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Toxicity of Dibutyl Phthalate-Contaminated Sediment to Laboratory- and Field-Colonized Estuarine Benthic Communities

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Dibutyl phthalate (DBP), one of a large class of alkyl esters of 1,2-benzene dicarboxylic acid, is used widely in the United States and other countries as a plasticizer for epoxy and PVC resin. Significant amounts of DBP commonly occur in the aquatic environment, including the sediment (Giam et al. 1978). Its octanol-water partition coefficient of 5.2 (US EPA 1979) indicates that sorption of DBP by sediment could be substantial in waters polluted by this chemical. Concentrations as high as 89 ppb have been reported in sediment samples from Chesapeake Bay (Peterson and Freeman 1982) and up to 15.5 ppm in those from the Rhine River (Schwartz et al. 1979).

Few studies have been conducted on the toxicity of waterborne DBP to saltwater animals and communities, none on exposures via sediment. Concentrations of 1 mg/L or less did not significantly affect larval development of the mud crab, Rhithropanopeus harrisii, but 10 mg/L was acutely toxic to larval grass shrimp, Palaemonetes pugio (Laughlin et al. 1977, 1978). In estuarine macrobenthic animal studies, numbers of individuals and species in communities exposed to DBP at 4 mg/L for 2 weeks were significantly less than those in control communities; abundance of the amphipod, Corophium acherusicum, was affected by 0.34 mg/L (Tagatz et al. 1983). In addition, Wofford et al. (1981) found that DBP was bioaccumulated by saltwater organisms after 24 hr of exposure. They reported that it was accumulated by oysters (Crassostrea virginica) and shrimp (Penaeus aztecus) 41.6 and 30.6 times, respectively, greater than the 500 µg/L concentration in water. To obtain information on the effects of DBP on estuarine communities exposed via the sediment, we investigated the responses of macrobenthic animals that colonized sand contaminated with this chemical in the laboratory and field.

MATERIALS AND METHODS

Effects of DBP on macrobenthic animals that colonized sand-filled boxes for 8 weeks in the laboratory (June 25 to August 20, 1984) and in the field (July 10 to September 4, 1984) were determined by comparing community structures in boxes that contained uncontaminated and DBP-contaminated sand. Laboratory boxes

were colonized by settling of planktonic larvae entrained in continuously-supplied unfiltered seawater from Santa Rosa Sound, Florida, whereas field boxes located in 3 meters of water in Santa Rosa Sound were colonized by naturally occurring animals. Salinity and temperature of Santa Rosa Sound water were recorded continuously at the laboratory. Salinity averaged 27.0 °/oo (14.5 to 33.0 °/oo) during the laboratory study and 25.5 °/oo (14.5 to 32.5 °/oo) during the field study; temperature averaged 28.5°C (25.5 to 31.0°C) during both studies.

In the laboratory study, eight triangular boxes each were used for the control and for three exposure concentrations of DBP (nominal values were 10, 100 and 1000 µg/g). One of the eight boxes was used only to supply sand samples for chemical analyses for DBP and those remaining were used for the comparison of benthic communities. The triangular boxes (32 cm X 32 cm X 45 cm X 6 cm deep), constructed of acrylic plastic, were filled to 5.5 cm depth with clean sand (control) or sand mixed with varying amounts of DBP (99+ % purity) and grouped in the form of a square in a 9 cm deep aquarium (Figure 1). The sand was collected more than 6 months previously from Santa Rosa Sound; 98% of the particles were from 0.26 to 1.00 mm in diameter. DBP (0.042, 0.42 or 4.2 ml) in 80 ml acetone was mixed by spoon with 2,725 cc of sand for each experimental box; an equal amount of acetone was blended similarly into control boxes. Water level in aquaria was maintained at 3.5 cm above the sand. Unfiltered seawater was delivered continuously to an overhead splitter box from which each of four adjacent standpipes supplied water at 1.5 L/min through a larger glass tube to the center of each aquarium. Water flowed from the aquarium through notched openings on all sides. Photoperiod was 12 h light:12 h dark; light energy at test surface was 6.4 $\mu E/m^2/s$.

Fifteen days after the start of the laboratory study, scuba divers placed 24 sand-filled boxes (32 cm X 32 cm X 6 cm deep) in six groups of four in Santa Rosa Sound. The boxes were positioned in the substratum so that their surfaces were level with the surrounding sand. Each group consisted of a control and nominal concentrations of DBP of 10, 100 and 1000 µg/g of sand. One of the six groups was used only to supply sand samples for chemical analyses of DBP. DBP (0.094, 0.94 or 9.4 ml) in 180 ml acetone was mixed by hand with 6,150 cc of sand (from source described previously) for each box. The same amount of acetone was blended into control boxes.

Core samples were taken with a modified 50 cc syringe (opening enlarged to full diameter) at the beginning of the tests, then weekly or biweekly from one laboratory and one field box representing each concentration. These were analyzed to determine whether the concentration of DBP in sand changed during the 8-week studies. In addition, water samples were taken after one day, then weekly from laboratory aquaria containing contaminated or uncontaminated boxes and analyzed for DBP that leached from the sand. Laboratory and field data are presented as nominal

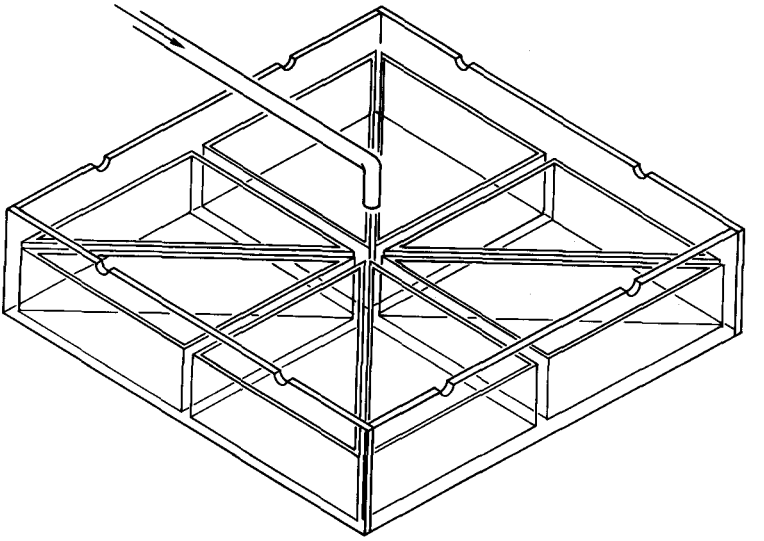

Figure 1. One of four aquaria containing eight triangular boxes used to test the effects of DBP in sediment on macrobenthic communities.

concentrations.

Sediment oxidation-reduction potential (Eh) was determined in laboratory and field boxes (when transferred to laboratory aquaria) at the end of the 8-week tests. For each study, measurements were taken at surface, 1, 2, 3 and 4 cm depths in two control sediments and two contaminated sediments representing each concentration, using a saturated calomel half-cell as a reference and a platinum indicating electrode calibrated with a standard quinhydrone solution.

After 8 weeks of colonization, laboratory and field communities (covered with acrylic plastic and transferred to the laboratory) were harvested. Animals retained by a 1-mm mesh sieve were preserved, counted, and identified.

The Kruskal-Wallis distribution free procedure was used to test for phthalate-exposure effects on numbers of individuals by phylum and on total numbers of individuals and species (all phyla combined). When an overall concentration effect was determined to be statistically significant ($\alpha = 0.05$), Miller's multiple comparison procedure based on average ranks, using an experimentwise error rate, 0.10, was applied to determine which concentration exposure effects differed from the control (Hollander and Wolfe 1973).

For determination of DBP concentrations in wet sand samples, 10 grams were extracted three times with 25 mL acetone by shaking. The acetone extracts were diluted to an appropriate volume with petroleum ether for gas-chromatographic analyses. For water samples, 1 liter was passed through a 6 cm by 0.5 cm i.d. glass column of Amberlite XAD-4 resin to trap DBP. The resin traps were eluted with 5 mL of acetone and the eluates were diluted to volume with petroleum ether for gas-chromatographic analyses. Average percentage recovery for sand fortified with DBP was 95; for water, 87. Concentrations of DBP in treatments were not adjusted for percentage recoveries. Detection limits were 0.01 µg/g for sand and 0.25 µg/L for water.

RESULTS AND DISCUSSION

Seven-day loss of DBP from sediments that contained initial nominal concentrations of 10, 100 and 1000 µg/g ranged from 43 to 69% in the laboratory and from 40 to 80% in the field. Early substantial loss agrees with the finding of Walker et al. (1984) that half the starting DBP concentrations in estuarine sediments disappeared within 3 days under laboratory conditions. In our studies, DBP remained in sediments even during the last 2 weeks of the tests. Of initial concentrations (10, 100 and 1000 µg/g), 19, 11 and 48%, respectively, persisted in laboratory sediments and 41, 4 and 19% persisted in field sediments. Analyses of laboratory water samples from all concentrations indicated that 30 to 53 µg DBP/L leached into water from contaminated sediments on the second day; none was detected after 7 or more days.

Eh profiles in laboratory and field boxes revealed that the highest DBP contamination resulted in anaerobic conditions and a more chemically reducing environment than did lower or no contamination (Figure 2). Eh decreased with increasing depth in control, 10 µg/g and 100 µg/g boxes, but almost all values were substantially above 150 mv. Reduction was most pronounced in boxes with 1000 µg DBP/g, and at 4-cm depth, Eh decreased to 74 mv in the field and to minus 36 mv in the laboratory.

A total of 706 animals representing 40 species of 7 phyla was collected from control boxes and boxes exposed to DBP in the laboratory (Table 1). Annelids (primarily *Mediomastus californiensis*), mollusks (primarily *Acteocina canaliculata*), arthropods (primarily *Corophium acherusicum*), and echinoderms (primarily *Leptosynapta* sp.) were the most abundant phyla.

In the laboratory, community structure was altered by the highest concentration of DBP in sediment (Table 1). The average number of species in the high exposure group was significantly less than the average number in the control group. Gray (1980) pointed out that the disappearance of rare species which often occurs in polluted areas is ecologically important because communities from unpolluted areas characteristically have many species represented by one or a few individuals. The echinoderm,

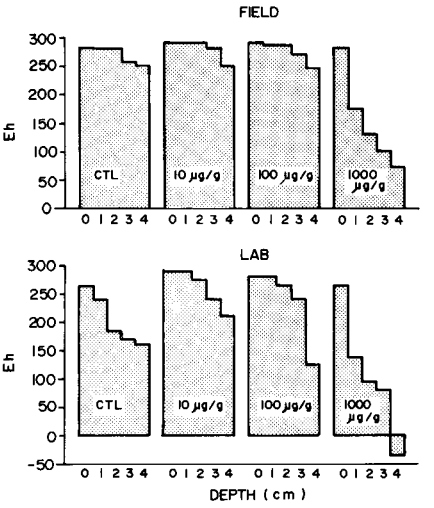

Figure 2. Field and laboratory Eh profiles (mv) in control boxes (sand) and in boxes containing DBP-contaminated sand. Each value is an average of two measurements at each depth.

Leptosynapta sp., was significantly affected and did not occur in boxes containing DBP at 1000 µg/g. Abundance of the capitellid worm, Capitellides teres, was non-significantly greater in 1000 µg/g boxes than in control boxes; however, other annelids collectively were significantly less abundant in this concentration than in the control. Difference in abundance trends between C. teres and other annelids may not have been due to difference in sensitivity to DBP. Dominance of another capitellid (Capitella capitata) in very polluted areas has been attributed to adaptable life-history characteristics, rather than to tolerance (Gray 1980).

A total of 784 animals representing 58 species of 9 phyla was collected from control boxes and from boxes that contained DBP in the field (Table 2). Annelids (primarily Hydroides dianthus), mollusks (primarily Crepidula maculosa), and chordates (primarily Branchiostoma caribaeum) were the most abundant phyla.

As in the laboratory, field community structure was significantly affected by the highest concentration of DBP in sediment (Table 2). However, only mollusks were significantly fewer in boxes containing 1000 µg DBP/g than in control boxes or boxes containing 10 µg/g.

Differences in the effects of DBP on phyla in laboratory and field benthic communities were probably partly due to species variation in sensitivity within phyla. Numbers of mollusks were affected

Table 1. Animals in Laboratory-colonized benthic communities collected from control boxes and from boxes with DBP-contaminated sand for 8 weeks. Replicates were pooled.

Taxon	Control	DBP (µg/g), nominal		
		10	100	1000
ANNELIDA				
Mediomastus californiensis	19	10	13	7
Capitellides teres	2	5	10	18
Myriochele oculata	14	8	10	2
Prionospio heterobranchia	11	6	5	5
Capitellides jonesi	4	2	3	4
Armandia maculata	2	2	3	3
Capitella capitata	8	0	0	1
Polydora socialis	2	3	4	0
Neanthes succinea	4	0	1	2
Nereis pelagica	3	1	0	2
Piromis sp.	1	3	0	1
Pomatoceros sp.	2	1	1	0
Hydroides dianthus	0	1	0	1
Owenia fusiformis	0	1	1	0
Ampharete americana	1	0	0	0
Amphictene sp.	1	0	0	0
Diopatra cuprea	0	1	0	0
Lumbrineris inflata	1	0	0	0
Ophiodromus obscurus	1	0	0	0
Prionospio steenstrupi	1	0	0	0
Scoloplos rubra	1	0	0	0
Tharyx marioni	0	1	0	0
Total annelids	78	45	51	46
MOLLUSCA				
Acteocina canaliculata	28	21	36	20
Laevicardium mortoni	7	12	8	10
Mulinia lateralis	5	7	6	2
Diastoma varium	2	0	4	2
Abra aequalis	1	3	0	2
Mitrella lunata	1	2	0	1
Musculus lateralis	0	0	0	3
Tagelus divisus	0	0	2	0
Atrina sp.	0	0	1	0
Lyonsia hyalina	0	0	1	0
Total mollusks	44	45	58	40
ARTHROPODA				
Corophium acherusicum	32	43	37	30
Panopeus herbstii	1	0	0	0
Total arthropods	33	43	37	30

Table 1. Continued.

Taxon	Control	DBP (µg/g), nominal 10	100	1000
ECHINODERMATA				
Leptosynapta sp.	28	28	35	0*
Unidentified Ophiuroidea	2	1	0	1
Total echinoderms	30	29	35	1*
CHORDATA				
Molgula manhattensis	10	6	6	21
COELENTERATA				
Unidentified Actiniaria	7	3	4	1
RHYNCHOCOELA				
Unidentified no. 1	1	1	0	0
Unidentified no. 2	1	0	0	0
Total nemerteans	2	1	0	0
ALL PHYLA				
Individuals per box	29.1	24.6	27.3	19.9
Standard error	2.4	3.4	3.4	2.6
Species per box	12.3	10.3	10.1	8.7*
Standard error	0.7	0.5	0.7	1.0

*Significantly different from control (α =0.10).

only in the field, but the dominant three species in the field differed from those in the laboratory. The sensitive echinoderm in laboratory communities, *Leptosynapta* sp., was not collected in field communities. Of the 68 species occurring in laboratory- or field-colonized communities, only 15 (22%) were common to both sites.

Concentrations of DBP that significantly affected abundance of macrobenthic organisms were two orders of magnitude lower for waterborne exposure (Tagatz et al. 1983) than for sediment-bound exposure. Numbers of chordates, mollusks, arthropods, and annelids in laboratory- or field-colonized benthic communities exposed to approximately 4 mg DBP/L water (measured) were significantly fewer than in control communities. In the present exposures via sediment (nominal), no significant effects occurred at 100 µg/g, and echinoderms and mollusks were the only phyla affected at 1000 µg/g. Although effective concentrations may be higher in sediment than in water, sedimentary sinks in nature often contain toxic substances at concentrations much higher than the concentrations in the overlying water (Schuytema et al. 1984).

Table 2. Animals in field-colonized benthic communities collected from control boxes and from boxes with DBP-contaminated sand for 8 weeks. Replicates were pooled.

Taxon	Control	DBP (µg/g), nominal		
		10	100	1000
ANNELIDA				
Hydroides dianthus	24	17	22	17
Poecilochaetus johnsoni	19	19	15	11
Axiothella mucosa	13	21	15	12
Prionospio heterobranchia	5	17	8	9
Pomatoceros sp.	9	3	2	4
Nereis pelagica	1	3	4	4
Scolelepis sp.	1	7	0	3
Dasybranchus lunulatus	3	0	1	5
Neanthes succinea	1	3	0	4
Laeonereis culveri	1	2	1	1
Capitella capitata	0	0	1	3
Pectinaria gouldii	0	1	1	1
Sabella micropthalma	0	2	1	0
Unidentified Nereidae	2	0	1	0
Leitoscoloplos fragilis	1	0	0	1
Armandia maculata	0	1	0	0
Capitellides teres	0	0	0	1
Chone sp.	0	0	1	0
Glycera americana	1	0	0	0
Glycinde solitaria	0	0	1	0
Linopherus sp.	0	1	0	0
Loimia viridis	0	1	0	0
Streblospio benedicti	0	1	0	0
Total annelids	81	99	74	76
MOLLUSCA				
Crepidula maculosa	32	23	16	17
Persicula sp.	12	19	9	5
Diastoma varium	16	7	5	5
Mitrella lunata	2	7	8	5
Nassarius vibex	5	2	8	2
Acteocina canaliculata	3	7	2	4
Marginella apicina	5	5	3	1
Pyramidella sp.	5	4	3	1
Anomalocardia auberiana	4	3	2	2
Polinices duplicatus	3	4	2	2
Laevicardium mortoni	3	2	3	1
Tellina sp.	2	0	0	2
Nassarius acutus	1	0	1	0
Olivella mutica	1	0	0	1
Triphora nigrocincta	0	2	0	0
Crepidula plana	0	0	1	0
Unidentified Nudibranchia	1	0	0	0
Total mollusks	95	85	63	48*

Table 2. Continued.

Taxon	Control	DBP (µg/g), nominal		
		10	100	1000
CHORDATA				
Branchiostoma caribaeum	24	21	29	31
Symphurus plagiusa	1	1	0	0
Total chordates	25	22	29	31
ARTHROPODA				
Bowmaniella sp.	4	3	2	0
Acanthohaustorius sp.	0	3	2	1
Pagurus sp.	2	1	2	0
Callinectes similis	1	2	1	0
Grandidierella bonnieroides	1	0	1	2
Killiapseudes sp.	1	2	1	0
Pinnixa sp.	0	2	1	0
Neopanope texana	1	1	0	0
Ampelisca sp.	0	1	0	0
Trachypenaeus similis	1	0	0	0
Total arthropods	11	15	10	3
PHORONIDA				
Phoronis architecta	5	4	1	1
COELENTERATA				
Unidentified Actiniaria	1	0	0	1
SIPUNCULA				
Golfingia sp.	0	1	0	0
Unidentified Sipuncula	1	0	0	0
Total sipunculids	1	1	0	0
ECHINODERMATA				
Unidentified Ophiuroidea	1	0	0	0
RHYNCHOCOELA				
Unidentified Rhynchocoela	0	0	0	1
ALL PHYLA				
Individuals per box	44.0	45.2	35.4	32.2
Standard error	4.6	5.8	2.7	2.1
Species per box	18.8	18.4	15.8	14.6
Standard error	1.1	1.1	1.2	1.3

* Significantly different from control ($\alpha = 0.10$).

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