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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

5000 ppm acetonitrile. Due to the relatively high solubility of MEP in water, it was possible to spike sediments with MEP without the use of acetonitrile. A 1.0 g l^{-1} solution of MEP was prepared in autoclaved milli-Q water. This concentration is below the reported water solubility of the unionized form of MEP, which is between 1.2 and 7.2 g l^{-1} (Peterson and Parkerton, 1999). The final concentration of MEP in the sediment was 4 μg g^{-1} sediment (wet weight). The amount of MEP was measured in sediment subsamples taken after 17, 47 and 65 h incubation in the dark at 22 ± 1 °C. A control experiment using autoclaved sediment spiked with the aqueous solution of MEP was also conducted to detect any loss of chemical not due to microbial degradation.

2.6. MPE extraction and derivatization procedure

An internal standard (a MPE not included in the incubation) was added to the vials containing the incubation subsamples and 10 ml acetonitrile. The samples were extracted into the acetonitrile on a vortex mixer and by sonication (5 min). After centrifugation (2500 rpm, 5 min), the acetonitrile was filtered (Whatman No. 1) and evaporated to dryness under a fumehood. The residues were dissolved in ethyl acetate (1.5 ml) by vortexing. The ethyl acetate was transferred to 2 ml amber glass autosampler vials with teflon-lined caps (Agilent, Mississauga, ON). The MPEs were derivatized to form methyl-ester analogs using trimethylsilyldiazomethane–methanol according to the procedure of Niino et al. (2002). Following derivatization, the ethyl acetate mixture was evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 0.5 ml toluene, and a 1 μl volume of this was injected into the GC/MS system.

2.7. Instrumental analysis

Methylated derivatives of MPEs were analyzed using an Agilent 6890 GC (Agilent, Mississauga, ON) equipped with an Agilent 5973 MS detector and with an Agilent cool on-column inlet. The GC was fitted with an HP-5MS 5% phenyl methyl siloxane-coated capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and a fused-silica deactivated guard column (5 m × 0.530 mm i.d.). The GC oven temperature was programmed as follows: during injection, the temperature was 50 °C, then after 2 min it was increased at 30 °C min^{-1} to 160 °C, followed by a slower temperature increase at 15 °C min^{-1} to a final temperature of 310 °C. The carrier gas was helium at a flow rate of 1.0 ml min^{-1}. The MS conditions for EI ionization of MPE derivatives were as follows: ion energy, 70 eV; ion source temperature, 230 °C; selected ions, *m/z* 91 (for the MBzP derivative) and *m/z* 163 (for all other MPE derivatives). For MPEs that were an isomeric mixture of congeners (MiHxP, MiNP, MiDP), the area of largest peak in the family of peaks appearing on the chromatogram was monitored. MPEs were quantified using calibration curves constructed by spiking autoclaved sediment with known amounts of MPEs, adding the internal standard, and taking the sample through the extraction and derivatization procedure. Curves were linear ($r^2 > 0.98$) for all MPEs over the range examined. The extraction efficiency was determined by comparing peak areas of 1 μg MEP, MBzP, MEHP and MnOP extracted from autoclaved sediment and derivatized with peak areas of derivatized standards. Extraction efficiency was 72%, 78%, 86% and 99% for MEHP, MnOP, MEP and MBzP, respectively.

2.8. Kinetic analysis

The kinetics of biodegradation of individual MPE congeners (first-order rate constant and half-life) were determined from linear regression of the slope (after the lag phase) on a plot of the logarithm of MPE concentration in subsamples of the sediment incubation versus time. The lag phase was defined as the period of time during which changes in MPE concentration were <10% of the mean concentration of the test chemical in the autoclaved control.

3. Results and discussion

3.1. Sediment characterization

The organic carbon content of the sediment was 2.90 ± 0.17% (mean ± SD, $n = 4$) for the marine sediments from False Creek and 10.8 ± 1.2% (mean ± SD, $n = 3$) for freshwater sediments from Buntzen Lake. The pH of both sediment types was 6 ± 0.2. The number of culturable bacteria was high in marine and freshwater sediment samples (i.e. $>10^8$ g^{-1} sediment, wet weight). The agar slides inoculated with autoclaved marine or freshwater sediment were blank, verifying the effectiveness of the sterilization procedure (Figure S1).

3.2. Effect of spiking solvent

Fig. 1 shows that MEP concentrations in the incubation mixtures at various exposure times throughout the biodegradation test did not vary with the acetonitrile concentrations used for spiking. This indicates that the acetonitrile concentration in the incubation had no significant effect on the degradation rate of MEP. Hence, acetonitrile was used as the vehicle for spiking the sediment with MPEs and the acetonitrile concentration was kept below 2000 ppm.

3.3. MPE biodegradation

After an initial lag phase, MPE concentrations followed an exponential decay pattern during incubations for both sediments tested while no significant change in concentration was observed in treatments with autoclaved sediments (Fig. 2). This indicates that microbial activity was

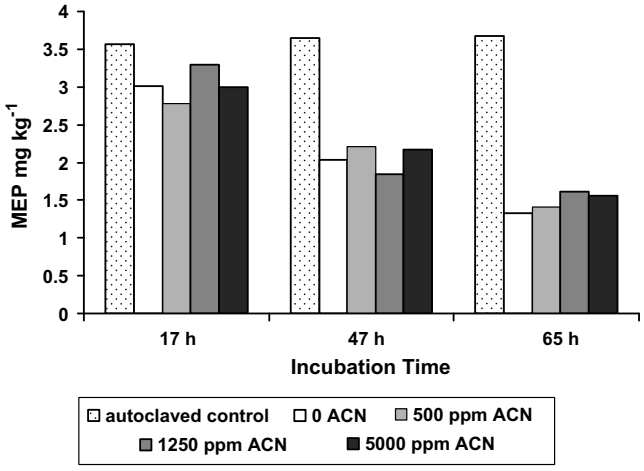

Fig. 1. Concentrations of MEP (mg kg^{-1} wet weight) in sediments at various times throughout the incubation period (22 °C in the dark) after spiking MEP dissolved in distilled water and various amounts of acetonitrile (ACN) producing ACN concentrations in the incubation mixture of 0, 500, 1250 or 5000 ppm. MEP incubated with autoclaved sediment (i.e. negative control) is also shown.

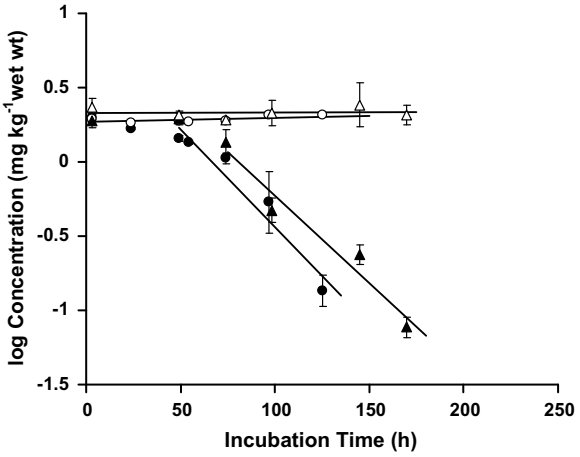

Fig. 2. 10-based logarithmic concentrations (mg kg^{-1} wet weight) of MEHP in marine (filled circles) and fresh water (filled triangles) sediments as a function of incubation time (h). Error bars represent one standard deviation, $n = 9$ for marine sediment, $n = 5$ for fresh water sediments. Open circles and triangles represent concentrations in autoclaved sediments (i.e. control).

responsible for the observed degradation of the MPEs. The lag phase varied from 18 to 70 h among individual incubations in marine sediments and between 4 and 70 h in freshwater sediments (Table 1). No relationship between the duration of the lag phase and congener type is apparent. Fig. 2 shows that after the initial lag phase MPE concentrations in sediments dropped quickly over time. First-order biodegradation half-lives ranged between 16 ± 2 h (mean ± SD) for MnBP to 26 ± 9 h (mean ± SD) for MEHP in marine sediments and between 26 ± 7 h (mean ± SD) for MnOP to 39 ± 6 h (mean + SD) for MiDP in freshwater sediments (Table 1).

Figure S2 shows that the degradation rates in freshwater and marine sediments are comparable. No significant differences ($P < 0.05$) were found between half-life times measured in freshwater and marine sediments for individual MPE congeners following an analysis of variance. The comparability of esterase activity among water bodies is not without precedent. Paris et al. (1984) observed that biodegradation rates of 2,4-D *n*-alkyl esters were similar among water sampled from five different sites and concluded that non-specific esterase activity occurs widely in microorganisms. The kinetics of microbial degradation of bisphenol A also appear similar in waters sampled throughout the USA and Europe, both freshwater and marine, and from light and heavily urbanized areas (Klecka et al., 2001).

Fig. 3 illustrates that half-lives of MPEs are comparable among congeners and that there is no apparent relationship between the half-life time and the length or extent of

Table 1
log K_{ow}, log *D*, mean measured degradation half-life time ($t_{1/2}$ in h) at 22 °C, range of measured degradation half-life times (in h) at 22 °C, number of independent experiments n and the mean measured degradation half-life time ($t_{1/2}$ in h) at 5 °C for various phthalate monoesters in natural marine and freshwater sediments

Chemical name	Abbreviation	log K_{ow}	log *D* (pH 6)	$t_{1/2}$ (h), 22 ± 1 °C (mean ± SD)	$t_{1/2}$ (h), range	*n*	Lag phase (h), range	$t_{1/2}$ (h), 5 ± 1 °C (mean ± SE)
Marine sediments								
Mono-ethyl phthalate	MEP	1.86	−0.79	35 ± 10	28–42	2	20–40	
Mono-butyl phthalate	MnBP	2.84	0.07	16 ± 2	14–18	4	24–50	150 ± 12
Mono-benzyl phthalate	MBzP	3.07	0.64	26 ± 12	12–43	5	18–50	188 ± 78
Mono-*iso*-hexyl phthalate	MiHxP	3.85	0.86	26 ± 4	12–24	5	22–33	
Monoethylhexyl phthalate	MEHP	4.73	1.66	26 ± 9	20–48	9	18–50	215 ± 13
Mono-*n*-octyl phthalate	MnOP	5.22	1.66	18 ± 4	14–24	8	18–50	225 ± 50
Mono-*iso*-nonyl phthalate	MiNP	5.30	1.99	23 ± 5	17–29	4	20–70	200 ± 44
Mono-*iso*-decyl phthalate	MiDP	5.79	2.45	25 ± 6	21–29	2	22–30	
Freshwater sediments								
Mono-butyl phthalate	MnBP	2.84	0.07	30 ± 16	12–40	3	4	
Mono-benzyl phthalate	MBzP	3.07	0.64	34 ± 10	25–45	3	4	
Monoethylhexyl phthalate	MEHP	4.73	1.66	29 ± 9	19–44	5	50–140	
Mono-*n*-octyl phthalate	MnOP	5.22	1.66	26 ± 7	19–34	5	50–70	
Mono-*iso*-nonyl phthalate	MiNP	5.30	1.99	39 ± 6	32–44	3	4	

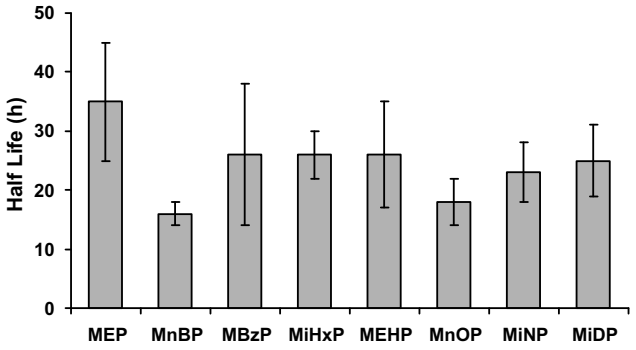

Fig. 3. Biodegradation half-life times (h) of MPEs arranged in the order of increasing molecular weight. Mean ± SD.

branching of the monoester alkyl-chain. Biodegradation profiles of DPEs observed by Yuan et al. (2002) and Howard et al. (1991) show that half-life times also do not vary with increasing K_{ow} for DPEs with a log K_{ow} up to 4.5. For DPEs with a log K_{ow} >4.5, the half-life times of DPEs increase markedly with increasing K_{ow}. Table 1 shows that while the calculated log K_{ow} of undissociated MPEs range between 1.86 and 5.79 (Peterson and Parkerton, 1999), log D values (i.e. ionization corrected log K_{ow}) for MPEs at the experimental pH of 6 only range between −0.79 and 2.45 (calculated by ChemAxon's Marvin Sketch® following Viswanadhan et al., 1989) and hence are much lower than their calculated log K_{ow}. The lack of an increase of the half-life time with increasing length of the alkyl-chain of the MPEs is therefore consistent with the observations for low molecular weight DPEs with a log K_{ow} below 4.5. The low effective K_{ow} of MPEs may translate in a low particle-water sorption coefficient and a relatively fast particle to water desorption rate. Desorption is therefore unlikely to be a rate limiting step in the biodegradation process for MPEs.

Figure S3 shows that half-lives measured in freshly collected and previously frozen sediments were approximately the same and an analysis of variance showed no statistically significant ($P < 0.05$) differences between the half-lives. This indicates that sediment storage in the freezer did not have an effect on the measurement of the biodegradation rate. Table 1 shows that half-life times in marine sediments incubated at 5 °C were approximately 8-fold greater than those measured at 22 ± 1 °C. Such temperature dependency of biodegradation is typical, and indicates that lower temperatures cause a lower microbial activity and hence a lower rate of MPE degradation.

The results indicate that MPEs appear to be readily degraded by microbial organisms in natural fresh water and marine sediment with half-life times ranging between approximately 225 h (at 5 °C) to a minimum value of 16 h (at 22 °C). These values are many times lower than half-life times used to identify persistent substances under current regulatory initiatives. These findings are consistent with those reported by Scholz (2003), who examined the ultimate biodegradation of MnBP, MiNP, MiBP, and a mixture of MnOP and MnDP to CO_2 and found that all test chemicals were completely and readily biodegradable after a lag phase of approximately 48 h.

The occurrence of a lag phase prior to degradation is a common observation in laboratory studies of the microbial biodegradation of chemicals, including this study, and can last up to 3 weeks (Staples et al., 1999; Chang et al., 1999; Ingerslev et al., 2000). The general view is that the lag phase reflects the time for specific populations of degraders to grow to a level where disappearance of a particular substrate can be detected (Chen and Alexander, 1989). It may also reflect the preferential consumption of other organic nutrients by the microorganisms, i.e. once the primary nutrient source is sufficiently reduced, degradation of the test chemical may begin (Ingerslev et al., 2000). Time required for enzyme induction (Aronson and Howard, 1999), lack of nutrients (Wiggins et al., 1987) and degrader predation (Wiggins et al., 1987) have also been proposed to cause the lag phase. While the cause of the lag phase remains unknown, the implication of lag phases on chemical persistence remains equally unclear. However, the role of the lag phase is worthy of investigation and consideration in environmental fate models.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2008.01.059.

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