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RELATIONSHIP BETWEEN BIODEGRADATION AND SORPTION OF PHTHALATE ESTERS AND THEIR METABOLITES IN NATURAL SEDIMENTS

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Abstract—Regulatory evaluations of commercial chemicals in Canada, the United States, the European Union, and other countries aim to identify biodegradation rates of chemicals in natural soils and sediments. However, commonly used biodegradation testing methods are limited in their capacity to determine biodegradation rates under natural environmental conditions. As a result, widely varying biodegradation rates have been reported for many very hydrophobic substances. This variability causes difficulties in regulatory evaluations, potentially leading to chemical misclassification. In the present study, the authors developed a model of the relationship between biodegradation, sorption, and hydrophobicity, and tested the model in experiments that measured the biodegradation rates of a range of di-phthalate esters (DPEs) and mono-phthalate esters (MPEs) in natural sediments. The results indicate that DPEs and MPEs have the inherent capacity to be quickly degraded by microbes in sediments at a common rate, but that DPE biodegradation rates in natural sediments decrease with increasing phthalate ester sorption to sediments. The results show that inherently biodegradable substances that are subject to a high degree of sorption can be expected to exhibit long half-lives in natural sediments. The model provides a potential methodology for assessing biodegradation rates in natural sediments from inherent biodegradation rates measured in screening tests by accounting for chemical sorption. The present study indicates that a reduced rate of biodegradation is due to a reduced fraction of freely dissolved chemical concentration in the interstitial water, and that the environmental significance of sorption-reduced biodegradation rates needs to be viewed in the context of risk in chemical evaluations. Environ. Toxicol. Chem.

Keywords—Phthalate ester Biodegradation Sorption Sediment Persistence

INTRODUCTION

Biodegradation, defined as the biologically mediated alteration of a chemical [1], is a key environmental process that is considered in the evaluation of chemical substances in Canada, the United States, the European Union, and other countries, and globally under international agreements including the United Nations Stockholm Convention on Persistent Organic Pollutants (UNSCPOP) and the United Nations Environmental Program Protocol on Long Range Transport of Air Pollutants [2,3]. The rate of biodegradation is expressed in terms of the half-life of the substance [4]. For a substance to be deemed persistent in sediments under the UNSCPOP, the half-life needs to be equal or greater than 180 d [2]. Methods to estimate the rate of biodegradation of chemicals in the environment can be classified into two broad categories: screening tests and simulation tests. Screening tests are used to measure the biodegradability of organic chemicals and are generally conducted using sewage inocula as the degrading microbial community. The Organization for Economic Cooperation and Development (OECD) has developed methodologies for a series of screening tests (e.g., OECD 301, 302, 304A) designed to assess the ready or inherent biodegradability of commercial chemicals. Results of screening tests are often considered to be limited in their ecological relevance because they employ unnatural matrices (e.g., sewage inocula), temperatures greater than those in most environments, and substrate concentrations that often exceed concentrations found in the environment [4,5]. Screening tests are generally used to classify chemicals into three categories: (1) chemicals with potential to be easily and rapidly biodegraded, (2) chemicals with potential for degradation under specific environmental circumstances, and (3) chemicals with no biodegradation potential [4]. Screening tests have been carried out to quantify the inherent and ready biodegradability of phthalates [6,7] and have shown that both lower molecular weight (LMW) and higher molecular weight (HMW) phthalates are readily biodegradable.

Simulation tests are designed to measure persistence and biodegradation rates under natural conditions in water, soil or sediment, and sludges [8]. The OECD provides guidelines that need to be followed in simulation tests for registration purposes [9]. Simulation tests are considered to be more environmentally realistic than screening tests [5] because they include factors that can influence the rate of biodegradation (e.g., oxygen availability, temperature, concentration of parent compound, length of incubation) [1]. The design of simulation tests reflects the fact that the biodegradability of a substance in the environment is not solely an inherent property of the chemical that can be easily measured in a test, but is dependent on environmental conditions such as microbial activity, soil and sediment conditions, contaminant concentrations, and temperature [4]. Differences between screening and simulation tests can produce large variations in reported biodegradation rates for chemicals and often contribute to uncertainty in regulatory evaluations.

All Supplemental Data may be found in the online version of this article.

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Disparity between biodegradation rates measured in screening tests and simulation tests is especially important for very hydrophobic organic substances that are often the focus of regulatory evaluations. For example, several authors have shown that di-2-ethylhexyl phthalate (DEHP), di-n-octyl phthalate (DnOP), and di-iso-nonyl phthalate (DiNP) esters do not readily degrade in sediment simulation tests [10–13], whereas others report degradation [14,15], including ready degradation in screening tests [6,7].

To improve methods used to characterize the persistence of phthalate esters and possibly other commercial chemicals in soils and sediments, we investigated the relationship between sorption and biodegradation of phthalate esters in natural sediments. We combined sorption and biodegradation models to develop a theoretical relationship between sorption and microbial biodegradation rate in natural sediments, and then tested the model by conducting simulation experiments with a range of phthalate esters in natural sediments in which depletion rates and metabolite formation rates were measured. Phthalate esters provide a unique family of test chemicals to investigate the relationship between hydrophobicity and biodegradation, because these chemicals are known to be biodegradable [15–20] yet vary greatly in their hydrophobicity and sorption to sediments [16]. Phthalate esters are also widely used in plastics and other products, produced in large quantities, and subject to regulatory evaluations in several jurisdictions around the world.

THEORY

For intracellular and extracellular microbial degradation (i.e., biodegradation) of chemicals to occur, it is generally considered that chemicals sorbed to sediment must first desorb into the aqueous phase before becoming bioavailable to microorganisms or extracellular enzymes [21–23]. Therefore, the process by which organic chemicals are biodegraded in natural systems can be conceptually 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 micropores), followed by biological uptake and transformation [24,25]. Desorption plays an important role in the biodegradation process and influences the environmental fate and ecotoxicity of sediment-bound contaminants [21,25–31]. A simplified description of this process can be developed based on a first-order kinetic equation, and the assumption that only freely dissolved compound in sediment (i.e., a mixture of water and particles) is available for transformation [25]

$$\frac{dM_t}{dt} = -k \times M_t = -k_i \times \phi \times M_t \quad (1)$$

Where M_t is the total chemical mass in the sediment (mol), which is the sum of the mass of chemical in the interstitial water (M_w) and particulate matter (M_p) of the sediment, t is time (d) and k is the apparent first-order biodegradation rate constant (d^{-1}). k_i is the inherent biodegradation rate constant (d^{-1}) (i.e., the biodegradation rate constant of the unbound or freely dissolved chemical in the sediment), and ϕ is the fraction of the total mass of chemical in the sediment interstitial water that is present in unbound form in the interstitial water.

$$\phi = \frac{M_w}{M_t} = \frac{M_w}{M_w + M_p} \quad (2)$$

If we can assume, as a first approximation, that the relationship between the chemical concentrations in dry weight particles (C_p in mol/kg dry wt) and in the water (C_w in mol/L) can be described by an equilibrium partition coefficient K_{pw} (in L/kg dry wt) and considering that C_p is M_p/W_p and C_w is M_w/V_w, where W_p and V_w are, respectively, the weight (kg) and volume (L) of the particulate and aqueous components of the sediment, it follows that Equation 2 can be rewritten as

$$\phi = \frac{1}{1 + K_{pw} \times \frac{W_p}{V_w}} \quad (3)$$

For many organic contaminants, K_{pw} can be approximated as $f_{OC} \times K_{OC}$ where f_{OC} is the organic carbon content (kg/kg) of the particles and K_{OC} is the chemical's organic carbon (OC)–water partition coefficient (L/kg OC) [32]. For some organic substances (e.g., organochlorines), K_{pw} (L/kg dry wt) can be related to the 1-octanol–water partition coefficient (K_{OW}) as $\alpha_{OC} \times f_{OC} \times K_{OW}$ where α_{OC} is the organic carbon to octanol proportionality constant (L/kg OC), which ranges between 0.14 and 0.89 with a mean recommended value of 0.35 L/kg OC [33]. Substitution into Equation 3 gives

$$\phi = \frac{1}{1 + \alpha_{OC} \times f_{OC} \times \frac{W_p}{V_w} \times K_{OW}} \quad (4)$$

Substituting Equation 4 into 1 then gives

$$\frac{dM_t}{dt} = -\frac{k_i}{1 + \alpha_{OC} \times f_{OC} \times \frac{W_p}{V_w} \times K_{OW}} \times M_t \quad (5)$$

where the apparent biodegradation rate constant k equals

$$k = \phi \times k_i = \frac{k_i}{1 + \alpha_{OC} \times f_{OC} \times \frac{W_p}{V_w} \times K_{OW}} \quad (6)$$

Equation 6 shows that for chemicals with a low K_{OW} for which $\alpha_{OC} \times f_{OC} \times (W_p/V_w) \times K_{OW} << 1$, the apparent and inherent biodegradation are the same as ϕ approaches 1.0. However, for chemicals with a high K_{OW}, for which $\alpha_{OC} \times f_{OC} \times (W_p/V_w) \times K_{OW} >> 1$, the apparent biodegradation rate constant is lower than the inherent biodegradation rate constant k_i. Figure 1 illustrates the theoretical relationship between the sorption coefficients or K_{OW} and the biodegradation rate constant for substances with similar inherent biodegradation rate constants according to Equation 6.

It is possible to test the hypothesized relationship between sorption and biodegradation by studying the biodegradation rates of chemicals that share similar biodegradation pathways. Di-phthalate esters (DPEs) are a family of chemical substances that may have similar inherent biodegradation rates: DPEs all share two ester bonds that can be biodegraded by microbial esterases to form mono-phthalate esters (MPEs) (Fig. 2a), as described in Staples et al. [16]. The MPEs can be further hydrolyzed [16,34] (Fig. 2b). The MPEs possess a much lower K_{OW} than their parent DPEs and, due to their pK_a of approximately 4.2 [34], are largely ionized at most environmental pH values (Fig. 2c). The log D, that is, the logarithm of the ratio of chemical concentration in octanol (C_O) and the combined concentration of the ionized ($C_{W,i}$) and neutral form ($C_{W,n}$) in the water (i.e., $\log D = \log [C_O/(C_{W,i} + C_{W,n})]$), which can be referred to as the pH-dependent log K_{OW} of MPEs at environmental pH levels varies from approximately -1.7 for DMP to 1.8 for DiNP (ChemSilico). Consequently, at neutral pH, MPEs are

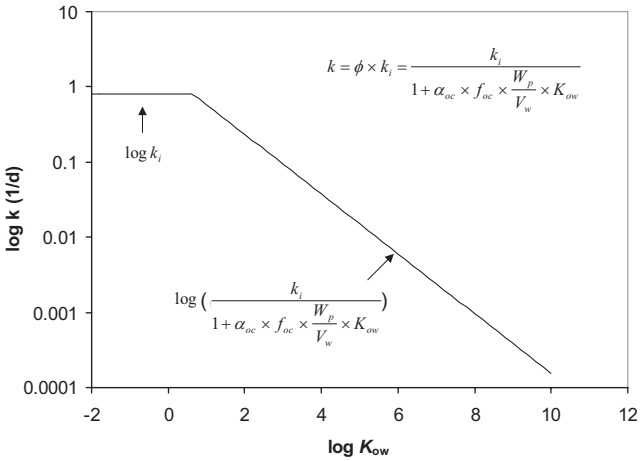

Fig. 1. General relationship between the logarithm of the apparent biodegradation rate constant (log k) and log K_{OW}, illustrating that for hydrophobic substances with low log K_{OW} (i.e., a low capacity to sorb to organic carbon of particulate matter, $\phi \approx 1$), the apparent (k) and inherent (k_i) biodegradation rate constants are equal, and that for substances with high K_{OW} (i.e., greater capacity to sorb to organic carbon of particulate matter, $\phi < 1$), the apparent biodegradation rate constants decrease with increasing K_{OW}.

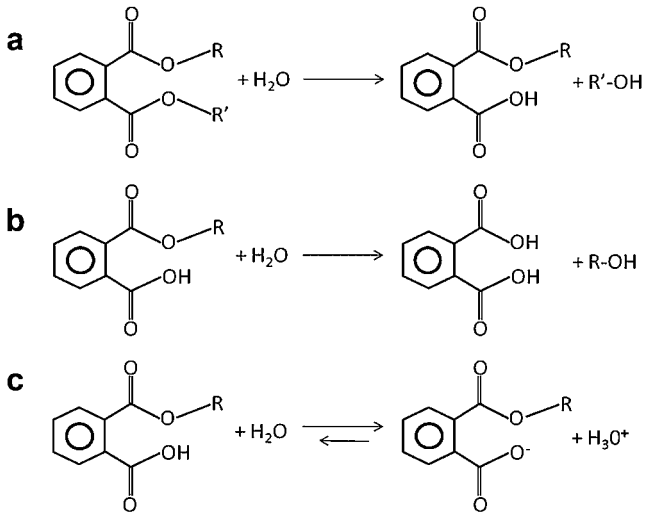

Fig. 2. Schematic diagram of the enzymatic hydrolysis of di-phthalate esters (DPEs) (**a**), mono-phthalate esters (MPEs) (**b**), and the dissociation of MPEs in water (**c**).

expected to be predominantly in the unbound form in the sediment (i.e., $\phi \approx 1$). Mono-phthalate esters exhibit similar microbial degradation half-lives of approximately 24 h [35], suggesting the existence of a common inherent degradation rate for the mono-esters. If DPEs also possess a common inherent biodegradation rate, it is possible to explore the role of sorption on the biodegradation rate constant, as described in Equation 6, by measuring the apparent biodegradation rate constant (log k) as a function of log K_{OW}, that is,

$$\log k = \log k_i - \log\left(\frac{W_p}{V_w}\right) - \log(\alpha_{OC} \times f_{OC}) - \log K_{OW} \quad (7)$$

for di-phthalates for which $\alpha_{OC} \times f_{OC} \times (W_p/V_w) \times K_{OW} >> 1$, whereas for DPEs for which $\alpha_{OC} \times f_{OC} \times (W_p/V_w) \times K_{OW} << 1$, log k is constant at log k_i.

MATERIALS AND METHODS

Chemicals

Di-ethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl-benzyl phthalate (BBP), DEHP, DiNP, di-iso-butyl phthalate (DiBP), and DnOP were purchased from Sigma-Aldrich. Deuterated compounds d_4-dimethyl phthalate (d_4-DMP) and d_4-DnOP were purchased from Cambridge Isotope Laboratories. Stock solutions were prepared in Spectro-grade distilled acetonitrile. Analytical grade solvents were used for cleaning glassware and sampling equipment.

Sediment and water samples

Surficial sediment was collected using a grab sampler from the marina south sampling station [36] in False Creek, an urban marine inlet located in Vancouver, BC, Canada. The top layer (0.5–1.0 cm) of each grab sample was removed with a metal spatula and pooled in a 2-L glass jar. False Creek water was collected in a 4-L amber glass bottle from approximately 1 m above the bottom for use as overlying water in the incubations. Samples were kept on ice in the field and stored at 4°C in the laboratory until use. Incubations were begun within 2 d of sample collection. The OC content of the False Creek sediment, determined according to Van Iperen and Helder [37], was $2.89 \pm 0.22\%$ ($n = 12$ determinations) and was unaltered after autoclaving ($2.92 \pm 0.28\%$, $n = 3$). The pH of the field sediments from False Creek was 8.0 ± 0.1 and W_p/V_w was held at 0.37 kg/L. Sterile sediment used for the negative control was prepared by autoclaving sediments and adding 300 µl of 1% mercuric chloride (Fisher Scientific). Easi-Cult TTC dip-slides (Orion Diagnostica) were used to confirm the sterility of the control sediments and the microbial activity of the test sediments. False Creek water was autoclaved using the same procedure. Glassware, metal lids, and sampling tools were baked at 400°C for 10 h, and then rinsed three times with distilled acetone, iso-octane, and dichloromethane. Metal lids were lined with aluminum foil that had been rinsed with the same cleaning solvents and baked at 350°C for 10 h.

Sediment spiking procedure

A spiking solution containing DEP, DnBP, BBP, DEHP, DiNP, d_4-DMP, and d_4-DnOP was prepared in acetonitrile to produce final concentrations in sediment that were approximately 100-fold greater than "background" DPE concentrations measured previously in these sediments [36,38]. Measured spiking concentrations were 3, 12, 10, 70, 170, 8, and 3 µg/g sediment (wet wt) for d_4-DMP, DEP, DnBP, BBP, DEHP, d_4-DnOP, and DiNP, respectively. The spiking solution was added to a 2-L glass flask and evaporated to near dryness in the fume hood to minimize the effect of the carrying solvent on sediment microorganisms. Sediment (500 g) was then added and stirred vigorously using an electric mixer for 12 h, after which an additional 1 kg of wet sediment was added and stirred for an additional 2 h.

Incubation procedure

Spiked sediment (30 g) and 10 ml of overlying False Creek water were transferred to 125-ml glass jars with foil-lined metal lids. Test and control samples were incubated in parallel at 12 to14°C in the dark for 144 d (test) and 96 d (control). To promote aerobic conditions, headspace gas exchange was conducted twice per week by removing the lids and gently swirling the jars' contents at 120 rpm in a rotary shaker for 5 min. This

was done in a Type II biosafety cabinet to prevent contamination by microorganisms or DPEs sorbed to airborne dust. Dissolved oxygen (YSI 58 laboratory dissolved oxygen meter, YSI) and temperature (Extech 42275 data logger, Extech Instruments) were measured throughout the incubation period. All incubations were performed in triplicate. On days 0, 0.5, 1, 2, 4, 8, 12, 24, 48, 96, and 144, three jars representing triplicate samples were removed from the incubation chamber, amended with 300 μl of 1% mercuric chloride to stop biodegradation, mixed on a vortex shaker for 5 s, and frozen at −20°C until extraction and analysis.

Sediment extraction and cleanup

The samples were thawed at room temperature. After stirring, a sediment subsample (2 g) was transferred to a cleaned and baked scintillation vial. Internal standard solution (50 μl containing 410 ng/μl DnOP and 35.3 ng/μl DiBP) was added and the sediment was extracted with 10 ml acetonitrile by vortexing for 10 s and sonicating for 5 min. The vials were centrifuged for 5 min at 1300 *g,* and the acetonitrile decanted and filtered through Whatman No. 1 filter paper. The extraction was repeated two more times. The volume of the pooled filtrate was reduced to approximately 1 ml using a rotary evaporator. The concentrated extract was transferred to an amber autosampler vial and stored at −40°C until being shipped to the Institute of Ocean Sciences, Sidney, British Columbia, Canada for analysis. Extraction efficiencies were determined in independent experiments where known amounts of DPEs were added to sterile sediments and a clean reference solvent (control), and then analyzed following extraction and cleanup.

Instrumental analysis

Sediment extracts containing the spiked DPEs were analyzed for DPEs using low-resolution gas chromatography–mass spectrometry (GC-LRMS) according to Mackintosh et al. [36], and for phthalate monoesters d_4-monomethyl- (d_4-MMP), mono-ethyl- (MEP), mono-n-butyl- (MBP), mono-2-ethylhexyl- (MEHP), d_4-mono-n-octyl- (d_4-MnOP), and mono-nonyl (MNP) phthalate by liquid chromatography electrospray–ionization mass spectrometry (LC/ESI-MS) according to Blair et al. [39]. Concentrations were expressed as the average mass of DPE or MPE in triplicate incubations per gram dry weight sediment (water content of sediment was, on average, 73% of the wet sediment wt).

Quality assurance/quality control

Procedural blanks were prepared in triplicate by performing the extraction procedure using solvents and glassware only (i.e., without sediment). Only DnBP and DEHP were detected in the procedural blanks, and for these congeners, sample concentrations were blank-corrected, and method detection limits (MDLs) were calculated as the mean ± 3 SD of the DPE concentration in the procedural blanks. For the other DPEs and for MPEs, limits of quantification (LOQs) were determined as the lowest detectable calibration standard (ng/ml) multiplied by the final volume of extraction solvent (ml), divided by the mass of extracted sediment (g). Extraction efficiencies of DPEs were determined in a pilot study in which sterilized sediments were spiked with known amounts of the analytes. Extraction efficiencies (± SD) were 25 ± 2, 45 ± 5, 86 ± 8, 82 ± 6, 82 ± 8, 103 ± 4, and 81 ± 15% for d_4-DMP, DEP, DnBP, BBP, DEHP, d_4-DnOP, and DiNP, respectively.

Data analysis

Apparent DPE biodegradation rate constants were determined by linear regression of the natural logarithm of the DPE concentration in the sediment versus time over the degradation phase of the incubation period, which in most cases lasted throughout the entire incubation period. Half-lives ($t_{1/2}$) were calculated as 0.693/k. Formation and biodegradation rates of the MPE metabolites were determined by fitting the MPE formation rate constant k_f and the MPE biodegradation rate constant k_d to a numerical integration of the equation

$$\frac{dC_{mpe}}{dt} = k_f \times C_{dpe} - k_d \times C_{mpe} \quad (8)$$

where C_{dpe} and C_{mpe} are the DPE and corresponding MPE concentrations, respectively, in the sediments. The C_{dpe} versus time function was determined through linear interpolation of measured concentrations of DPEs in the sediments throughout the incubation period. The calculations—carried out in an Excel spreadsheet—used the solver function to determine a k_f and k_d that produced the best agreement between observed and calculated C_{mpe} as characterized by the minimum sum of squared differences between observed and calculated C_{mpe}.

RESULTS AND DISCUSSION

Incubations

Dip-slides on days 48 and 96 of incubation showed microbial colony formation on dip-slides exposed to the test sediments. No microbial colonies were observed on dip-slides exposed to the sterilized control sediments. This indicates that microbial activity was maintained throughout the test while no microbial activity was present in the control sediments.

Biodegradation

As illustrated in Figure 3a for DEP, concentrations of d_4-DMP, DEP, DBP, and BBP in the test sediment samples dropped during the first 21 d of the incubation period. No significant decline in concentration with time was observed after that period. Biodegradation rates were determined from the concentration time curve during the degradation phase. Concentrations of d_4-DMP, DEP, DBP, and BBP in the control sediments did not show a statistically significant decline over the duration of the incubation period, as demonstrated by the slopes of the linear regression of ln concentration versus time relationships, which were not significantly different from 0 ($p > 0.05$). Differences in the ln concentration versus time curves between sterilized (control) and nonsterilized (test) sediments indicate that a high rate of microbial transformation occurred for these phthalates. Figure 3b illustrates that concentrations of the corresponding mono-ester metabolites in the sediments increased rapidly as a result of the high initial rate of DPE breakdown, then peaked when the MPE formation rate matched the MPE breakdown rate, and fell in the later part of the incubation period when the MPE breakdown rate exceeded the MPE formation rate.

Figure 3c shows that concentrations of DEHP declined slowly. Linear regression of the natural logarithm of the concentration with time showed that the decrease in concentration was statistically significant ($p = 0.03$) compared to control sediments. However, no statistically significant decline in the logarithm of concentration of d4-DnOP ($p = 0.14$) and DiNP ($p = 0.99$) in the sediment was observed. Figure 3d illustrates that a small but statistically significant increase in the concen-

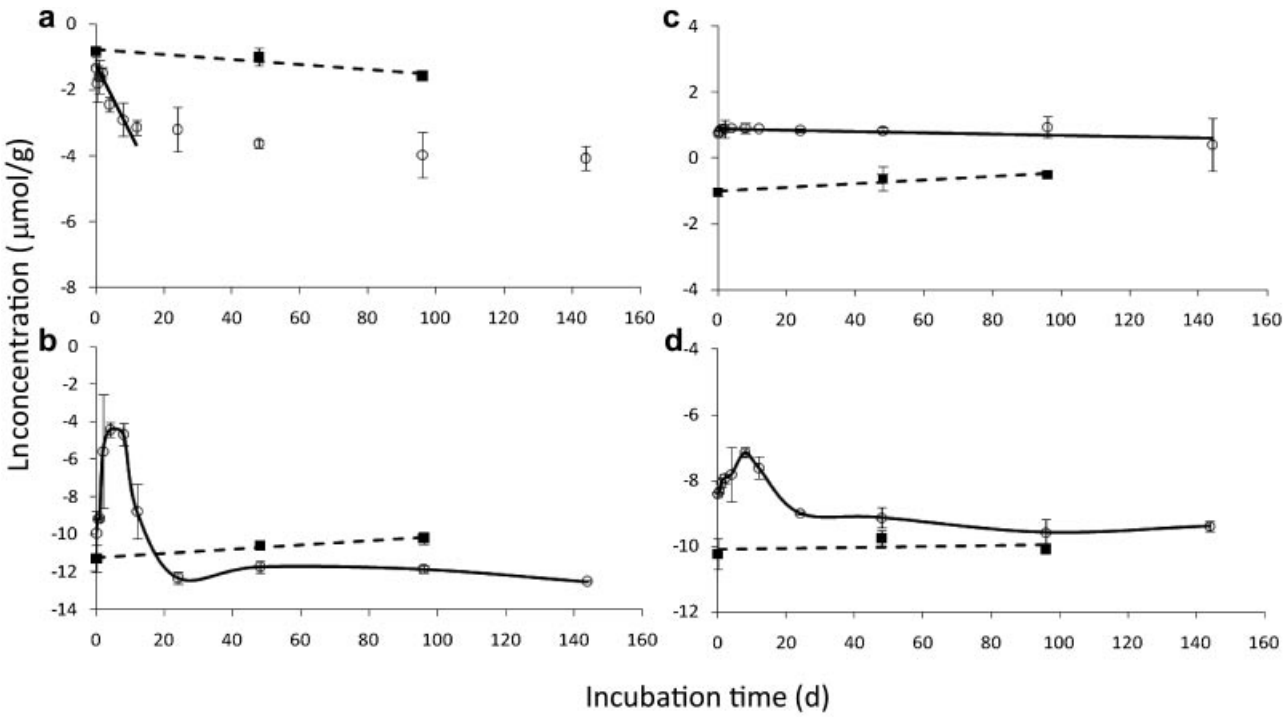

Fig. 3. Natural logarithm of the concentration (μmol/g dry wt) of di-ethyl phthalate ester (DEP) (**a**), mono-ethyl phthalate ester (MEP) (**b**), di-2-ethylhexyl phthalate ester (DEHP) (**c**), and mono-2-ethylhexyl phthalate ester (MEHP) (**d**) in test (solid line) and control (dashed line) sediments versus incubation time (day). Errors bars are standard deviations.

trations of the corresponding MPE metabolite MEHP occurred during the initial phase of the incubation period. Similar observations were made for MiNP. These findings indicate that, under the conditions used in this experiment, the high molecular weight DPEs appear to degrade more slowly than the low molecular weight DPEs, and that the MPE metabolites are quickly degraded regardless of whether they are high or low molecular weight phthalates.

Table 1 shows that the apparent biodegradation half-life times for the DPEs ranged from approximately 3 d for DMP to 347 d for DEHP, and more than one year for DiNP. Figure 4 shows that, consistent with the theory outlined (Fig. 1), apparent biodegradation rate constants of DPEs decrease with increasing K_{OW}. Linear regression of log k versus log K_{OW} shows a statistically significant correlation ($p = 0.0097$, $r^2 = 0.88$), which improved ($p = 0.008$, $r^2 = 0.93$) if the only non di-alkylated phthalate ester (BBP) was removed from the correlation, that is,

$$\log k = -0.40\ (\pm 0.08) \times \log K_{OW} + 0.16\ (\pm 0.52) \quad (9)$$

where standard errors are presented in parentheses.

Table 1. Log K_{OW}, the measured apparent biodegradation rate constant k (d^{-1}), and the corresponding half-life time (d) for the di-phthalate esters examined in the present study

Di-phthalate ester (DPE)	log K_{OW}	k (d^{-1})[a]	$t_{1/2}$ (d)
Di-methyl phthalate ester (DMP)	1.78	0.28 (±0.07)	2.5
Di-ethyl phthalate ester (DEP)	2.74	0.15 (±0.05)	4.6
Di-n-butyl phthalate ester (DnBP)	4.52	0.015 (±0.009)	46
Butyl-benzyl phthalate ester (BBP)	4.98	0.24 (±0.07)	2.9
Di-2-ethylhexyl phthalate ester (DEHP)	8.08	0.002 (±0.0008)	347
Di-n-octyl phthalate ester (DnOP)	8.08	0.004*	173
Di-iso-nonyl phthalate ester (DiNP)	8.98	0.00006*	12,000

[a] Standard errors in parentheses.
* A measured depletion rate that was not statistically different from zero ($p > 0.05$).

The correlation indicates the possible existence of a common inherent biodegradation rate constant among the di-alkyl-phthalate esters in the nonsorbed or freely dissolved form. This inherent biodegradation rate constant can be estimated from the biodegradation rate constant ($0.28 \pm 0.07\ d^{-1}$) of DMP, the low molecular weight di-alkyl-phthalate ester, which corresponds to a half-life time of 0.693/0.28 or 2.5 d. Di-methyl-phthalate has the smallest capacity to sorb because of its relatively low log K_{OW} of 1.78. Equation 4 estimates an unbound fraction for DMP in the False Creek sediments of 0.84 based on a measured 2.7% organic carbon content, a 1:3 particle/water distribution, and an assumed sorptive capacity of organic carbon that is 35% of that of octanol [33]. Using this value of the unbound fraction, the actual inherent biodegradation rate constant of DMP is estimated to be $0.28\ d^{-1}/0.84$ or $0.33\ d^{-1}$, corresponding to an inherent half-life time of approximately 2 d.

Table 2 shows that biodegradation half-lives of the MPEs ranged between 0.34 to 1.5 d, close to the $t_{1/2}$ values of 1 to 2 d reported by Otton et al. [35] in sediments from the same site collected at a different time. Figure 4 shows that the apparent biodegradation rate constant k of the MPEs does not follow a statistically significant relationship with log D and ranges between 0.45 and $2.0\ d^{-1}$, with a mean value of $1.1\ d^{-1}$ for all MPEs. Biodegradation rates of the mono-esters are not expected to be affected by sorption because the mono-esters are largely ionized at environmental pH levels (pK_a of 4.2) and log D varies from −1.7 for MMP to 1.79 for MiNP. Because MPEs are highly bioavailable, the apparent biodegradation rate constant reflects the inherent biodegradation rate constant. The observation in both the present and a previous study [35], that all MPEs are degraded at a common rate, indicates that the structure of the R group (Fig. 2b) does not affect the rate of biodegradation in sediment. Figure 4 illustrates that the degradability of all MPEs ($t_{1/2} = 0.45$ to 2.0 d) is not significantly different from the inherent degradability of DPEs derived from

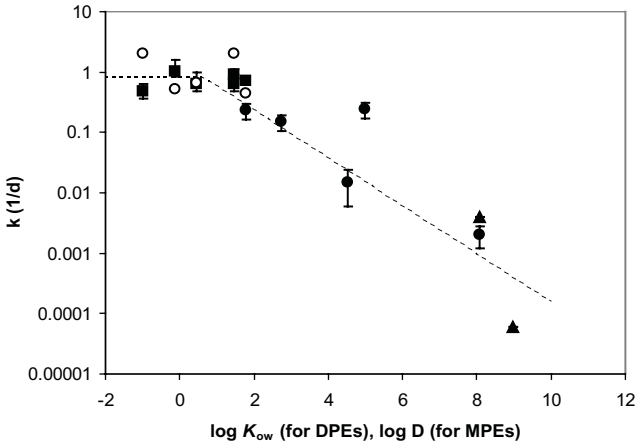

Fig. 4. The apparent biodegradation rate constants k (d^{-1}) of di-phthalate esters (DPEs) determined from statistically significant depletion rates (●) and statistically insignificant depletion rates (▲) (Table 1), and mono-phthalate esters (MPEs) determined in the present study (○) (Table 2) and in Otton et al. [35] (■) as a function of log K_{OW} (for DPEs) and log D at pH = 8.1 (for MPEs). The dashed line presents the general relationship between the biodegradation rate constant and log K_{OW} (for DPEs) and log D (for MPEs).

the biodegradation rate of DMP ($t_{1/2}$ of DMP = 2.0 d). This suggests that DPEs may be as degradable by microorganisms as their mono-ester metabolites, but that sorption to particulate matter reduces the bioavailability of the DPEs and thereby lowers the degradation rate constant. This means that very hydrophobic substances, such as the DEHP, DnOP, and DiNP in the present study, can have long half-life times of one year or more in sediments, while these phthalate esters in bioavailable form are highly biodegradable.

The findings and the model (Eqns. 5 and 6) help to explain the considerable variation in reported half-life times of phthalate esters in screening and simulation degradation tests for the very hydrophobic phthalate esters. They may also help to formalize the relationship between sorption and biodegradation noted by several authors. For example, Staples et al. [16] concluded that the greater degree of biodegradation observed for low molecular weight phthalates compared to high molecular weight phthalates may reflect the greater sorptive tendency of the high molecular weight phthalates. Liang et al. [17] published a review of DPE biodegradation measurements from tests employing microorganisms isolated from select environmental media, and cultured in synthetic media. The review showed that, in vitro, a broad range of high and low molecular weight DPEs can be degraded at a relatively rapid rate by bacteria isolated from different freshwater and marine sediments, soils, and sludges. These results are consistent with the findings of the present study, indicating that all DPEs are highly biodegradable. However, sorption can affect the fraction of DPEs in sediments and soils that are available for reaction. The importance of bioavailability in determining biodegradation rates has also been noted for other classes of hydrophobic substances, such as polycyclic aromatic hydrocarbons [27–31]. Clearly, careful consideration is required before extrapolating screening test results to predictions of biodegradation in the environment. The present study shows that this is especially applicable to very hydrophobic substances that have a very high capacity to sorb to organic carbon of particulate matter. Equation 6 may be helpful in extrapolating the results from screening tests, in which conditions are optimized to obtain unimpeded rates of biodegradation, to natural conditions where sorption to sediments and soils are important factors that slow down apparent biodegradation rates.

Modeling biodegradation

Supplemental Data, Table S1 shows that the inherent biodegradation half-life in natural sediments of the di-alkyl-phthalate esters of 2 d determined in the present simulation test study falls within the range of half-life times reported in screening tests. This indicates that screening tests are very useful in estimating inherent biodegradation rates in natural sediments, and that by using Equation 6, it may be possible to estimate corresponding half-life times in natural sediments from the half-life times determined in screening tests. For example, assuming a chemical equilibrium between water and natural sediments with an organic carbon content of 1%, a 1:1 particle/water distribution volume (W_p/V_w), and a sorptive capacity of organic carbon that is 35% of that of octanol [33], DnBP is expected to exhibit a half-life time of approximately 167 d, which is due to an inherent half-life time estimate of 1 d measured in a screening test (Supplemental Data, Table S1) and a fraction of unbound chemical in the sediment of 0.006, producing an apparent half-life time in natural sediments at equilibrium of 1/0.006 or 167 d. The measured half-life time of DnBP in this simulation study was shorter than that, but measured under conditions that were likely not sufficient to achieve an equilibrium between water and sediments. For very hydrophobic substances ($\log K_{OW} > 6$), the model predicts the fraction of chemical available for degradation to be very low, hence producing long half-lives. For these substances, sorption is expected to have a large effect on the biodegradation rate in sediments. Factors affecting the degree of sorption can be expected to have a significant impact on the biodegradation rate. For example, the high apparent sorption capacity of carbonaceous or black carbon [26] may cause low biodegradation rates of hydrophobic chemicals in sediments that contain significant amounts of this type of carbon. In addition, the

Table 2. Log D (at pH = 8.1[a]), the formation rate constant k_f (d^{-1}), the apparent biodegradation rate constant k_d (d^{-1}), and the corresponding half-life (d) of several mono-phthalate esters determined in the present study and in Otton et al. [35]

Mono-phthalate ester (MPE)	log D	k_f (d^{-1})	k_d (d^{-1})	$t_{1/2}$ (d) (the present study)	$t_{1/2}$ (d)[b] (Otton et al. [35])
Mono-methyl phthalate ester (MMP)	−1.70			3.0	
Mono-ethyl phthalate ester (MEP)	−0.99	0.16	2.03	0.34	1.5 (±0.42)
Mono-n-butyl phthalate ester (MBP)	−0.13	0.015	0.52	1.3	0.67 (±0.08)
Butyl-benzyl phthalate ester (BBP)	0.44	0.24	0.67	1.0	1.1 (±0.50)
Mono-2-ethylhexyl phthalate ester (MEHP)	1.46	0.0021	2.03	0.34	1.1 (±0.38)
Mono-n-octyl phthalate ester (MnOP)	1.46				0.75 (±0.17)
Mono-iso-nonyl phthalate ester (MiNP)	1.79	0.00003	0.45	1.54	0.96 (±0.21)

[a] Calculated by ChemSilico.
[b] Standard errors in parentheses.

degree to which substances are in equilibrium between interstitial water and sediment particulate matter can affect the biodegradation rate. This is relevant in simulation experiments where the exposure time of sediments with test chemicals is often less than that required to reach an equilibrium or a steady-state sediment–water concentration distribution similar to that in the field.

Although it is unlikely that one model, such as Equation 6, is able to capture the factors influencing sorption and biodegradation, the simple model may be useful in evaluating the general persistence of hydrophobic organic chemicals in sediments. Inherent biodegradation rate constants, derived from screening tests, can be used in the models to estimate biodegradation rate constants in natural sediments, by multiplying the inherent biodegradation rate constant with the fraction of freely dissolved chemical concentration in the sediment.

One of the implications of the present study is that many very hydrophobic organic substances are expected to have a low rate of biodegradation in natural sediments, despite their inherent biodegradability. While a low rate of biodegradation is usually considered to be an undesirable chemical property, it is important to note that the high rate of sorption that causes persistence also limits uptake of the chemical into biota. This reduction in exposure due to sorption results in a reduction in risk. Therefore, biodegradation rates may be best considered in the context of risk, rather than as an independent line of evidence during the process of chemical evaluation.

SUPPLEMENTAL DATA

Table S1. (70 KB PDF)

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