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# Biodegradation of mono-alkyl phthalate esters in natural sediments

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**Abstract**

Mono-alkyl phthalate esters (MPEs) are primary metabolites of di-alkyl phthalate esters (DPEs), a family of industrial chemicals widely used in the production of soft polyvinyl chloride and a large range of other products. To better understand the long term fate of DPEs in the environment, we measured the biodegradation kinetics of eight MPEs (-ethyl, -*n*-butyl, -benzyl, -*i*-hexyl, -2-ethyl-hexyl, -*n*-octyl, -*i*-nonyl, and -*i*-decyl monoesters) in marine and freshwater sediments collected from three locations in the Greater Vancouver area. After a lag period in which no apparent biodegradation occurred, all MPEs tested showed degradation rates in both marine and freshwater sediments at 22 °C with half-lives ranging between 16 and 39 h. Half-lives increased approximately 8-fold in incubations performed at 5 °C. Biodegradation rates did not differ between marine and freshwater sediments. Half-lives did not show a relationship with increasing alkyl chain length. We conclude that MPEs can be quickly degraded in natural sediments and that the similarity in MPE degradation kinetics among sediment types suggests a wide occurrence of nonspecific esterases in microorganisms from various locations, as has been reported previously.


*Keywords:* Phthalate monoester; Degradation; Sediment; Environmental persistence; Lag phase

## 1. Introduction

Di-alkyl phthalate esters (DPEs) are a family of chemicals widely used in the manufacture of plastics (e.g. polyvinyl chloride, polystyrene, polyvinyl acetate) and many other industrial and consumer products including adhesives, paints, lacquers, and medical and personal care products. Their high global production, estimated at more than 5.2 million tonnes per annum (Parkerton and Konkel, 2001), makes the environmental fate of DPEs of considerable interest. Because DPEs are additives and are not chemically bound to the products (e.g. polymers) to which they are added, they have the potential to migrate from consumer products into the environment. Once in the environment, DPEs are subject to biodegradation by microorganisms in soil, water and in freshwater and marine sediments under both aerobic and anaerobic conditions (Saeger and Tucker, 1976; Ejlertsson et al., 1997; Staples et al., 1997; Cartwright et al., 2000; Hashizume et al., 2002; Yuan et al., 2002; Chang et al., 2004).

The pathway of microbial degradation of DPEs includes ester hydrolysis to the corresponding mono-alkyl phthalate acid ester (MPE). MPEs can undergo further enzymatic ester hydrolysis to form phthalic acid, which is further broken down to benzoic acid, and ultimately to carbon dioxide (Xu et al., 2006). Microbial degradation rates of DPEs vary substantially (Sugatt et al., 1984; Staples et al., 1997; Cartwright et al., 2000; Yuan et al., 2002; Chang et al., 2004). For example, diethyl phthalate (log $K_{ow} = 2.38$) has a degradation half-life in soil of approximately 0.8 day, whereas only 10% of the more lipophilic di-(2-ethylhexyl) phthalate (log $K_{ow} = 7.50$) is removed from the same soil after a

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70 day incubation (Cartwright et al., 2000). Scholz (2003) used $CO_2$ evaluation to measure ultimate biodegradation rates of mono-*n*-butyl phthalate (MnBP), mono-*iso*-butyl phthalate (MiBP), mono-2-ethylhexyl-phthalate (MEHP), mono-isononyl phthalate (MiNP), mono-*n*-hexyl/*n*-octyl/*n*-decyl-phthalate ($M_{6/8/10}P$) and mono-*n*-octyl/*n*-decyl-phthalate ($M_{8/10}P$) and found all MPEs to be readily biodegradable. Microbial degradation rates of MPEs in natural sediments have not been reported to date as far as we know. In this paper, we investigate the degradation half-lives of eight MPE congeners in marine and freshwater sediments. The measured rates are important information in assessments of the overall fate of DPEs and their reaction products in the environment.

## 2. Methods

### 2.1. Chemicals

Mono-ethyl phthalate (MEP), mono-benzyl phthalate (MBzP), mono-*iso*-hexyl phthalate (MiHxP), mono-ethyl-hexyl phthalate (MEHP), mono-*n*-octyl phthalate (MnOP), mono-*iso*-nonyl phthalate (MiNP) and mono-*iso*-decyl phthalate (MiDP) were gifts from Dr. T.F. Parkerton (ExxonMobil Biomedical Sciences, Annandale, NJ). They were synthesized by Chemsyn Science Laboratories (Lenexa, KS) and had 97–99% purity as esters as determined by HPLC. Mono-*n*-butyl phthalate (MnBP) was synthesized with 97% purity by BASF (Mount Olive, NJ) and was a gift from Dr. K.A. Robillard (Eastman Kodak, Rochester, NY). MiHxP, MiNP and MiDP were isomeric mixtures of alcohols; the named MPE was the dominant component. Individual stock solutions were prepared in glass-distilled acetonitrile (Caledon Laboratories Ltd., Georgetown, ON) and stored at 4 °C in the dark. Spiking solutions were prepared in acetonitrile from these stocks. The purity of all other solvents was HPLC grade. Trimethylsilyldiazomethane was purchased from Sigma-Aldrich (Mississauga, ON).

### 2.2. Collection of sediment samples

Surface sediment samples were collected using a petit ponar grab sampler from two locations in False Creek (called 'North Central' and 'Marina South' in Mackintosh et al., 2004), an urbanized marine inlet in Vancouver. Freshwater sediments were collected from Buntzen Lake (Buntzen Lake Recreation Area, north of the City of Port Moody). The top layer (0.5–1.0 cm) of each grab sample was transferred to cleaned 250 ml glass jars with foil-lined lids. The cleaning procedure for the jars, spatulas and foil for lining the jar lids was as described earlier (Lin et al., 2003). The filled jars were immediately placed on ice for transport to the laboratory. Sediments were either used for incubations immediately or were stored at −20 °C in the dark until use. The pH of the sediments was measured in the field using pHydrion pH-indicator strips (Micro Essential Laboratory, Brooklyn, NY).

### 2.3. Preparation and characterization of sediments

Frozen sediments were thawed at room temperature and any pebbles or vegetative material were removed. Autoclaved sediments were used as controls to measure any degradation or loss of chemical not due to microbial degradation. They were prepared by autoclaving three consecutive times at 120 °C for 20 min, followed by 24 h cooling periods at 22 ± 1 °C. The total organic carbon content and moisture content of the sediments were measured at the Institute of Ocean Sciences (Sidney, BC) using a Control Equipment Corporation 440 Elemental Analyzer according to Van Iperen and Helder (1985). Estimates of the number of bacteria culturable on agar under aerobic conditions were obtained using EasiCult® dip-slides (Orion Diagnostica, Espoo, Finland). The agar dipslides were inoculated by dipping into a 1:2000 dilution of sediment with sterilized milli-Q water.

### 2.4. Incubation procedure

Samples of sediment (4.0 g wet weight) were transferred to new solvent-rinsed 20 ml glass scintillation vials (VWR International, Mississauga, ON). Samples from autoclaved sediment were used for the no-biodegradation control to determine loss of MPEs by processes other than biodegradation. A small volume (8 μl) of a 1 g $l^{-1}$ MPE solution in acetonitrile was then added to the sediment slurry and gently mixed on a vortex mixer. Sediments were exposed to one to three MPEs at a time in the dark. The final concentration of each MPE tested was 2 μg $g^{-1}$ sediment (wet weight). The incubation vials were capped with foil-lined caps and wrapped in foil to eliminate the potential of photolysis. The proportion of headspace air-to-sediment at the beginning of the incubation was 4.5:1, based on the volume of sediment in the scintillation vial. The spiked sediments were incubated in triplicate at 22 ± 1 °C and at 5 ± 1 °C. At various time points, 0.5 g subsamples were removed and transferred to clean glass scintillation vials for analysis. A 10 ml volume of acetonitrile was added to 0.5 g subsamples to stop biodegradation. The sediments were not agitated or actively oxygenated during the incubations, except when removing subsamples. Incubation experiments were repeated up to nine times. Incubations of MEP, MEHP and MnOP were done with both freshly collected sediments and freezer stored sediments to investigate the possible effect of freezing on the biodegradation rate.

### 2.5. Effect of spiking solvent

To examine the potential effects of the spiking solvent acetonitrile on the biodegradation rate, the biodegradation rate of mono-ethyl phthalate (MEP) was measured in incubations of marine sediment containing 0, 500, 1250 or