E-05	1.5E+04	2.1E-03	337
DnOP	3.9E-05	1.3E-05	5.5E+04	4.1E-03	-
DiNP	1.8E-06	6.3E-07	1.2E+06	5.0E-05	-

[a] calculated using eq. (8)
[b] calculated using eq. (11)
[c] calculated using eq. (3)

were generally within an order of magnitude of the observed value (Table 8). For HMW phthalates, compared with measured k_t values, the model appears to under-predict biodegradation rate constants by two to three orders of magnitude; however, a direct comparison between predicted, and measured biodegradation rate constants for HMW DPEs is difficult as measured k_t values for DnOP and DiNP were not statistically different from 0 (no observed degradation) and DEHP degradation may have been over-estimated in the experiment. The log model predicted rate constants were also plotted agains log K_{ow} (Figure 17).

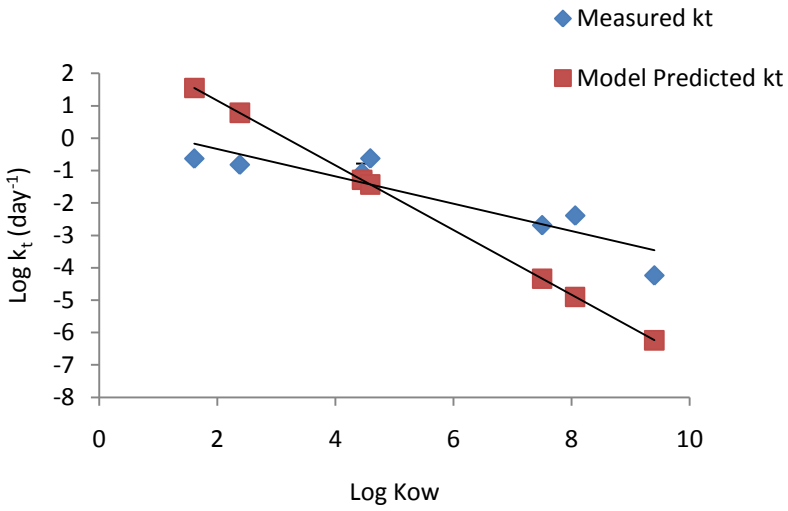


Figure 17. Comparison of measured and model predicted log k_t versus log K_{ow}.

While the model may not accurately predict the actual k_t values (especially for HMW compounds), predicted and measured biodegradation rate constants show a similar relationship with log K_{ow} (i.e., they both decrease with increasing log K_{ow}) (Figure 17). This comparison between empirical and modeled biodegradation rate constants highlights the important effect that partitioning characteristics (i.e., K_{ow}) have on the rate of DPE biodegradation. A possible explanation for the difference between measured and modelled k_t values is that the model assumed that test media (sediment and water) are in equilibrium, which may not be the case in test sediments.

4.4 Factors that Modify Phthalate Biodegradation

A wide range of physiochemical and biological factors that were not included in the model are capable of affecting the rate of phthalate biodegradation in a variety of

environmental media and test conditions (Johnson & Heitkamp, 1984; Chang et al., 2005). Several of these are discussed below:

Concentration of test chemical – Several studies have reported a positive relationship between the rate constant (assuming first order kinetics) of primary degradation and increasing test concentrations for phthalates. Johnson et al. (1984) found that higher molecular weight phthalates (DEHP, DiOP, and DiNP) degraded faster when spiked at higher concentrations in freshwater sediments. This has also been observed in tests involving many other organic compounds (Wesnigk et al., 2001). This observation has implications for the current biodegradation study, as HMW DPEs were spiked to concentrations between 16.1 and 834 µg/g dw, approximately 200 to 500 times higher than previously observed in background False Creek sediments (Mackintosh, 2002). DiNP and d4DnOP were spiked to 16.1 and 36.7 µg/g dw, respectively, while DEHP was spiked to a significantly higher concentration (834 µg/g dw). It is possible that DEHP was the only HMW DPE that exhibited statistically significant degradation because the high spiking concentration resulted in a greater amount of dissolved (bioavailable) compound, which resulted in a detectable rate constant (k_t) in this study. Given the inherent biodegradability of DnOP and DiNP (Sugatt et al., 1984; Wu et al., 2010) it is possible that the dissolved fraction of these DPEs degraded during the incubation, however the concentration of dissolved DPE formed at a rate that was not detectable by the experimental design.

The opposite concern, that DEHP was spiked to such a high concentration that it may have had an inhibitory effect on the biodegrading community, is likely unfounded as:

- The presence of an active degrading community is verified by the observed biodegradation of lower molecular weight DPEs, and the observed colony growth on positive control dip slides; and
- Cartwright et al. (2000) found that treatment of soil with 100mg/g (more than 100 times greater than the highest concentration from the current study) DEHP had no significant effect on the number of culturable bacteria in soil.

Temperature – As would be expected, biologically mediated degradation occurs fastest at temperatures that favour bacterial growth and activity. Several studies have indicated that the rate of biodegradation increases with incubation temperature between 4 and 28^{o}C (Johnson et al., 1984). This has been confirmed for DPEs by Ritsema et al. (1989) and Chang et al., 2005 who determined that the rate of biodegradation for DEP, DnBP and DEHP decreased when incubation temperature was below 10^{o}C or above 40^{o}C. Using a temperature lower than that recommended in standard test methodologies likely affected observed rates of biodegradation, however, if test conclusions are to be extrapolated to make recommendations in natural temperate systems, the test temperature should reflect natural conditions.

Oxygen Availability – Aerobic degradation of organic chemicals typically occurs faster than anaerobic biodegradation (Howard et al., 1991). This has been confirmed for phthalates by several studies including Chauret et al., (1996) and Yuan et al., (2002).

Ejlertsson et al. (1997) hypothesized that a potential explanation for this observation is that aerobic bacteria use emulsifiers and/or solubilizers to increase bioavailability of hydrophobic substances differently than their anaerobic counterparts. Differences in the ability of microorganisms to degrade DPEs was accounted for by attempting to mimic natural sediment conditions. The measures taken to approximate natural conditions included promoting the formation of an aerobic surficial sediment layer by performing headspace gas exchange, agitating samples (gently) on a rotary shaker, and maintaining an environmentally realistic incubation temperature. These conditions allowed rapid degradation of the LMW DPEs, indicating that conditions for degradation were favourable.

Length and complexity of ester side chain – In older (pre-1990) phthalate biodegradation literature there are several studies that observed degradation rates decrease with increasing length and complexity of the side chains in phthalates (O'Grady et al., 1985; Painter and Jones, 1990). Johnson et al. (1984) concluded that the length and complexity of the diester alkyl group is the most important factor in determining the rate of biodegradation. They emphasised the importance of molecular structure in predicting environmental persistence of organic chemicals. Given that higher molecular weight phthalates have been demonstrated to be inherently biodegradable in screening tests, they are able to cross cellular membranes and can be ingested by microorganisms, it is unlikely that steric effects are limiting biodegradation of the higher molecular weight DPEs. More recent biodegradation literature (Ejlertsson et al., 1997; Zeng et al, 2004) recognized that the partitioning characteristics (e.g., $K_{ow,}$ K_{oc}) and aqueous solubilities

which are correlated with length and complexity of ester side chains are likely factors that limit biodegradation of the higher molecular weight phthalates under natural conditions.

Affinity to black carbon – Enhanced affinity of DPEs has been observed for a super-sorbent organic carbon fraction known as carbonaceous geosorbents (CGs), commonly referred to as black carbon. The presence of CGs may significantly limit the quantity of a hydrophobic compound that is dissolved in the aqueous sediment matrix, subsequently limiting the fraction of compound that is bioavailable for degradation (Cornelissen et al., 2005). Xu and Li (2008) investigated the absorption behavior of DnBP on marine sediment, and determined that CGs had an adsorption capacity more than twice as great as that of the remaining organic material in raw sediments. This phenomenon has been demonstrated for other hydrophobic chemicals; PAHs for example have been found to sorb more strongly to sediments than would be predicted by K_{ow} (Accardi-Dey and Gshwend, 2002).

The likely presence of CGs in False Creek sediments, could help explain both the slower than predicted biodegradation rate constants for DMP and DEP, and the lack of observed degradation for d4DnOP an dDiNP. With regards to DEHP, it is possible that the high spiking concentration exceeded the adsorptive capacity of the black carbon component of test sediments resulting in increased sorption to other (less sorbent) forms of organic carbon. This may have contributed to the observed degradation DEHP.

5: Conclusions and Recommendations

To better understand the environmental fate of DPEs in a temperate marine system, a simulation test incorporating environmentally realistic conditions was conducted to measure the biodegradation rates of seven DPEs, as well as monitor the formation and degradation of their primary monoester metabolites. Experimental results revealed a significant difference between the biodegradation behaviour of LMW and HMW DPEs. LMW congeners (methyl, ethyl, *n*-butyl, butylbenzyl phthalate diesters) degraded rapidly with sediment half-lives of 3.0, 4.5, <8, and 3.0 days, respectively. The formation of transient monoester metabolites was consistent with the pattern of the parent DPE biodegradation, (and upon the disappearance of parent compound), these were also rapidly degraded. The opposite was true for HMW DPE congeners, which exhibited very slow (2-ethyl-hexyl phthalate had a sediment half-life of 340 days) or no significant biodegradation (*n*-octyl and *i*-nonyl phthalate diesters) over the 144 day incubation period. Biodegradation rate constants (k_t) were determined to have a significant relationship with K_{ow}, indicating that partitioning of DPE into the aqueous phase likely plays a key role limiting the rate of biodegradation for HMW congeners.

A simple model derived from a first order kinetic equation and equilibrium partitioning was used to describe the possible role of sorption on biodegradation rate constants. The model was tested by determining the degree of K_{ow} dependence of the biodegradation rate constants. The measured degradation rate constants were found to show a K_{ow} dependence that was consistent with the model. From this it was concluded

that sorption plays a key role in the biodegradation of DPEs in sediments. Acceptance of the model would indicate that many high K_{ow} substances with a high inherent biodegradation capacity are likely to be very poorly degraded in natural sediments because only a small fraction of the chemical is bioavailable. The results of the modelling exercise indicated that LMW DPEs were predicted to have a significant dissolved fraction (10-99%), whereas < 0.01% of HMW DPEs was predicted to be dissolved in the aqueous phase.

The culmination of the different analyses, (empirical and theoretical) indicate that the likely reason for the clear distinction between LMW and HMW DPE biodegradation behaviour is that HMW DPEs are strongly adsorbed to the organic fraction of sediment rendering them unavailable to microorganisms for biodegradation.

Biodegradation results presented herein indicate that DPE congeners with a log K_{ow} of approximately 7 and higher (in this experiment DEHP, DnOP, and DiNP), should not be expected to biodegrade significantly in sediments under natural conditions. These compounds are likely to meet the CEPA definition of "Persistent".

The biodegradation rates obtained in this study can be used in evaluations of the environmental fate of DPEs in False Creek. Over the past decade, the REM toxicology laboratory under Frank Gobas has conducted several detailed studies on the partitioning behaviour and bioaccumulation potential of phthalates in False Creek. The biodegradation results presented herein will be viewed in conjunction with previous work to parameterize a multimedia fate model for the inlet, and provide a comprehensive assessment of environmental fate for phthalates in False Creek.

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