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Simultaneous Accumulations of Naphthalene, a PCB Mixture, and Benzo(a)pyrene, by the Oyster, *Crassostrea virginica*

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Organic contaminants in the marine environment may not only affect productivity of marine organisms but may ultimately affect the health of humans. The accumulation and fate of marine contaminants hazardous to human health should, therefore, be studied in those marine species consumed by humans.

The objective of the present investigation was to assess accumulations of organic contaminants when the oyster Crassostrea virginica was simultaneously exposed to several contaminants. Polychlorinated biphenyls (PCBs), naphthalene, and benzo(a)pyrene were selected for study due to their toxicity and ubiquitous distribution in the marine environment. The objective included considering the possible significance of contaminant accumulations to humans by using a commercially important organism, and possible antagonistic-synergistic effects of multiple contaminants. The bioavailability of contaminants presented as dissolved versus particulate matter was also investigated.

MATERIALS AND METHODS

In order to introduce organic contaminants, (i.e. naphthalene, a PCB mixture, and benzo(a)pyrene to oysters via ingested particulate matter, detrital type matter was produced from Spartina alterniflora (smooth cord grass) leaves. Fresh, green leaves, cut into 2 to 5 cm lengths, were blended with 24 $^{0}/_{oo}$ Instant Ocean[1], until no intact leaves were visible. This slurry was passed through a 2.5 cm thick pad of dacron filter material resulting in the production of cell-like particles 5 to 30u in diameter.

Concentration of detrital material to be exposed to organic contaminants was adjusted to one million particles per ml. All

[1]The use of any trade name herein does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1. Concentrations of organic contaminants used to label detritus-like particulate matter and as dissolved organics for investigating rates of contaminant accumulations by C. virginica. Radioisotopically labelled compounds are expressed as weight by using manufacturer's stated specific activity.

Treatment	Particulate Matter			Dissolved Contaminant	
	ug added	Final Conc. ng/ml[a]	% Accumulation Efficiency[b]	ug added	Conc. ng/ml[c]
^{14}C naphthalene[d]	500	34.6	41	500	83
^{14}C PCBs[e]	60	10.1	100	60	10
^{3}H benzo(a)pyrene[f]	6	1.2	103	6	1[g]
^{14}C naphthalene[d]	500	20.5	25	500	83
PCBs, unlabelled	60	10.0[c]	--	60	10
^{3}H benzo(a)pyrene[f]	6	1.3	108	6	1[g]
naphthalene, unlabelled	500	83.3[c]	--	500	83
^{14}C PCBs[e]	60	12.0	101	60	10
^{3}H benzo(a)pyrene[f]	6	1.3	108	6	1[g]

[a] Based on radioactivity of cells

[b] (g after sorption x 100) ÷ g added

[c] Assumed that 100% of added contaminant was present

[d] [1(4,5,8) - ^{14}C] naphthalene, 5mCi/mmol (Amersham)

[e] [^{14}C(U)] PCBs, 54% chlorine, 31.3mCi/mmol (New England Nuclear)

[f] [G-^{3}H] benzo(a)pyrene, 40 Ci/mmol (Amersham)

[g] Concentrations are based on a mixture of stable and radioisotopic compounds (1:1)

particle counts were done with a Sedgwick-Rafter counting cell (Rand et al. 1975). A 600 ml suspension of particles was poured into each of five beakers and gently stirred with a magnetic spin bar. Radioactively labeled and unlabeled contaminants were allowed to adsorb to particulate material within respective beakers at initial concentrations shown in Table 1. Contaminants consisted of [1(4,5,8)^{14}C] naphthalene (5 mCi/mmol-Amersham), [^{14}C(U)] poly-chlorinated biphenyls, 54% chlorine, isomeric mix (31.3 mCi/mmol-Amersham) and G^{3}H benzo(a)pyrene (40 Ci/mmol-New England Nuclear), unlabeled PCBs (Arochlor 1254) and unlabeled naphthalene (Fisher Scientific). All studies were conducted at 25°C. After 15 hours, samples were taken to determine remaining radioactivity in the particulate and dissolved phases of each treatment. Since only an average of 1% of any given radioisotope remained in solution (Table 1), labeled particles were not rinsed prior to being fed to oysters.

Contaminated suspensions were added to 5.4 liters of 24 $^{o}/_{oo}$ seawater in each plastic tub. Each treatment contained six oysters that had not been fed for three days prior to initiation of this study. Gentle agitation was created by a magnetic spin-bar during the 15 hours that oysters were allowed to feed. Samples of gill, mantle, labial palps, and digestive diverticula were excised from each oyster and analyzed by liquid scintillation spectroscopy. Results reported are means of single analyses of each tissue excised from four to six oysters per treatment.

Fecal material collected from each treatment group was digested with Beckman BTS-450, and Beckman Ready-Solv cocktail was added before determining radioactivty. Radioactivity of all samples (water, suspension, tissues and feces) was determined with Packard Tri-Carb Model 3255 liquid scintillation spectrophotometer. Acceptability of the labeled suspension by the oysters was indicated by the presence of radioactively labeled green feces after 15 hours.

A comparison of the accumulation of contaminants by absorption from water with uptake by ingestion of the organic contaminants by oysters was undertaken. All experimental parameters were the same for the dissolved chemical treatments as for the particulate treatments (Table 1), with the exception that no food was provided to oysters during exposure. Additionally, prior to exposure, approximately 4 cm of the shell edges opposite the hinge of each oyster were broken off to permit continuous contact with the medium in which the oysters were immersed. Water and tissue samples were processed and analyzed by the same techniques as in the particulate study.

All accumulation data were subjected to T-tests for matched pairs with a microcomputer program (Tandy Corporation).

RESULTS AND DISCUSSION

Although comminuted S. alterniflora leaves readily sorbed organic contaminants dissolved in artificial seawater, rates of uptake for naphthalene were lower than for other contaminants. (Table 1) If detrital material was exposed to each organic contaminant individually, for 15 hours, either the PCB mixture or benzo(a)pyrene was accumulated at uptake efficiencies of 100% and 103%, respectively, relative to the amount of compound originally added while apparent efficiency for naphthalene accumulation was 41%. However, in the presence of all three contaminants, the apparent efficiency of naphthalene sorption was 25%. Accumulation efficiencies for PCBs and benzo(a)pyrene ranged between 101-108% in the simultaneous presence of all three organic contaminants. After 15 hours, 97 to 99% of each contaminant was sorbed in the detrital fraction relative to respective amounts dissolved in ambient aqueous media.

Accumulations of ^{14}C labeled naphthalene by oysters fed detritus-like particulate matter, manufactured from ground S. alterniflora leaves, or from contaminants dissolved in ambient water, were antagonistically affected by the simultaneous exposure to multiple contaminants and were tissue specific (Figure 1). Tissue concentrations of naphthalene from oysters exposed

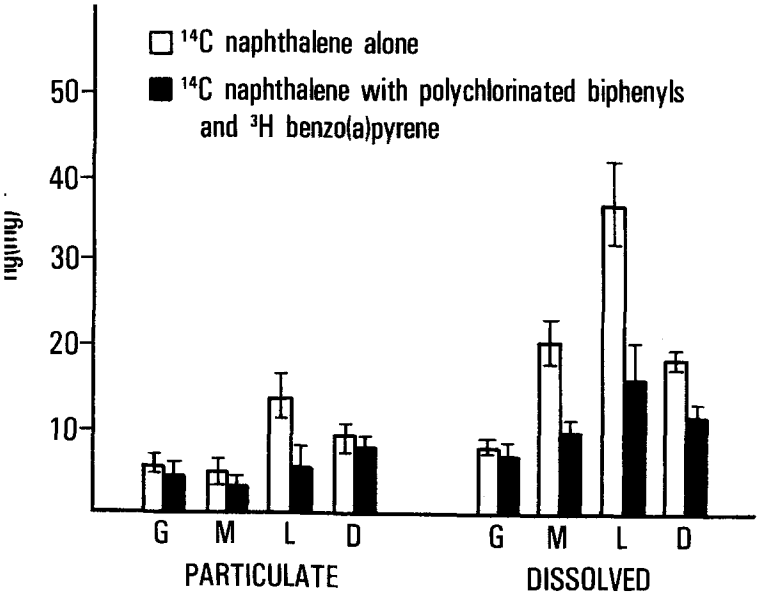

Figure 1. Concentrations of ^{14}C naphthalene accumulated by gills (G), mantle (M), labial palps (L), and digestive diverticula (D) excised from C. virginica exposed to particulate and dissolved organic contaminants. Values (ng/mg tissues/dry wt) are means and standard deviations based on 5 to 6 oysters.

to naphthalene sorbed to ground S. alterniflora leaves ranged from 5.4 ng/mg in mantle tissue to 14 ng/mg in labial palps. Concentrations of naphthalene in tissues from oysters exposed simultaneously to naphthalene, a PCB mixture and benzo(a)pyrene had lower average concentrations of naphthalene than tissues from single contaminant exposures. The largest decrease ($P \leq 0.05$) in naphthalene occurred in labial palps, decreasing from an average concentration of 14 to 5.8 ng/mg. Although concentrations of naphthalene in oysters exposed to dissolved organics were generally higher than concentrations from respective tissues from oysters fed contaminated detrital material, accumulation of dissolved naphthalene was also depressed by the presence of other organic contaminants (Figure 1). As in the case of contaminants from ingested detrital material, the greatest suppression of naphthalene accumulation occurred in labial palps ($P \leq 0.05$), decreasing from 36.6 to 16 ng/mg.

Unlike observed accumulations of naphthalene, accumulations of a ^{14}C labeled PCB mixture by selected oyster tissues were not always antagonistically affected by simultaneous exposures to three organic contaminants (Figure 2). Among oysters exposed to contaminants sorbed to detrital material, simultaneous exposure to three contaminants resulted in no effect ($P > 0.05$) on accumulations in mantle, labial palp, gill, or digestive diverticula. Accumulations of a PCB mixture by oyster tissue following exposure to all three dissolved organic contaminants, however, resulted in significantly lower ($P \leq 0.05$) PCB accumulations in all tissues except gills. In contrast to naphthalene accumulation, PCB accumulation following exposure to a dissolved PCB mixture was generally not different from accumulations obtained from ingested particulate matter. However, average PCB concentrations from both particulate and dissolved contaminant sources in oyster tissues were significantly lower ($P \leq 0.05$) than those measured for naphthalene (4.6 ng/mg versus 14.4).

Tissue accumulations of radiolabeled benzo(a)pyrene were not significantly affected ($P > 0.05$) by the simultaneous presence of other organic contaminants (Figure 3). Among oysters fed detrital material, gill tissue accumulated significantly ($P \leq 0.05$) more benzo(a)pyrene than mantle, labial palps, or digestive diverticular tissues. When oysters ingested particulate matter sorbed with the mixed organic contaminants, benzo(a)pyrene concentrations in all tissues monitored were not significantly different ($P > 0.05$) than when oysters were exposed only to benzo(a)pyrene. Similarly, when oysters were exposed to dissolved organics, simultaneous exposure to

naphthalene and PCB's did not result in significant changes ($P > 0.05$) in tissue concentrations of benzo(a)pyrene. Concentrations of benzo(a)pyrene accumulated by oyster tissues from particulate matter were significantly lower ($P \leq 0.05$) than average concentrations in tissues of oysters exposed to dissolved organics. Furthermore, the amount of benzo(a)pyrene accumulated by selected oyster tissues was significantly less ($P \leq 0.05$) than amounts of either naphthalene or PCB's accumulated by respective tissues.

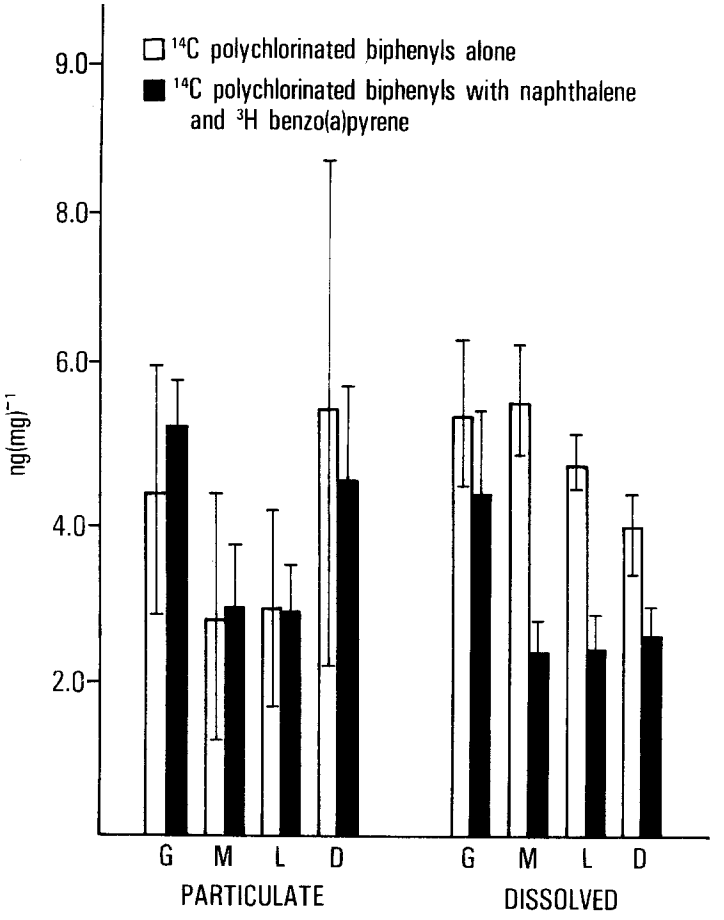

Figure 2. Concentrations of ^{14}C PBC's taken up by gills (G), mantle (M), labial palps (L), and digestive diverticula (D) excised from C. virginica exposed to particulate and dissolved organic contaminants. Values (ng/mg tissue dry wt) are means and standard deviations based on 4 to 6 oysters.

A comminuted S. alterniflora suspension was an adequate means for subjecting oysters to organic contaminants via ingestion

but allowed an apparent loss of some naphthalene. Considering that 1% or less of naphthalene present after 15 hours was in solution and only 25 and 41% of the added compound was detected in the particulate fractions, it appears that naphthalene was lost from these treatments, probably by evaporation. Hydrocarbon losses from solution during accumulation studies were previously reported e.g. Neff et al. 1976), Anderson et al. 1974b). By contrast, no such losses were observed in benzo(a)pyrene or PCB treatments in the present study.

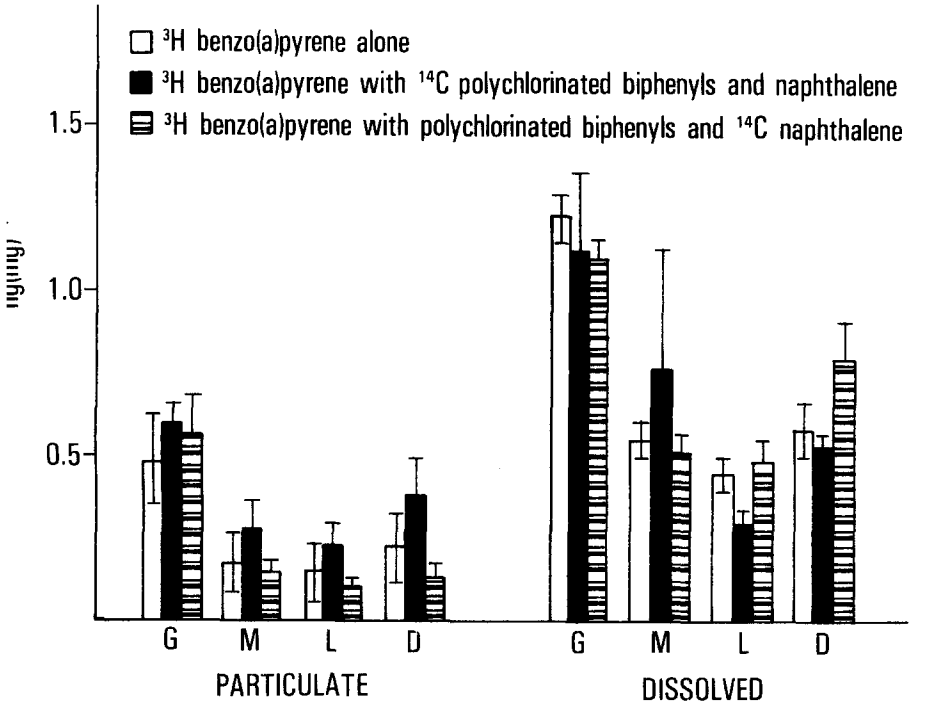

Figure 3. Concentration of ^{3}H benzo(a)pyrene taken up by gills (G), mantle (M), labial palps (L), and digestive diverticula (D) excised from C. virginica exposed to particulate and dissolved organic contaminants. Values (ng/mg tissue dry wt) are means and standard deviations based on 5 to 6 oysters.

Although the biological significance of organic contaminant ingestion versus direct sorption from ambient water has not been determined, direct sorption from water has usually been considered the most rapid and predominant mode of accumulation (Stegeman, Teal 1973, Stegeman 1974). Using the blue crab Callinectes sapidus, Lee et al. (1976) compared rates of benzo(a)pyrene accumulation from water and food and found that all tissues assayed accumulated significantly more contaminant from food. Results from the present study suggested that relative accumulation of dissolved versus particulate contaminants may be dependent upon contaminant species. While naphthalene

and benzo(a)pyrene were accumulated in significantly greater amounts in dissolved rather than particulate form, there were no significant differences ($P > 0.05$) in amounts of accumulation between particulate and dissolved forms for PCB mixture (Figures 1, 2 and 3).

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