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Environmental and Chemical Factors Influencing the Biodegradation of Phthalic Acid Esters in Freshwater Sediments

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ABSTRACT

A wide range of environmental and chemical factors influenced the biodegradation of simple and complex phthalic acid esters in an aquatic environment. The length and configuration of the alkyl phthalate diester significantly affected the primary biodegradation of di-n-butyl (DBP), di-2-ethylhexyl (DEHP), di-isooctyl (DIOP), and di-isononyl (DNIP) phthalate. After 14 days incubation in aerobic sediments at 22°C, less than 2% of the branched-chain alkyl phthalates—DEHP, DIOP and DINP (at μg concentrations)—were biodegraded, compared with 85% of the linear alkyl DBP. Primary biodegradation of DEHP, DIOP and DINP was significantly greater at high concentrations (> μg litre^{-1}) and high temperatures (> 22°C) in freshwater sediments. Pre-exposure of the sediments to DBP, DEHP, DIOP and DINP did not influence their biodegradation. The addition of organic nutrients significantly affected the primary biodegradation of DBP with varied results, depending on the nutrient, its concentration, and the time of addition. Neither inorganic nitrogen nor phosphorus alone or in combination influenced the degradation of DBP. The complex alkyl phthalate DEHP in sediments biodegraded under anaerobic conditions; even though the process was slow, both primary and ultimate degradation did occur.

INTRODUCTION

Phthalic acid esters (PAEs) have many applications in modern society. They are chiefly used as plasticisers with synthetic polymers, such as polyvinyl chloride (PVC), to impart flexibility, workability, and extensibility to the final product (Graham, 1973). Their widespread use has made PAEs a common aquatic contaminant in the environment (Mayer *et al.*, 1972; Zitko, 1972; Stalling *et al.*, 1973; Mathur, 1974*a*; Johnson *et al.*, 1977; Giam *et al.*, 1978; Schwartz *et al.*, 1979; Pierce *et al.*, 1980).

Laboratory efforts to estimate the probable fate of PAEs in the environment resulted in numerous biodegradation studies and myriads of test procedures: pure culture, enriched culture, activated sludge, river die-away and sediment microcosms (Mathur, 1974*a*; Engelhardt *et al.*, 1975; Johnson & Lulves, 1975; Saeger & Tucker, 1976; Kurane *et al.*, 1979; Wylie *et al.*, 1982). These investigations suggested that diverse microbiota degrade many PAEs. The degradative pathway of each is similar: initial hydroxylation and hydrolysis of the alkyl portions of the diester, rapid decarboxylation of the phthalic acid moiety, and ultimately ring cleavage and oxidation of the catechol residue (Johnson & Lulves, 1975; Keyser *et al.*, 1976). These laboratory tests focused on the biodegradability of PAEs and, to a limited extent, on the environment where it occurred. They essentially omitted environmental and chemical factors that may determine the fate and persistence of PAEs in natural ecosystems. The objective of the present study was to determine how these factors influenced the biodegradation of simple and complex PAEs in freshwater sediments. The environmental and chemical factors investigated were: (1) type of ester side-chain; (2) oxygen tension; (3) concentration of the test chemical; (4) temperature; (5) organic and inorganic nutrient enrichment; and (6) pre-exposure of PAE to indigenous microbiota.

MATERIALS AND METHODS

Chemicals

Carbonyl-[^{14}C] labelled di-*n*-butyl phthalate (DBP), 1·53 mCi mM^{-1}, was purchased from Mallinckrodt Co., St Louis, Missouri; carbonyl-[^{14}C] and ring-[^{14}C] labelled di-2-ethylhexyl phthalate (DEHP), 13·36 and 10·52 mCi mM^{-1}, respectively, were purchased from Pathfinder

Laboratories Inc., St Louis, Missouri; and carbonyl-[^{14}C] labelled di-isooctyl phthalate (DIOP) and di-isononyl phthalate (DINP), 9·59 mCi mM^{-1} each, were donated by Exxon Research and Engineering, Linden, New Jersey. Purity of the chemicals exceeded 99 % as determined by gas–liquid and thin-layer chromatography. Acetone, which was used as a carrier solvent, did not exceed 0·1 % of the total volume. The rationale for the test concentration of PAEs was based on DBP, DEHP and DIOP residues in freshwater and marine sediments (Mayer *et al.*, 1972; Schwartz *et al.*, 1979). In the present study, we considered μg litre^{-1} (or μg kg^{-1}) concentrations of PAEs to be environmentally relevant.

Freshwater sediments and microbial degradation

Sediment and water samples were taken from Little Dixie Lake, an 83-ha impoundment located in an agricultural watershed 16 km east of Columbia, Missouri. Chemical characteristics of the lake water, which were reported by Johnson (1982), are similar to those found in other Missouri reservoirs (Jones, 1977). Sediment samples (pH 7·6 $\pm$ 0·2, total organic carbon 8·0 $\pm$ 0·7 %) were collected from the littoral zone with an Ekman dredge. The upper 3 cm of sediment at the water:soil interphase were removed, homogenised at low speed in a blender, and stored aerobically in the dark at either 12 or 22 °C.

In the basic biodegradation test, a 100-ml volume of lake water and sediment (9:1, wt/wt) was placed in a 250-ml Erlenmeyer flask (or reaction beaker in the flow-through respirometer), sealed tightly with a rubber stopper, and incubated with different [^{14}C]-phthalate compounds. Biodegradation was monitored by trapping and assaying $^{14}CO_2$ evolved from inoculated sediments. Primary degradation (i.e. ester cleavage) was determined by using carbonyl [^{14}C]-labelled phthalates. The process was a two-step reaction: initial hydrolysis of the ester linkage followed by rapid decarboxylation of the exposed labelled carbonyl groups of the phthalic acid moiety. The first major degradation product, the monoester, was not monitored by this radiometric procedure. Previous research (Johnson & Lulves, 1975) clearly established that the monoester does not persist. Decarboxylation of the free carbonyl group of the phthalic acid moiety occurred rapidly after hydrolysis of the diester with release of the detectable [^{14}C] as $^{14}CO_2$. Therefore the use of $^{14}CO_2$ as an indicator of primary degradation of the phthalates is valid. The ring-labelled compound DEHP ([^{14}C]-phthalic acid moiety) was used to

determine ultimate degradation (i.e. complete mineralisation) of a complex phthalate. We used two types of radiorespirometer: a static type (Skaar *et al.*, 1981) modified after Gledhill (1975), and a flow-through type (Johnson, 1980). Labelled CO_2 was trapped with either 0·2 N KOH or monoethanolamine–ethylene glycol (3:7, v/v); duplicate 1-ml samples of these trapping solutions were counted with an appropriate scintillation cocktail (Johnson, 1980). Radioactivity was measured with a Beckman LS-230 liquid scintillation counter; counts were corrected for trapping efficiency, quench and background radiation. In the static respirometer aerobic conditions were maintained by aerating (swirling) and venting the samples semi-weekly. In the flow-through respirometer, a flow of either purified air or gaseous nitrogen was used to obtain aerobic or anaerobic environments. All samples were conducted in triplicate at 22 °C (unless otherwise indicated), incubated in the dark for 28 days, and sampled at 3, 7, 21 and 28 days.

All biodegradation experiments were conducted as described above with the following variables:

(1) *Multiple concentrations.* Concentrations of PAEs ranging from $\mu g\,litre^{-1}$ to $mg\,litre^{-1}$ were prepared by mixing labelled and unlabelled PAEs together. A new specific activity was calculated for each compound based on 10-fold dilutions (Table 4, later). The effects of chemical concentration on the primary biodegradation of DBP, DEHP, DIOP and DINP were determined.

(2) *Multiple temperatures.* All sediment microcosms were acclimated to the test temperature for 1 week before the degradation test. All multiple temperature experiments were performed simultaneously with similar sediments. The biodegradation of DBP, DEHP, DIOP and DINP was determined at 12, 22 and 28 °C; only DBP and DEHP were tested at 5 °C.

(3) *Organic and inorganic nutrient enrichment.* For organic enrichment of sediments in biodegradation tests a common carbohydrate and readily available organic nitrogen source—glucose and peptone (Bacto-dextrose and Bacto-peptone, Difco Laboratories, Detroit, Michigan)—at concentrations of 0·01, 0·1 and 1 % were used. Organic nutrients at 0·01 % were added on day 3 during the test after $^{14}CO_2$ sampling, and monitored on days 7 and 14. Inorganic nitrogen, 7·5 mg $litre^{-1}$ as KNO_3, and phosphorus, 0·5 mg $litre^{-1}$ as KH_2PO_4, were added. The normal

N:P (15:1) ratio for Little Dixie Lake was maintained during inorganic enrichment; these values represented a 10-fold increase over natural concentrations (Johnson, 1982).

(4) *Pre-exposure.* All sediments were pre-exposed to the test PAE for 28 days and then incubated with the [^{14}C]-labelled phthalate for an additional 28 days. Controls consisted of untreated and carrier-solvent treated sediments; acetone was used as solvent of all PAEs. Each compound tested was at the same concentration as during the pre-exposure period.

Statistical analysis

Results were reported as total percent degradation of the PAE substrate introduced; however, statistical analysis was performed on untransformed data. Using the Statistical Analysis System (SAS, 1979) computer package, we determined differences among treatments over time within each experiment by two-way analysis of variance and least-significant difference tests and regression coefficient analysis with 't' test comparison. Unless otherwise indicated, statements of statistical significance imply $P \leq 0{\cdot}05$.

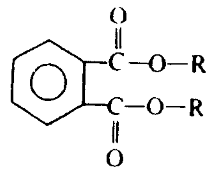
RESULTS AND DISCUSSION

Ester side-chain complexity

The length and configuration of the alkyl group of the diester significantly influenced the biodegradation of the four phthalates DBP, DEHP, DIOP and DINP when they were incubated in aerobic freshwater sediments at 22 °C (Tables 1 and 2). The butyl ester (DBP) with its short, linear 4-carbon alkyl groups was rapidly degraded—nearly 85 % in 14 days. Significantly, the addition of another four or five carbons to the alkyl group, e.g. di-ethylhexyl, di-isooctyl or di-isononyl—all branched chains—dramatically increased the persistence of the phthalates in sediments. For example, after 14 days of aerobic incubation, less than 2 % of these PAEs were biodegraded as compared with 85 % of DBP. Even after an additional 14 days of incubation in the sediments, 94 % or more of these compounds remained unrecovered as CO_2. Although there were significant differences ($P = \leq 0{\cdot}01$) in the biodegradation rates among the

TABLE 1
Phthalic Acid Ester Structures

Phthalic acid moiety



Compound	*Abbreviation*	*Mol. wt.*	*Ester moiety*
Di-*n*-butyl phthalate	DBP	278	$R = -CH_2-CH_2-CH_2-CH_3$
Di-2-ethylhexyl phthalate	DEHP	390	$R = -CH_2-CH(-CH_2-CH_3)-CH_2-CH_2-CH_2-CH_3$
Di-isooctyl phthalate	DIOP	390	$R = -CH_2-CH_2-CH_2-CH_2-CH_2-CH(CH_3)-CH_3$
Di-isononyl phthalate	DINP	416	$R = -CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH(CH_3)-CH_3$

TABLE 2
Influence of Ester Side-Chain Complexity on the Primary Biodegradation of DBP, DEHP, DIOP and DINP in Freshwater Sediments[a]

Compound	*Time (days)*	
	14	*28*
DBP	84·6 (2·1)[b]	NR[c]
DEHP	1·85 (0·21)	5·90 (0·53)[d]
DIOP	1·28 (0·02)	2·66 (0·15)[d]
DINP	0·54 (0·04)	1·11 (0·10)[d]

[a] Values are reported as total percent biodegradation of inoculum, mean ±SD (in parentheses), all samples in triplicate, temperature 22 °C. Inoculum of [^{14}C]-carbonyl phthalate (μg litre^{-1}) was: DBP, 82; DEHP, 18·2; DIOP, 18·3; and DINP, 19·7.
[b] At 14 days incubation DBP degradation was significantly different from that of DEHP, DIOP and DINP (ANOVA, $P = \leq 0{\cdot}05$).
[c] Not reported.
[d] Regression analysis showed significant difference ($P = \leq 0{\cdot}01$) of degradation rates over 28-day incubation period; each 28-day sample was significantly different from 14-day sample (ANOVA, $P = \leq 0{\cdot}05$).

long-chain esters, the absolute amount of degradation was similar: DEHP, 6%; DIOP, 3%; and DINP, 1%. Johnson & Lulves (1975) showed that degradation of DEHP in sediments does vary among samples collected at different times or seasons of the year. In the present series from Little Dixie Lake, the degradation of DEHP never exceeded 25%, after 28 days incubation at 22 °C.

The length and configuration of the alkyl diester chains of these phthalates appeared to significantly influence their persistence in freshwater ecosystems. Swisher's (1970) generalisation relating chemical surfactant structures to biodegradation rates seemed to apply here; microbial degradation of PAEs in these freshwater sediments was promoted by alkyl group linearity but deterred by alkyl group-branching.

Aerobiosis and anaerobiosis—ultimate biodegradation

Ring cleavage of the branched-chain diester DEHP occurred in both aerobic and anaerobic sediments at 22 °C (Table 3). In aerobic sediment 13·8% of the ring-[^{14}C] labelled DEHP biodegraded in 28 days, as compared with 9·9% (estimated) under anaerobic conditions. (Certainly

the use of anaerobic $^{14}CO_2$ data must be evaluated with caution because methanogenesis in anaerobic sediments may result in losses of CO_2. We did not account for this possible loss in the present study.) The rate of ring cleavage of DEHP over time differed significantly ($P \leq 0{\cdot}01$) between aerobic and anaerobic sediments. Under aerobic conditions, 5% of the ring-labelled DEHP was degraded during the first week; however, the rate decreased by the fourth week. Under anaerobic conditions, 1% of the ring-labelled DEHP was degraded during the first week, and degradation tended to follow a linear pattern, increasing with time to over 4% by the fourth week ($P = \leq 0{\cdot}01$).

TABLE 3
Ultimate Biodegradation of the Long-Chain Phthalate DEHP in Freshwater Sediments Under Either Aerobic or Anaerobic Conditions[a]

	Time (days)			
	7	*14*	*21*	*28*
Aerobic	5·05 (2·54)	9·06 (3·96)	12·08 (5·37)	13·79 (6·49)[b,c]
Anaerobic	1·00 (0·15)	3·18 (0·49)	5·73 (0·58)	9·86 (1·21)[b]

[a] Values are reported as total percent biodegradation of inoculum; mean ±SD in parentheses, all samples in triplicate, temperature 22°C. [^{14}C]-labelled DEHP original concentration was 14·3 μg litre^{-1}.

[b] Regression analysis indicated slope difference ($P = \leq 0{\cdot}05$).

[c] Significant difference between day 7 and day 28 samples (ANOVA, $P = \leq 0{\cdot}05$).

In these experiments we documented for the first time the biodegradation of a complex long-chain alkyl phthalate under anaerobic conditions in freshwater sediments: the process was slow but primary and ultimate degradation nevertheless did occur. Also, we found that DIOP and DINP in anaerobic sediments degraded slowly, with less than 1% of the parent compound appearing as CO_2 in 28 days. (Data not shown in tables or figures; we detected only primary degradation because of the carbonyl labelling.) These differences in biodegradation probably resulted from the effects of oxygen on both the adsorptive characteristics of the chemical and the enzymatic activities at different steps in the degradative pathway. For example, Sodergren (1982) reported that binding adsorption of DEHP on sediment and surfaces decreased its

bioavailability. The decreasing aerobic degradation rates of the ring-[^{14}C]-labelled DEHP may indicate binding or adsorption of the substituted benzene intermediates, such as catechol and protocatechuate, which occurred after ester hydrolysis and decarboxylation of the phthalic acid moiety. If this were true, $^{14}CO_2$ evolution from the carbonyl-[^{14}C]-labelled DEHP would be unaffected and the degradation of ring-[^{14}C]-labelled DEHP to $^{14}CO_2$ would be decreased due to adsorption of the substituted benzene intermediates. The increasing degradation that was observed for the anaerobic ring-[^{14}C]-labelled DEHP may be explained enzymatically. It is known that specific esterases (Kirsch, 1971) and dioxygenases (Keyser *et al.*, 1976) are involved in the ester hydrolysis and ring cleavage of PAEs. Because dioxygenative ring cleavage is both NADH- and oxygen-dependent, the increasing rate of ring cleavage in the absence of oxygen may indicate the presence of an inducible, non-oxygen-dependent enzyme system, resulting in a different degradative pathway. Clearly, further research is needed to determine the specific mechanism or mechanisms by which chemical binding, adsorption, shifting microbial populations and enzymatic activities affect PAE biodegradation.

Experiments with multiple concentrations

Biodegradation of the long-chain phthalates DEHP, DIOP and DINP over time was significantly greater at very high concentrations (i.e. 10 mg litre^{-1}) than at the lower concentrations (Table 4). Cumulative degradation values of all three compounds were similar within the low to high range (i.e. from about 18 μg litre^{-1} to about 1·8 mg litre^{-1}). The small differences were attributed to good replication within the experiment; in most instances the standard deviation among compounds was low. In contrast, the concentration of DBP had no detectable effect on its biodegradation rate in sediment.

These results indicated that complex long-chain PAEs degraded slowly at low concentrations. Similarly, Boethling & Alexander (1979) found that the biodegradability of certain xenobiotic chemicals (2,4-dichlorophenoxyacetate, 1-naphthyl-N-methyl carbamate, *p*-chlorobenzoate and chloracetate) decreased markedly at realistically low environmental concentrations. Thus it would be misleading to predict PAE persistence in the environment from conventional laboratory procedures that test PAE concentrations as high as 6000 mg litre^{-1} (Kurane *et al.*, 1979; Mathur, 1974*b*).

TABLE 4
Effect of Different Chemical Concentrations of DBP, DEHP, DIOP and DINP on their Primary Biodegradation in Freshwater Sediments[a]

Compound	*Concentration (mg litre^{-1} in parentheses)*[c]	*Time (days)*[b]			
		7	*14*	*21*	*28*
DBP	High (8·2)	64·6 (3·4)	72·6 (4·0)	NR	NR
	Medium (0·82)	63·2 (3·1)	70·1 (2·6)	NR	NR
	Low (0·082)	64·1 (3·1)	70·9 (2·0)	NR	NR
DEHP	Very high (10·0)	3·70 (0·3)	9·08 (0·4)	14·64 (0·4)	19·79 (0·6)[c,d]
	High (1·82)	1·73 (0·1)	4·29 (0·2)	6·46 (0·3)	8·47 (0·5)[d]
	Medium (0·182)	1·83 (0·1)	4·59 (0·1)	6·99 (0·2)	9·29 (0·3)[d]
	Low (0·018 2)	2·24 (0·5)	5·19 (0·6)	7·76 (0·7)	9·98 (0·9)[d]
DIOP	Very high (10·0)	2·77 (0·2)	4·27 (0·4)	6·84 (0·8)	9·62 (1·4)[c,d]
	High (1·83)	0·88 (0·1)	1·53 (0·1)	2·56 (0·4)	3·77 (0·7)[d]
	Medium (0·183)	0·97 (0·1)	1·76 (0·1)	2·94 (0·1)	4·44 (0·3)[d]
	Low (0·018 3)	0·93 (0·01)	1·66 (0·01)	3·00 (0·2)	4·89 (0·3)[d]
DINP	Very high (10·0)	0·48 (0·02)	0·81 (0·03)	1·15 (0·1)	1·58 (0·01)[c,d]
	High (1·97)	0·30 (0·05)	0·56 (0·07)	0·84 (0·08)	1·15 (0·08)[c]
	Medium (0·197)	0·29 (0·03)	0·57 (0·04)	0·91 (0·02)	1·30 (0·03)[c]
	Low (0·019 7)	0·36 (0·08)	0·70 (0·15)	1·08 (0·18)	1·46 (0·20)[c]

[a] Values are reported as total percent biodegradation of inoculum; mean ± SD in parentheses; all samples in triplicate; temperature 22 °C.
[b] NR = not reported.
[c] Regression analysis indicated slope difference ($P = \leq 0{\cdot}05$).
[d] Significant difference between day 7 and day 28 samples (ANOVA, $P = \leq 0{\cdot}05$).

Multiple temperature experiments

As expected, temperature had a significant effect on the biodegradation of both simple and complex phthalates (Fig. 1). For example, by day 7 about 16 % of the DBP biodegraded at 5 °C, 56 % at 12 °C, 73 % at 22 °C and 86 % at 28 °C. By day 14, primary biodegradation of DBP was complete at the highest temperature. During days 14 to 28 (Fig. 1), degradation of DEHP, DIOP and DINP was also significantly greater at 28 °C than at lower temperatures. This difference was two-fold to six-fold depending on the day and the PAE. Compared with the values for DBP, these differences were small.

These data confirmed the result of Mathur (1974*b*), who reported that dioctyl phthalate and DEHP were used by soil enrichment cultures at 22 and 32 °C but not at 4 and 10 °C. Mathur also observed, as we did, that

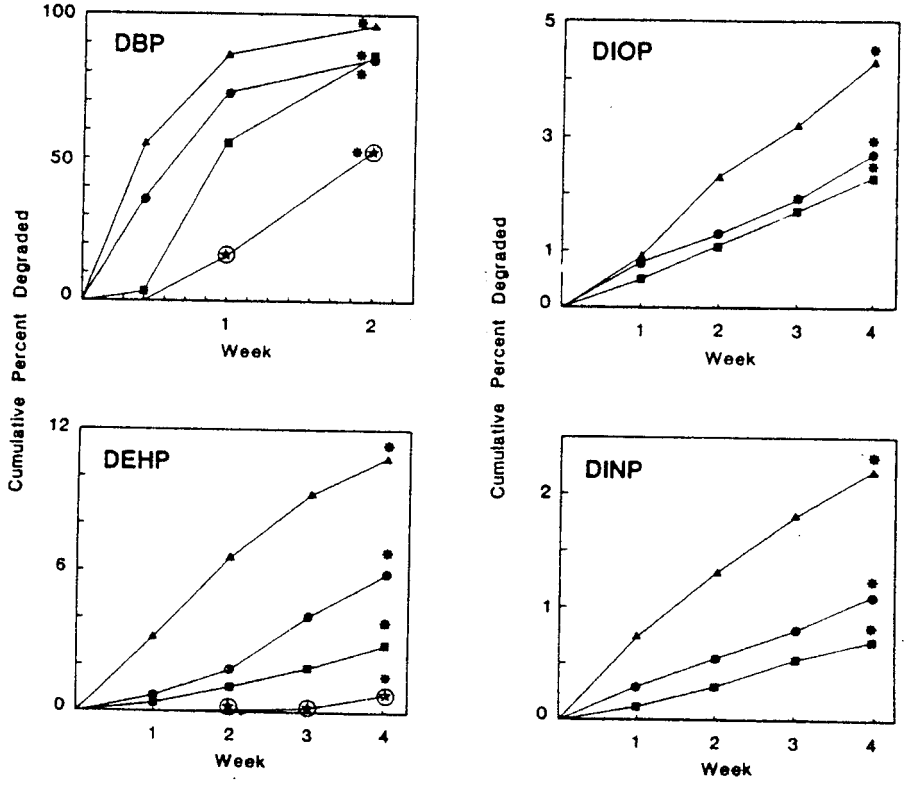


Fig. 1. Effect of different temperatures on the primary biodegradation of DBP, DEHP, DIOP and DINP in freshwater sediments. Values are reported as total percent biodegradation of inoculum. All samples are in triplicate at a temperature of 22 °C. Significant differences (*) ($P = \leq 0{\cdot}05$); differences between regression coefficients were determined by t test ($P = \leq 0{\cdot}05$). There were no significant differences between the 22° and 28° slopes for DBP. Symbol designations: 5 °C (⊛); 12 °C (■); 22 °C (●); and 28 °C (▲).

DBP degraded at temperatures ranging from 4 up to 21 °C at rates directly proportional to temperatures. These differences may be caused by a divergence of the microbial flora upon acclimation at different temperatures, as well as a difference between the relative persistence of DEHP, DIOP and DINP compared with DBP. Possibly the enzymatic degradation of DBP is more easily initiated at lower temperatures than comparable activities necessary for degradation of the more complex PAEs. The differences, however, may be a matter of bioavailability—DBP is more water-soluble than the long-chain PAEs (Pierce *et al.*, 1980).

Nutrient enrichment experiments

The addition of organic nutrients significantly influenced the biodegradation rate of DBP in sediments. The results varied, depending on the nutrient, its concentration and the time of addition (Fig. 2). For example, the degradation rates of DBP were significantly depressed by the addition of 0·1 and 1·0% glucose on day 0 (Fig. 2), but stimulated by the addition of 0·01% glucose on day 3. We speculated that the reduction was probably a diaxic-like response: the more readily available glucose was selectively used by the sediment microbiota in preference to the xenobiotic moiety DBP. The addition of glucose on day 3 may have stimulated the growth of hydrosoil microbiota (as do also the 0·1 and 1·0% glucose treatments) before the glucose was rapidly used, leaving an increasingly active microbial population (and one that was perhaps less perturbed than the population exposed to a higher concentration of glucose) with a depleted carbon source. Presumably this active population contained a greater number of microbiota capable of degrading DBP; thus biodegradation by days 7 and 14 was significantly greater than in the controls. The role of organic nitrogen is unclear; however, peptone at a concentration of 0·1% on day 0 did stimulate the biodegradation of DBP.

Lewis & Holm (1981) reported similar diaxic-like responses; the biodegradation rate of diethyl phthalate in axenic cultures of *Brevibacterium* sp., isolated from *Aufwuchs*, was depressed after conditions of glucose at 100 and 1000 mg litre^{-1}. Similarly, Chou & Bohonos (1979) found that the presence of readily assimilated microbial nutrients (glucose and yeast extracts) inhibited the formation and maintenance of mixed culture-degrading systems.

Neither the addition of inorganic nitrogen nor phosphorus alone or in combination significantly influenced the degradation of DBP in sediments. However, we observed that sediments from the more eutrophic waters tended to degrade DBP and DEHP more rapidly. Under similar conditions of temperature, PAE concentration and dissolved oxygen, hydrosoil samples taken from ponds at the Columbia National Fisheries Research Laboratory (N:P concentration, 1100:100 g kg^{-1}) degraded DBP and DEHP about 1·5–4 times faster (Johnson & Lulves, 1975; Johnson, 1980) than samples from Little Dixie Lake (N:P concentration, 678:52 g kg^{-1}).

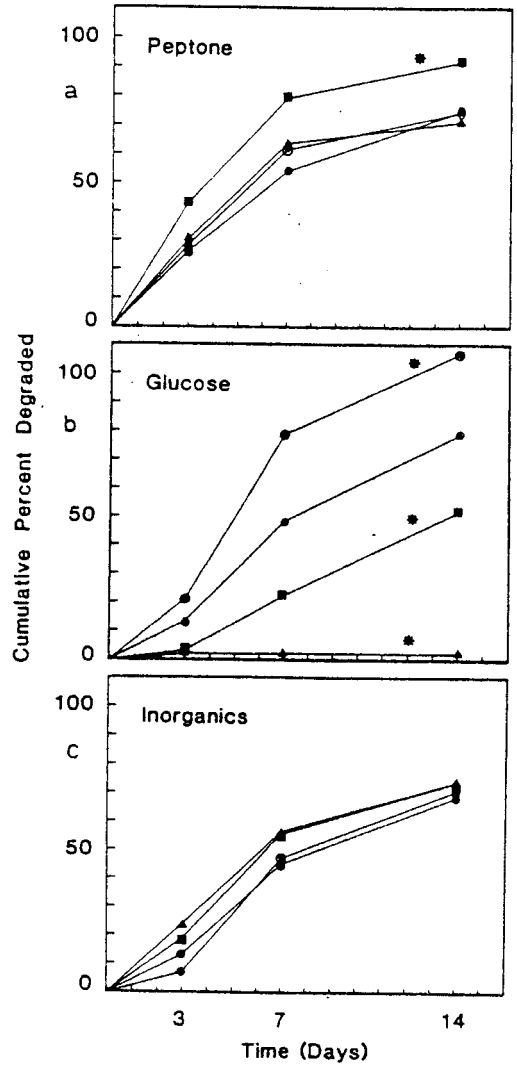


Fig. 2. Effects of organic and inorganic enrichment on the primary biodegradation of DBP in freshwater sediments. All values are reported as total percent biodegradation of DBP. All samples are in triplicate at a temperature of 22 °C. Significant differences (*) ($P = \leq 0{\cdot}05$); differences between regression coefficients were determined by t test ($P = \leq 0{\cdot}05$). Symbol designations: (a) peptone and (b) glucose control = ●, 1 % added on day 0 = ▲, 0·1 % added on day 0 = ■, 0·01 % added on day 3 = ⊛; (c) inorganic control = ●, nitrogen = ⊛, phosphorus = ■, nitrogen and phosphorus = ▲.

Pre-exposure experiments

Pre-exposure of freshwater sediments to DBP, DEHP, DIOP and DINP produced no detectable adaptation in the microbiota to enhance or otherwise alter their capacity to degrade phthalates (Table 5). Even pre-exposure of the more readily degradable phthalate DBP did not significantly affect its rate of biodegradation. Unexpectedly, we observed that sediment pre-exposed to the carrier solvent acetone (0·1%, v/v) significantly decreased the biodegradation of DEHP and DINP during the 28-day incubation period (Table 5) and decreased the degradation of DIOP at the 21- and 28-day sampling periods. The degradation decrease varied from 1- to 18-fold (Table 5).

TABLE 5
Effects of 28-Day Phthalate Pre-Exposure on Primary Biodegradation of DBP, DEHP, DIOP and DINP in Freshwater Sediments[a]

Compound and pre-exposure	*Time (days)*			
	7	*14*	*21*	*28*
DBP				
DBP	56·9 (3·1)	68·4 (2·3)	NR	NR
Acetone	58·3 (4·7)	69·0 (2·9)	NR	NR
Control	59·7 (6·9)	71·1 (2·1)	NR	NR
DEHP				
DEHP	2·5 (0·3)	5·6 (0·4)	8·2 (0·5)	10·2 (0·6)
Acetone	2·2 (0·5)	4·9 (0·8)	7·1 (1·0)	9·0 (1·0)
Control	11·7 (1·1)	17·4 (1·1)	21·1 (0·9)	23·9 (0·8)[b]
DIOP				
DIOP	0·5 (0·1)	1·2 (0·1)	1·8 (0·1)	2·3 (0·1)
Acetone	0·6 (0·2)	1·4 (0·2)	2·0 (0·3)	2·5 (0·4)
Control	0·5 (0·1)	1·2 (0·1)	2·1 (0·3)	3·3 (0·7)[b]
DINP				
DINP	0·5 (0·1)	0·9 (0·2)	1·5 (0·3)	1·9 (0·4)
Acetone	0·2 (0·1)	0·5 (0·1)	1·1 (0·1)	1·4 (0·1)
Control	3·6 (3·1)	6·2 (4·6)	7·5 (4·8)	8·1 (4·8)[d]

[a] Values are reported as total percent biodegradation of inoculum; mean ± SD in parentheses; all samples in triplicate; temperature 22 °C. Inocula of [^{14}C]-carbonyl phthalate (μg litre^{-1}) were as follows: DBP, 82; DEHP, 18·2; DIOP, 18·3; DINP, 19·7. Controls received neither phthalate nor acetone, NR = not reported.

[b] Regression analysis, significant difference $P = 0{\cdot}05$. Samples compared with pre-exposed and acetone controls.

Reduced degradation could be explained by 'isotope dilution' (Spain *et al.*, 1980) of the radio-labelled PAE inoculum by undegraded chemicals from the pre-exposure treatment. This hypothesis, in our judgment, seemed improbable, given the results of our multiple concentration experiments (Table 4). We determined that a 10-fold increase over the first three concentrations tested produced no significant change in the biodegradation rates of PAEs—actually these degradation rates increased at the highest concentration (Table 4). We speculated that pre-exposure of the sediments to an additional readily available carbon source (i.e. acetone) caused a burst of microbial activity that may have depleted one or more essential nutrients in the sediment. The slower biodegradation rates of the PAEs during subsequent exposure would then be a direct result of nutrient limitation.

Phthalates and their degradation products do not appear to be acutely toxic to sediment microbiota. When Mutz & Jones (1977) introduced DEHP and its degradation products, phthalic acid and 2-ethylhexanol, at the very high concentrations of 1 and 100 mg litre^{-1} into freshwater sediment, they found no perturbation of vital C, N or S activities, and no significant changes in microbial numbers or physiological activity after the treatment.

Certainly the low degradation rates of the complex phthalates DEHP, DIOP and DINP in sediments made the evaluation of pre-exposure experiment difficult; these results must be viewed with care. However, these studies do not support the conclusions of Pierce *et al.* (1980) that an 'ecosystem in recent contact with phthalates should degrade the substrate much faster than those where the ester has been introduced for the first time'.

CONCLUSIONS

The primary biodegradation of PAEs, although influenced by several environmental factors, was chiefly determined by the complexity of the diester alkyl group. Under various experimental conditions, the biodegradation of PAEs consistently had an inverse relation to length and branching of their alkyl groups. PAEs were ranked as follows according to their potential biodegradability: DBP > DEHP > DIOP > DINP. Other PAEs with alkyl groups of varying lengths and complexities would be expected to follow this pattern. These data stressed

the importance of molecular structure in predicting the environmental persistence of chemicals with a common moiety.

The detection of PAEs in aquatic environments is understandable, given their widespread use, the effect of temperature and chemical concentration on biodegradability, and the recalcitrant nature of PAEs with complex alkyl groups. As judged by our laboratory data, optimal biodegradation of long-chain PAEs occurred at high concentration in nutrient-rich aquatic systems with temperatures above 22°C. Such environmental conditions are typical of sewage-treatment ponds, wetlands, eutrophic lakes, and enriched streams during summer. Thus PAEs might be expected to accumulate in sediments during winter, especially at northern latitudes, because of low temperatures. Biodegradation of complex PAEs was slow at environmentally realistic (low) concentrations. Pre-exposure of sediments to a phthalate did not influence its biodegradability.

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