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Biodepuration of Polynuclear Aromatic Hydrocarbons from a Bivalve Mollusc, *Mercenaria mercenaria L.*

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■ *Mercenaria mercenaria*, exposed in vitro for 48 h to nine parent polynuclear aromatic hydrocarbons (PAHs) found in waste crankcase oil (WCCO) and analyzed by multiparametric analysis over a 45-day depuration period in an activated carbon-filtration aquaria system, did not depurate PAHs, but rather maintained them at detectable levels. Uptake of PAHs was shown to be directly related to clam weight. A cluster analysis of empirical results reaffirmed a biostabilization in PAH groupings in clam tissue over a 45-day depuration period and exhibited no evidence of a decreasing trend in total PAHs when subjected to ANOVA. Due to the commercial importance of hard-shell clams, the practices of clam depuration and clam relaying are reviewed in light of potential long-term public health exposures to low-level xenobiotics and the implications for human consumers.

Introduction

Oil pollution of our coastlines and estuaries continues to be a chronic global environmental problem. A considerable quantity of the petroleum hydrocarbons, estimated at 0.86 million metric tons annually (*1*) entering the marine environment from the land via storm and sanitary sewers and urban and river runoff, can be attributed to waste crankcase oil (WCCO) (*2, 3*). Polynuclear aromatic hydrocarbons (PAHs) are found in WCCO (*4, 5*). Impacts from waste petroleum hydrocarbons may be considerable when one takes account of the fact that the estuaries and coastal seas receiving these wastes, despite comprising only 10% of the total global marine environment, support approximately 99% of the total world fish population and shellfish production (*6*). WCCO contains heavy metals and volatile organics, as well as a variety of PAHs formed by the high-temperature combustion in automobile engines (*7*). PAHs are a class of compounds that are toxic, carcinogenic, and mutagenic. Table I shows the distribution of 10 common PAHs found in WCCO as well as other media. The presence of PAHs in the natural environment is a global phenomenon (*8*) with absolute concentrations being highest for samples taken in the vicinity of human activity and lowest for samples taken from more primitive environments. Mueller et al. (*9*) noted that urban runoff, which contains a considerable quantity of WCCO, is responsible for a substantial portion of the petroleum-related hydrocarbons entering the marine environment of New York.

The availability of petroleum-related pollution from PAHs to commercially important marine organisms is of major concern to many coastal ecologists (*10*). Chronic petroleum pollution of commercial shellfish species has been suggested to be so widespread that carcinogen contamination of seafoods may occur not only in obviously polluted waters, but also in waters deemed suitable for commercial seafood production (*11*).

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The shellfish industry in New York has been adversely affected in recent years by a host of anthropogenic pollutants as well as the overharvesting of available shellfish stocks. Because of the possibility of contamination by a variety of pollutant sources, the clamming industry has developed several practices to aid in the rejuvenation of shellfish harvesting. One practice, called "relaying", involves the removal of clams from "contaminated" natural waters and placement of these organisms in "certified" natural waters to allow depuration, or self-cleansing of bacteria, and then commercial harvesting of the transplanted clams for human consumption. Bacteriological standards for the human consumption of shellfish are assumed to have been met by clams depurated in certified waters (waters that meet coliform test standards and water temperatures of 50 °F or better) for 21 days. However, PAHs, such as those in WCCO, react similarly to other lipophilic compounds and accumulate in biological tissue. Moreover, PAHs may not be subject to the normal metabolic processes exhibited by filter-feeding bivalves. Thus, the potential persistence of compounds such as PAHs beyond a 21-day bacteriological depuration period is highly probable (*12*).

It is also possible that shellfish and other organisms taken from areas with a history of oil contamination have levels of compounds that, in turn, "bioactivate" other complex compounds found in WCCO into mutagens and carcinogens (*13*). Neither the mechanism nor the quantitation of partitioning of PAHs between aqueous media and various types of lipid tissue has been fully explored, and the long-term public health risks of ingestion of seafood containing subtoxic levels of chronically contributed xenobiotic contaminants such as PAHs is lacking (*14*). Since seafood is a major source of global food, the impact of bioavailable, bioconcentratable, petroleum-related compounds such as PAHs to human health was a major concern of this investigation. In an effort to investigate the ability of naturally occurring bivalves to cleanse themselves after exposure to a variety of PAHs (all found in WCCO), a laboratory experiment utilizing the bivalve mollusc *Mercenaria mercenaria* was designed.

Experimental Section

Nine parent PAHs frequently exhibited in WCCO, yet only a small part of the host of PAHs found in any petroleum product (*4, 32*) was of interest in this study: naphthalene, biphenyl, fluorene, phenanthrene, fluoranthene, pyrene, chrysene, benzo[*a*]pyrene, and benz[*a*]anthracene. In order to establish the aqueous stability of the nine PAHs, a UV–fluorescence spectroscopy synchronous excitation technique (*15, 16*) was used to prescreen total PAHs added to a control synthetic seawater aquarium tank (no clams) over 45 days.

Laboratory exposure of bivalve molluscs (*Mercenaria mercenaria L.*) to the nine reference PAHs was carried out in three additional 20-gall (75.7-L) glass aquarium tanks each with 20 clams. A 1.147 ppm PAH standard acetone solution (Supelco Co., Inc.) in seawater (Instant Ocean Aquarium Systems, Inc., Eastlake, OH) was added to each

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Table I. Mean Percentage Distribution of Waste Crankcase Oil PAH Characterizations[a]

PAH	Peake and Parker (4) WCCO	Hoffman et al. (32) urban runoff	atmos fallout	STP[c]	sediment	Tanacredi[b] WCCO
naphthalene	ND	1.3	0.7	32.0	0.5	45.2
biphenyl	ND	0.8	0.5	1.7	0.4	4.4
fluorene	6.0	2.7	2.9	2.4	1.2	4.4
phenanthrene	32.1	10.0	15.7	4.7	7.1	13.8
fluoranthene	17.9	25.2	33.5	3.0	17.9	4.5
pyrene	27.5	12.6	10.3	2.9	18.3	ND
benz[a]anthracene	3.6	11.6	8.7	0.2	7.4	4.7
chrysene	10.2	15.8	3.4	0.7	13.2	21.4
benzo[a]pyrene	1.4	4.8	3.0	0.2	17.3	1.5
anthracene	1.3	NT	NT	NT	NT	NT

[a] ND, none detected. NT, not tested. [b] WCCO from oil change in 1978 Ford with 107 200 miles and a previous oil change after 4023 miles. 10W/40 SAE oil analyzed by HPLC for this investigation. [c] STP, sewage treatment plant.

Table II. Summary of HPLC Findings. PAHs Extracted and Distributed in *M. mercenaria* from Exposure Tanks

	Tank 2 (Postexposure Sample Taken)						
	2 h	10 h	2 day	14 day	30 day	45 day	total
Naph	101.0	22.0	55.0	29.0	21.0	0.0	228.0
Bi	51.3	34.8	79.2	54.1	64.0	73.3	356.7
Fl	61.2	20.7	32.7	47.3	48.1	65.2	275.2
Phen	1040.0	94.7	169.0	390.0	633.0	392.0	2718.7
Flor	1320.0	241.0	199.0	376.0	663.0	335.0	3134.0
Pyr	470.0	131.0	61.9	205.0	201.0	203.0	1271.9
BaA	25.9	18.8	25.5	27.3	33.5	13.4	144.4
Chr	166.0	78.3	26.5	61.3	48.4	100.0	480.5
BaP	260.0	72.2	46.6	97.8	99.4	67.8	643.8
PAH totals	3495.4	713.5	695.4	1287.8	1811.4	1249.7	9253.2

	Tank 3 (Postexposure Sample Taken)						
	2 h	2 day	7 day	14 day	30 day	45 day	total
Naph	0.0	ND	57.0	117.0	52.0	48.0	274.0
Bi	84.1	87.2	114.0	72.0	45.5	68.5	471.3
Fl	39.3	68.1	62.2	42.4	34.3	45.2	291.5
Phen	161.0	548.0	766.0	316.0	221.0	415.0	2427.0
Flor	265.0	521.0	757.0	239.0	511.0	730.0	3023.0
Pyr	201.0	300.0	ND[b]	128.0	126.0	243.0	998.0
BaA	20.9	25.5	4.7	16.8	19.8	19.0	106.7
Chr	31.6	255.0	13.9	81.7	222.0	15.7	619.9
BaP	52.3	147.0	52.8	127.0	43.5	211.0	633.6
PAH totals	855.2	1951.8	1827.6	1139.9	1275.1	1795.4	8845.0

	Tank 4 (Postexposure Sample Taken)						
	2 h	10 h	2 day	7 day	14 day	30 day	total
Naph	114.0	95.3	ND	438.0	0.0	119.0	766.3
Bi	63.0	48.7	76.9	285.0	65.6	48.0	587.2
Fl	58.0	39.8	32.3	89.8	75.1	51.0	346.0
Phen	368.0	892.0	193.0	442.0	612.0	300.0	2807.0
Flor	195.0	1010.0	364.0	423.0	779.0	1450.0	4221.0
Pyr	113.0	223.0	104.0	28.2	359.0	97.2	924.4
BaA	33.0	43.0	20.5	15.8	33.3	9.7	155.3
Chr	877.0	163.0	93.8	9.9	204.0	90.5	1438.2
BaP	131.0	368.0	313.0	48.6	250.0	67.5	1178.1
PAH totals	1952.0	2882.8	1197.5	1780.3	2378.0	2232.9	12423.5

[a] All values in nanograms per gram of wet tissue weight. [b] ND, not detected.

tank. Filter systems were operated initially without any carbon for 48 h in order to afford maximum uptake and exposure of PAHs to clams. "Mother-stock" clams were maintained in separate tanks from which the experimental exposure clams were randomly selected. Because the stage in the life cycle of organisms at the time of analysis may influence hydrocarbon distribution, all clams were 7–8 years old, or in the commercial "cherrystone" age group, though this age differentiation can be variable from estuary to estuary. The mean wet weight (MWW) for exposure clams was 32.9 ± 5.14 g. All tanks were maintained in the dark and kept at constant temperature (21.5 ± 1 °C), pH (8.2), and salinity (*S*‰) (27 ppt). All parameters were monitored every other day.

Two clams, selected randomly from each tank, were established as "background organism" samples, shucked, and extracted. The remaining clams were left in the exposure tanks for the 48-h PAH exposure time. Carbon filters were added to the filter pumps only after the 48-h exposure period. Two clams were sequentially, yet randomly, removed for HPLC analysis (Table II). Each two-clam sample from each of the three exposure tanks

represented the pooled clam sample for that particular sample "depuration" time. Water samples of exposure tanks were periodically analyzed for quality assurance/quality control (QA/QC) purposes by GC–MS after carbon filtration and during the clam depuration period.

All exposure aquaria had Instant Ocean seawater previously recirculated continuously for 60 days through activated charcoal, 250 L/h pump rate, Eheim filtration pump systems (Ehiem Filter Co., Inc.) prior to clam exposure experiments. All water samples were extracted with methylene chloride in separatory funnels. All organism samples were Soxhlet extracted for 8 h in research grade hexane. (Fisher Scientific, Inc.)

The sample extracts were rotary evaporated to 35 mL. In order to reduce a variety of interferences, sample extracts were applied to a chromatographic column (1% water-deactivated silica/alumina gel) and the benzene–methanol elutriate was collected for analysis. All extracts were concentrated to 1–2 mL and subjected to either HPLC (clams) or GC-FID/GC–MS (water, activated-carbon extracts) analysis. Clam specimens were rinsed several times in distilled water, blotted dry, and weighed for total wet tissue weight (TWTW). Original clam tissue was stored in a freezer at −10 °C. Extraction for analysis started within 48–52 h of removal from exposure tanks. Three times the TWTW of anhydrous sodium sulfate was added to each two-clam sample. The sample was blended and ground for 2 min at high speed. Tissue homogenates were placed in hexane-prewashed Soxhlet thimbles and Soxhlet extracted for 8 h at approximately 20 cycles/h.

Water and periodic control clam samples were analyzed by gas chromatography on a Perkin-Elmer Sigma 3 and a Hewlett-Packard 5880A gas chromatograph, both equipped with capillary glass columns and flame ionization detectors (FID). The columns were 0.25 mm × 30 m DB-1 glass capillaries attached to a spitless injector. Temperature programming was initiated at 80 °C (held for 2 min), and programmed temperature increases of 4 °C/min were maintained to a final temperature of 310 °C and held for 12 min isothermal. Injector port temperature was 260 °C and detector temperature was 310 °C. All peak resolution and identification were accomplished by comparison of retention times for eluted compounds to retention times of eluted internal standard PAHs.

The GC–MS analysis conducted on water and on invertebrate food extracts was done by using a computerized Perkin-Elmer GC–MS equipped with a 6 ft × 0.1 in i.d. glass capillary column packed with 1% SP-1000 on Carbopack B (60/80 mesh). The GC–MS was capable of scanning from 20 to 260 amu every 7 s, utilizing 70-V electron energy in the electron impact ionization mode.

HPLC analyzes of exposure clam extracts were conducted on a Hewlett-Packard 1084B HPLC (U.S. EPA ID 167831), with a 25 cm × 4.6 mm i.d. Vydac 201TP column. A calibration range for the HPLC–UV detector system was prepared and analyzed by using Supelco PAH mixture 610M. The solvent and oven temperatures were 30 °C. The gradient used was 50% acetonitrile and 50% water for the first 2 min, then increased to 100% acetonitrile by 15 min, and held there for 5 min. The UV–fluorescence detector was set at 254 nm, the instrument flow rate was 1.50 mL/min, and a 25-μL injection size was used on each sample run. When chromatograms posed matrix interferences, direct quantification of the analyses was made impossible by either area estimation or by peak height measurements. Under these circumstances the sample was diluted by one-fourth and analyzed by a method of standard additions. The linearity of the range was evaluated for each compound by comparing the center standard to the upper and lower standards and calculating the percent relative error. The relative percent error for each parameter was consistently less than 15% for all compounds and was considered acceptable.

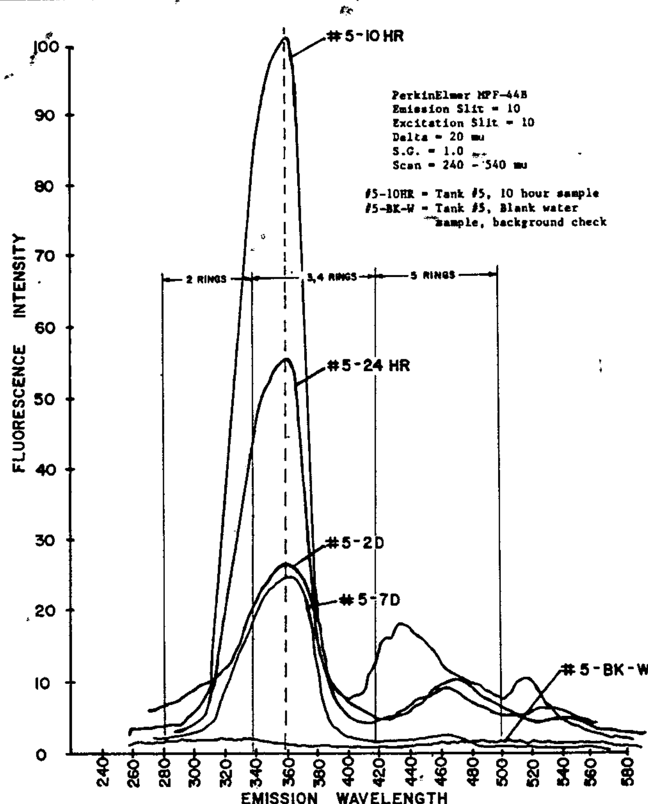


Figure 1. Synchronous fluorescence excitation emission spectra for control tank 5 water samples.

A synchronous excitation (SE) fluorescence spectroscopic method was used, which was previously described by Frank (*17*) and Frank and Gruenfeld (*18*). All UV–fluorescence analyses were conducted on a Perkin-Elmer MPF-3 UV–fluorescence spectrophotometer with two independent monochromators, a 150-W xenon arc light source, and 10-mm path length. Synchronous excitation of the individual water sample was accomplished by exciting it at 20-nm wavelength intervals between 240 and 480 nm. The excitation wavelength was maintained 20 nm less than the emission wavelength during the synchronous scan.

Results

The control tank (T5) contained PAH-contaminated water, no clams, and no carbon filtration system. Results of UV–fluorescence analysis (Figure 1) were corroborated by GC/GC–MS quantification of the nine PAH standard concentration of 1.147 ppm added initially to the control tank T5 water. This was done to establish total PAH concentration stability over a 45-day clam depuration period as a control reference for bioavailability to clams in exposure tanks (T2, T3, T4). The total PAH concentrations retained in nanograms per gram of wet tissue weight and detected by HPLC in organisms over the 45-day depuration period are shown in Figure 2. For each interval there were three replicates, each composed of two clams, providing for six clams per depuration time sample. Measured concentrations from each two-clam sample were then averaged for further analysis, yielding 18 sample data points for each PAH (Table II and Figure 2).

The means and standard deviations (probable error) for the averaged PAH values (N = 18) exhibited a diverse

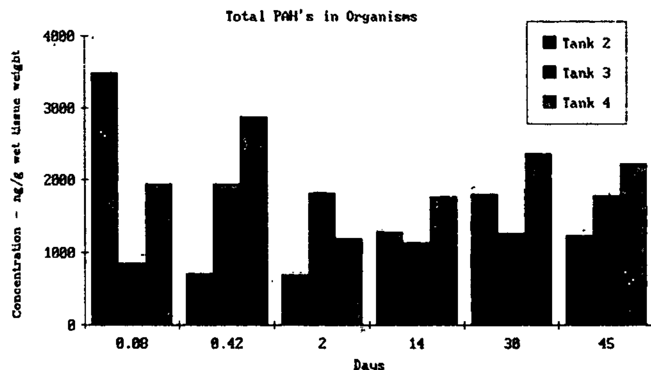


Figure 2. Days of depuration in activated-carbon-filtered aquaria.

pattern of standard deviation values, prompting some concern regarding the stability of statistical results for individual PAHs. Furthermore, it was strongly suspected that the results from some PAHs were significantly correlated, possibly due to the similarity in chemical structure. Specifically, the mean values of each compound in Figure 3 exhibited a general clustering of compounds across depuration times samples, and Figure 4 shows this clustering is consistently observable within each exposure tank. Nevertheless, it was also clear that both inter- and intratank variability exists in measured concentrations. Though tanks 2–4 were exposure tank replicates, intratank clam extracts exhibited a variance between amounts of each PAH individually retained (Figure 4). Further work on the specific mechanism driving the depuration kinetics of PAHs in clam tissue is clearly required.

To address the above concerns, a hierarchical cluster analysis was performed on the known PAHs. Basically, this type of analysis classifies PAHs with similar patterns and levels of nanogram per gram concentrations across the eight sequential time samples (2 h to 45 days). Technically, the objective measure of similarity used is the cosine of the angle between two octuple vectors representing the nanogram per gram levels per time sample for two compounds. PAH pairs with smaller angles (i.e., cosines near 1.0) are considered to be more similar. Note that this is an empirical, rather than analytical, grouping process and may not replicate for other types of organisms. The cluster analysis method is then used on a 9 × 9 similarity matrix to construct groups with high internal similarities of PAHs and low external similarities to PAHs in other groups.

When this similarity matrix, given in Table III, was analyzed with the unweighted pair group average (UPGMA) method (*19, 20*), three groups emerged between a rescaled distance 10 and 15 of the dendrograms in Table III (Phen + Flor + Pyr + BaP + BaA), (Naph + Bi + Fl), and (Chr). This grouping is somewhat parallel to a logical grouping based on molecular weight, solubility, and ring structure. In particular, group I compounds (Naph + Bi + Fl) have the highest solubilities, lowest molecular weights, and two-ring structures; group II compounds (Phen + Flor + Pyr) have intermediate values for solubility and molecular weight, and three- or four-ring structures; finally, group III compounds (BaA + Chr + BaP) have the lowest solubilities, the highest weights and four- or five-ring structures. Given this parallel to the empirical results, and the small sample size, the logical grouping probably makes more sense. Therefore, results were pooled within these groups for the following analyses.

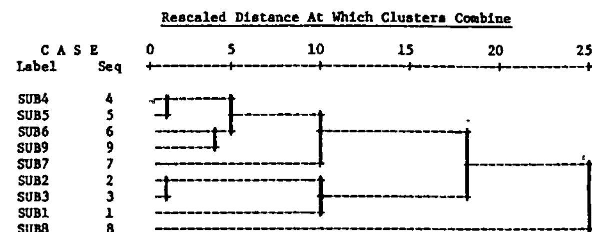
In Figure 5, a graphic representation is given of depuration rates in the three PAH logical clusters. The locally weighted regression technique is used to smooth the actual data points to enhance interpretability of the trend. An

Table III. Hierarchical Cluster Analysis Summary of All Results

A. Cosine Similarity Coefficient Matrix

Variable	SUB1	SUB2	SUB3	SUB4	SUB5	SUB6	SUB7	SUB8
SUB2	.8496							
SUB3	.6775	.8974						
SUB4	.5467	.7250	.8976					
SUB5	.5258	.6471	.8398	.9044				
SUB6	.3371	.5811	.8305	.8709	.82			
SUB7	.5004	.5905	.7748	.7172	.88	.7091		
SUB8	.4102	.4558	.6274	.5430	.45	.5355	.5513	
SUB9	.3947	.5873	.7634	.8490	.80	.8515	.6990	.5466

B. Dendrogram



C. Cluster Membership of Cases Using Average Linkage (UPGMA)

Label	Case	Number of Clusters						
		8	7	6	5	4	3	2
SUB1	1	1	1	1	1	1	1	1
SUB2	2	2	2	2	2	1	1	1
SUB3	3	3	2	2	2	1	1	1
SUB4	4	4	3	3	3	2	2	1
SUB5	5	4	3	3	3	2	2	1
SUB6	6	5	4	4	3	2	2	1
SUB7	7	6	5	5	4	3	2	1
SUB8	8	7	6	6	5	4	3	2
SUB9	9	8	7	4	3	2	2	1

examination of the smoothed data trends reveals no evidence of a decrease in total PAHs from clam tissue. Group II shows the highest overall readings although there is a slight dip in the trend at intervals 2 and 4. Group III shows some downward trend, but levels off at interval 4. Group I readings are uniformly low throughout the time sample sequence. It is important to note that the total sample is dominated by Group II (Phen + Flo + Pyr) since this group accounts for the majority of the detected PAHs (Figure 3).

A statistical analysis was also performed to test the null hypothesis that the nanogram per gram concentration maintained a uniform value across time intervals (i.e., H°: $\mu_1 = \mu_2 = ... = \mu_8$). The null hypothesis was rejected only for biphenyl at $\alpha = .05$. This result was probably due to an unusually high reading for the 7-day sampling, which was 100% higher than the next largest reading. Thus, the ANOVA results show no evidence of a downward trend across the eight time intervals, which corresponds to the graphical results in Figure 5. However, average PAH concentrations for every PAH did test as significantly different from 0. These findings strongly suggest that PAHs (1) do accumulate in clam tissues and (2) do not depurate significantly within a period of 45 days. Thus, the nine PAHs at the detectable bioavailability values were sequestered and appear to have been stabilized in clam tissue over the entire 45-day depuration period. Detectable levels of all nine reference PAHs were recovered in clam tissues after the full 45-day period. Clams in all exposure tanks were observed with extended siphons so that normal pumping or filtering activity by the clam species continued after exposure.

Discussion

The majority of previous investigations have shown that marine organisms are able to release petroleum hydrocarbons from their tissues rapidly when they were returned to petroleum-free environments, (*21, 22*). Boehm and

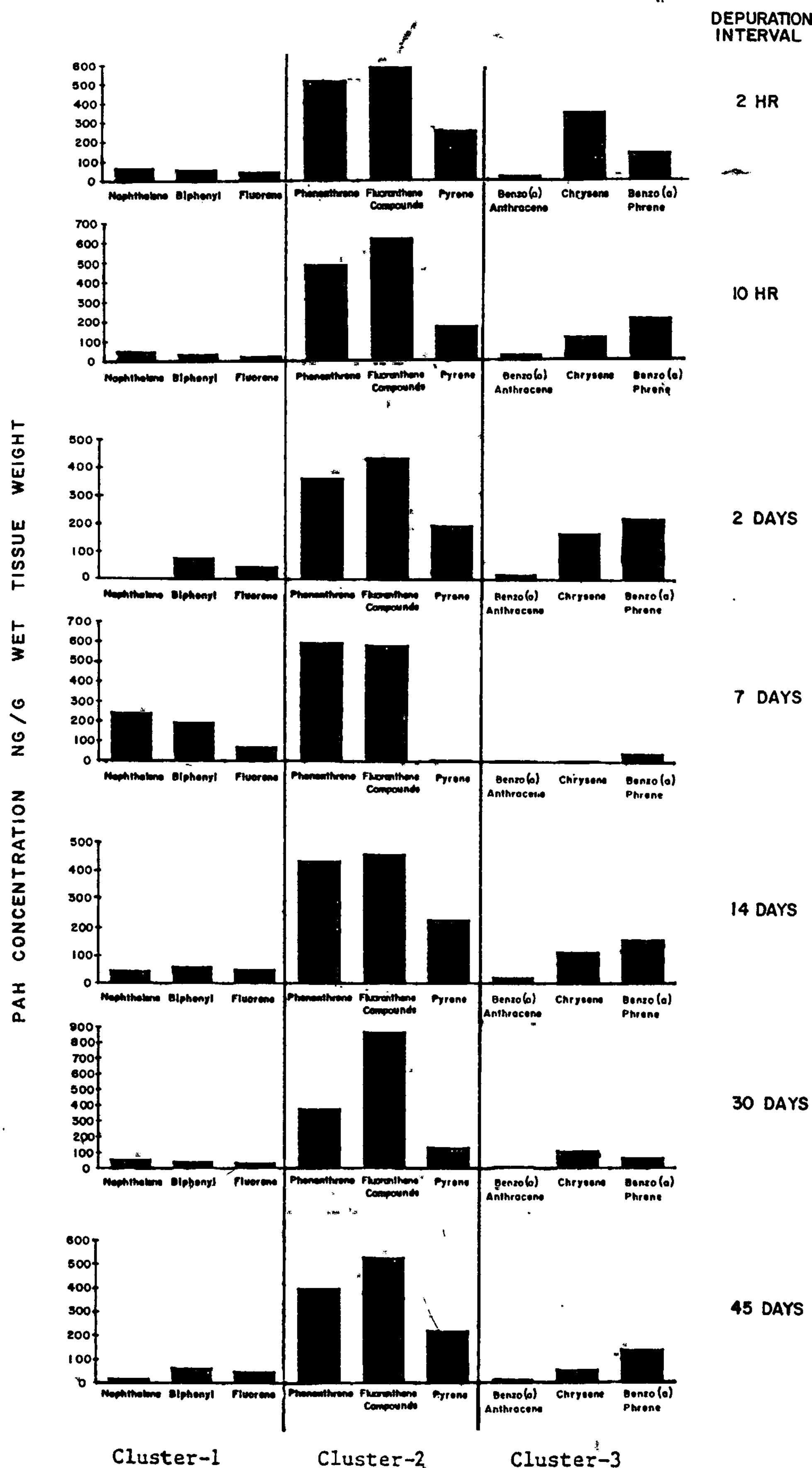


Figure 3. Mean PAH values per compound per time. Clusters I-III are, respectively, groups I-III.

Quinn (23), however, have reported that *M. mercenaria L.* chronically exposed to petroleum hydrocarbons accumulated them to high levels in their tissues and failed to release them when returned to clean seawater over a 120-day depuration period. They suggested that hydrocarbons released from molluscs exposed to xenobiotics for long periods of time would be extremely slow due to (1) an accumulation of hydrocarbons in a more stable tissue compartment (e.g., lipids) and (2) the organisms being in a positive nutritional balance. In this investigation, clams

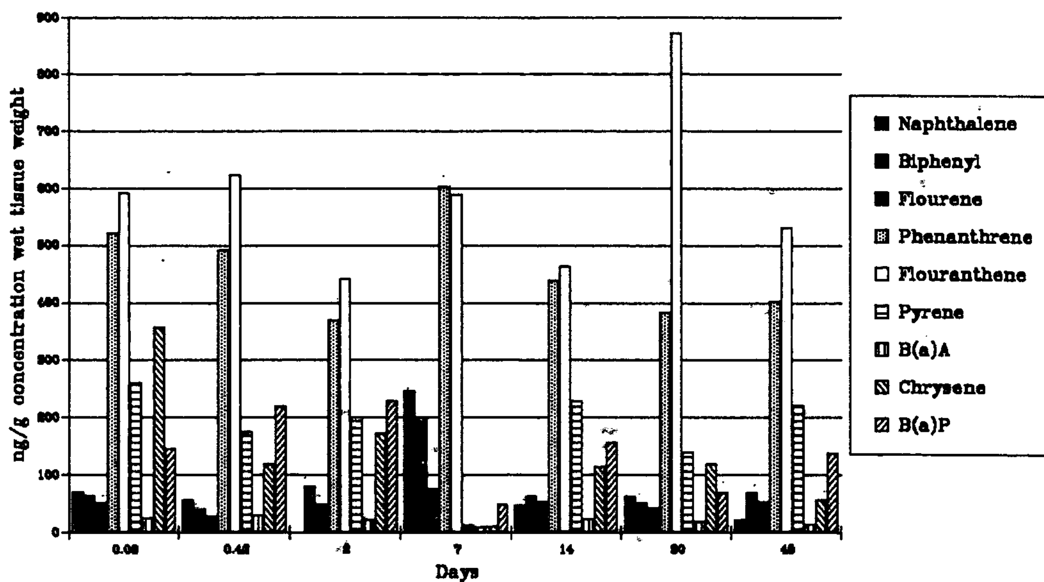


Figure 4. Mean values of organism exposure in tanks 2–4.

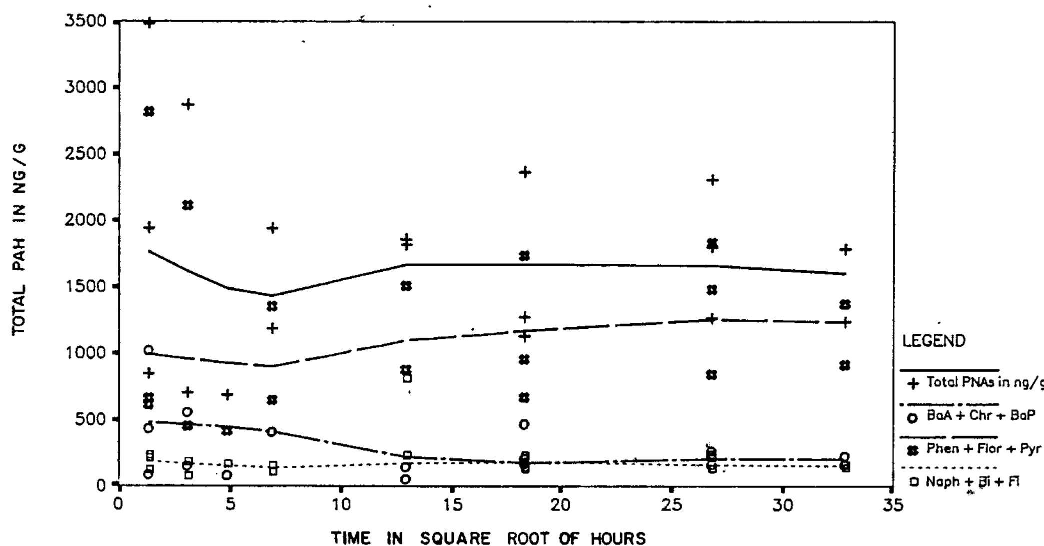


Figure 5. PAHs in clam tissue over time by use of locally weighted regression for smoothing.

exposed to the nine PAHs, mostly found in WCCO and associated with urban-influenced estuarine ecosystems, were not supplementally fed over the depuration period and yet the accumulated PAHs were still not depurated. The nine PAHs were immediately sequestered (i.e., within 48 h) and remained at relatively constant concentrations. Elimination of PAHs over the 45-day depuration period was not achieved. The results lend evidence to PAH maintenance being initiated in hard-shell clams over relatively short term periods. Unlike other marine taxa, this "sequestering" in molluscs may support the apparent inability of molluscs to metabolize PAHs to more water soluble and thus easily secreted polar metabolites. This increased "stabilization" and accumulation of parent PAHs is in agreement with Brown and Pancirov (*24*) and may, as they have suggested, show a long-term selective uptake of these specific aromatics. With PAH adsorptive ability and low water solubility, investigations have suggested that estuarine colloids contribute considerably to materials available for ingestion by filter-feeding bivalves (*25*).

These findings are also in agreement with those of Stein, Hom, and Varanasi (*26*), who showed that one PAH, BaP, reached steady-state concentrations in tissue by the first day of exposure in a fish (*Parophrys retulus*, English sole), known to be able to metabolize BaP. Food-web transfer is a major concern in the commercial fishing industry, one that may be warranted in light of the results of this investigation.

The generalization noted by Fowler (*27*) that there is an initial rapid discharge of PAHs, followed by a much slower loss of those PAHs associated with a more stable compartment within the organism, appears to be borne out to some degree in this work, but not according to statistical analysis of data in Figure 5, for example. Clams in this research had been maintained in carbon-filtered clean waters for over 1 year prior to initial exposure to the nine PAHs for 48 h, so the clams cannot be considered to be "chronically exposed" to PAHs. In addition, chromatographic profile background control checks of nonexposed background clams did not reveal any of the nine parent PAHs. There was no evidence that clams contaminated with PAHs for 48 h were able to rapidly eliminate these compounds in 1–5 days as observed by Lee et al. (*22*).

There is little knowledge of the effects on humans from ingestion of petroleum hydrocarbons, especially PAHs at subtoxic levels. Though previous research has emphasized the effects of single PAHs, it is possible that parent PAH byproducts and substituted PAHs may play a more important role in the environment, with some organisms acting as reserves for these compounds (*28*). Dunn and

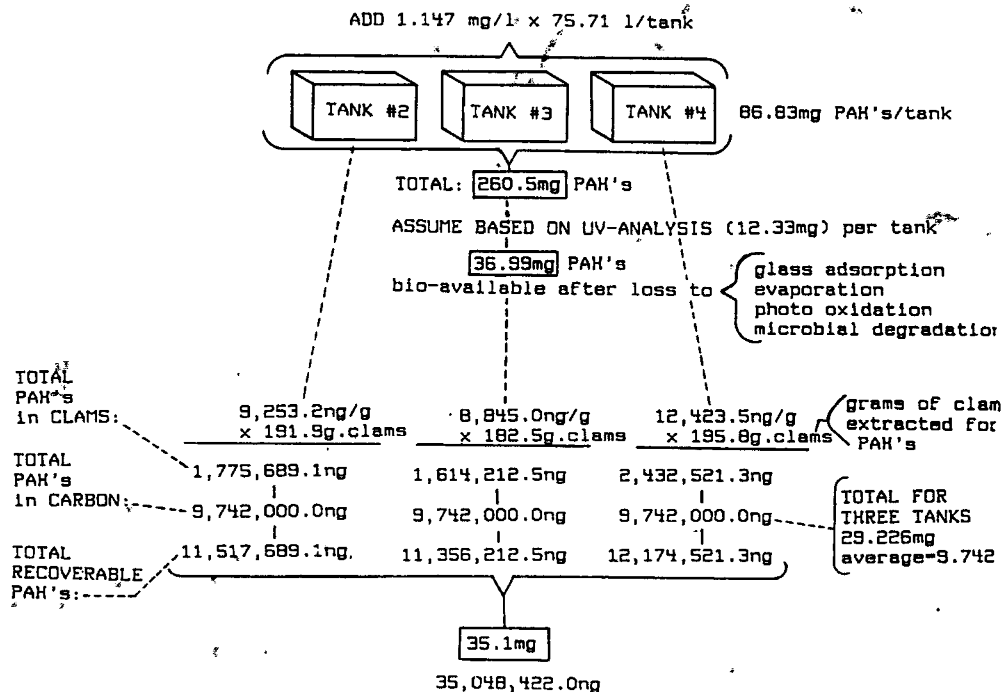


Figure 6. PAH bioavailability to clams in aquaria.

Fee (*11*) noted that commercial seafood contained low-level PAH contamination, and Pruel et al. (*29*) noted that there was a consistent level of PAH content in commercially sold *M. mercenaria* ranging from 1.5 to 17.0 ng/g based upon 100-g wet tissue weight for analysis. *Mercenaria* may very well be considered an "estuarine quality" indicator based upon its PAH levels. Values of PAHs after exposure, acute or chronic, are important if we are to attempt to establish a public health exposure threshold for PAHs in bivalves.

Depuration has been described by several investigators as being different for different hydrocarbons and different organisms. Neff and Anderson (*28*) demonstrated depuration of hydrocarbons from exposure to no. 2 fuel oil for 8 h although small quantities of substituted naphthalenes remained in oyster tissues 672 h (28 days) after exposure. Naphthalene and phenanthrene percent distribution values in WCCO were higher than any of the other seven PAHs (Table I). In the literature, however, they consistantly provide low (naphthalene) and high (phenanthrene) values in clam tissue (*29, 30*). Lee et al. (*22*) noted that *Mytilus edulis* took up 20 μg of [^{14}C]naphthalene and [^{3}H]-3,4-benzo[*a*]pyrene from a seawater solution but after 24 h in fresh seawater had discharged 80% of these compounds. This investigation exhibited that 94% of the subsequently recoverable PAH initial concentrations were retained in the closed aquarium system; most previous work utilized a seawater flow-through system or a considerably less sophisticated exposure system (Figure 6). The PAHs in this investigation were either sequestered by siphoning organisms or adsorbed to glass or activated carbon filters (Figure 7).

Similar to work of Dunn and Stitch (*31*), BaP in this investigation was sequestered by *M. mercenaria* at a low concentration and biostabilized by not depurating over the 45-day depuration period. Phenanthrene and fluoranthene consistently exhibited higher values in clam extracts throughout the sampling period, and though this result has been expressed in sediment values of previous investigations (*32, 33*), its significance for bivalves is unclear. As noted by Hoffman et al. (*32*), urban street runoff PAHs (WCCO) are similar in composition to atmospheric fallout PAHs, but different from municipal effluent PAHs. Urban

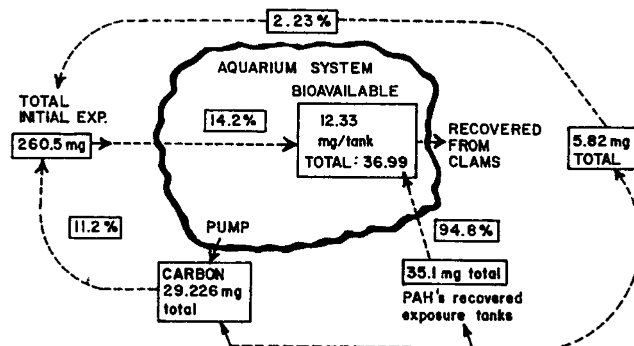


Figure 7. Summary of percentage distribution of PAHs in aquaria.

street runoff is a major source of high molecular weight PAHs to the estuary with a contribution of over 70% of the high molecular weight PAHs.

Jackim and Wilson (*34*) exposed *Mya arenaria* to ^{14}C-labeled no. 2 fuel oil, and testing concentrations of six PAHs after $5^1/_2$ weeks concluded that depuration rates were appreciably slower after chronic exposure than after an acute exposure. These observations are not in agreement with the present work since once a stable residual equilibrates, depuration of the range of PAHs, even after an acute 48-h exposure time, did not occur. Previous depuration experiments involved removal of clams from either "polluted" waters and transferring the clams to "clean" waters in continuous flow-through filtered seawater systems for depuration of contaminants. The use of carbon filtration systems in this experiment eliminated the need to move organisms at any time during their depuration periods, thus avoiding any metabolic stress to the clam or their pumping rates. In addition, a "closed-system aquarium" provides an ideal observation environment for in situ exposure and mass balance tracking.

An ancillary finding from this study was that heavier clams tended to collect higher concentrations of PAHs. The initial accumulation by bivalves of PAHs influenced by clam weight with possible correlations of PAH associations based upon lipid content is in general agreement with Fong (*35*) and supports the uses of bivalves as sentinel organisms for monitoring xenobiotics in coastal waters (*36*).

Pearson correlation coefficients were computed between combined weight of the two-clam sample and the PAH concentration; eight out of nine were positive. Fluorene and benz[a]anthracene had the highest correlations, 0.57 and 0.47, respectively; both were significant at the 0.05 level with a two-tailed test.

Phenanthrene, fluoranthene, and pyrene were detected in clam tissues at higher concentrations per sample time in relation to the other six PAHs. In most cases, these three PAHs exhibited the highest values for the sample under study at any particular depuration time. The most recoverable PAH from clam tissue extracts was fluoranthene, and BaA was the quantitatively least recovered PAH. Neff and Anderson (28) found phenanthrene was accumulated most rapidly by *Rangia cuneata* and released most slowly. For this compound, this investigation appears to be in agreement. Most previous investigations have concentrated on the water-soluable fraction (WSF) of petroleum products, specifically naphthalene and phenanthrene. Farrington et al. (37) found that phenanthrene, fluoranthene, and pyrene in bivalves were indicative of waste petroleum input. A propensity to bioaccumulate a particular PAH may reflect PAH bioavailability, previous exposure extent and duration, and PAH variable volatility and solubility.

Conclusions

The most important implication of the present study is that consumers of bivalve molluscs chronically exposed to persistent levels of PAHs at or above ambient PAH concentrations in urban estuaries may be at higher than normal health risk. Programs in highly urbanized estuarine ecosystems advocating the relaying or transferring of bivalves from "marginal waters" for ultimate commercial harvesting to "approved waters", while well intentioned, may be unduly exposing the shellfish-consuming public to higher than normal levels of PAHs. Even though individual clams may have "low or acceptable" PAH levels (levels difficult to establish and for which there are no present U.S. FDA or WHO threshold standards), cumulative effects of consumption could provide the necessary threshold concentrations of these carcinogenic/mutagenic PAHs in human lipid tissue potentially resulting in long-term health concerns. The usual 21-day depuration or relay period for bacteriological quality improvement in bivalve molluscs appears to be inappropriate and insufficient for chemical depuration. Due to the relative stability of PAHs in clam tissue under ideal depuration conditions, in situ depuration environments may never afford acceptable exposure concentrations of PAHs for consumers of shellfish from urban systems.

The following are recommended:

(1) Clam relay programs in urbanized estuarine systems should be reevaluated in light of potentially increased long-term public health risk from xenobiotics.

(2) Research should be undertaken to investigate the complexity of a myriad of compounds bioaccumulated by bivalve molluscs and their level of biomaintenance in lipid tissue compartments.

(3) Future investigations should concentrate on trophic transfer of low-level, chronic concentrations of a complex group of petroleum-derived compounds attributable to WCCO PAHs because WCCO has been established as the major contributer of PAHs in urban estuarine ecosystems.

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Registry No. Naphthalene, 91-20-3; biphenyl, 92-52-4; fluorene, 86-73-7; phenanthrene, 85-01-8; fluoranthene, 206-44-0; pyrene, 129-00-0; benz[a]anthracene, 56-55-3; chrysene, 218-01-9; benzo[a]pyrene, 50-32-8.

Literature Cited

(1) *World Resources: A Guide to the Global Environment: 1990–1991*; World Resources Institute: Oxford University Press: New York, 1990; p 186.
(2) *Oil in the Sea; Inputs, Fates and Effects*; National Academy of Science, National Research Council, National Academy Press: Washington, DC, 1985.
(3) Kneip, T. J.; Custshall, N. H.; Field, R.; Hart, F. C.; Lioy, P. J.; Mancini, J.; Mueller, J. A.; Sobotowski, C.; Szelligowski, J. In *Ecological Stress and the New York Bight*; Mayer, G., Ed.; Estuarine Research Federation: Columbia, SC, 1982; pp 145–161.
(4) Peake, E.; Parker, K. In *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*, Fourth International Symposium; Battelle Press: Columbus, OH, 1980; p 1025.
(5) Tanacredi, J. T. *J.—Water Pollut. Control Fed.* **1977**, *49*, 216.
(6) Oil and Hazardous Spills in U. S. Waters: 1971–1982. Council on Environmental Quality Report. Table A048 *Water Quality*; 1983; p 311.
(7) Pruell, R. J.; Quinn, J. G. *Environ. Pollut.* **1988**, *49*, 89–97.
(8) LaFlamme, R. E.; Hites, R. *Geochim. Cosmochim. Acta* **1977**, *42*, 289–303.
(9) Mueller, J. A.; Garrish, T. A.; Casey, M. C. Contaminant Inputs to the Hudson-Raritan Estuary. NOAA Technical Memorandum OMPA-I21, 1982.
(10) Smith, C. M.; Hackney, C. T. *Estuaries* **1989**, *12*, 42–48.
(11) Dunn, B. P.; Fee, J. *J. Fish. Res. Board Can.* **1979**, *36*, 1469–1476.
(12) Boehm, P. D.; Quinn, J. G. *Mar. Biol.* **1977**, *44*, 227–233.
(13) Dobrowski, C. J., Jr.; Epifano, C. E. *Can. J. Fish. Aquat. Sci.* **1980**, *37*, 2318–2322.
(14) Segar, D. A.; Davis, P. G. Contamination of Populated Estuaries and Adjacent Coastal Ocean—A Global Review. NOAA Technical Memo. NOS, OMA11, 1984.
(15) Frank, U. *Toxicol. Environ. Chem. Rev.* **1978**, 73.
(16) Frank, U.; Stainken, D.; Gruenfeld, M. In Proceedings of USCG/USEPA/API National Conference on Control of Hazardous Material Spills, Los Angeles, CA; 1979; p 323.
(17) Frank, U. In *Proceedings, Joint Conference on Prevention and Control of Oil Pollution*; American Petroleum Institute: San Francisco, CA, 1975; pp 87–91.
(18) Frank, U.; Gruenfeld, M. Determination of Petroleum Oils in Sediments by Fluorescence Spectroscopy Spectroscopy and NMR. Abstracts of the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy; U.S. EPA/API; 1971; Paper 400.
(19) Anderberg, M. R. *Cluster Analysis for Application*; Academic Press: New York, 1973.
(20) Everitt, B. S. *Cluster Analysis*, 2nd ed.; Heineman Books: London, 1980.
(21) Stegeman, J. J.; Teal, J. M. *Mar. Biol.* **1973**, *22*, 37–44.
(22) Lee, R. F.; Gardner, W. S.; Anderson, J. W.; Blaylock, J. W.; Barwell-Clarke, J. *Environ. Sci. Technol.* **1978**, *12*, 832–838.
(23) Boehm, P. D.; Quinn, J. B. *Estuarine Coastal Mar. Sci.* **1976**, 93–105.

(24) Brown, R. A.; Pancirov, R. J. *Environ. Sci. Technol.* **1979**, *13*, 878–879.
(25) Wijayaratne, R. D.; Means, J. C. *Mar. Environ. Res.* **1984**, *11*, 77–89.
(26) Stein, J. E.; Hom, T.; Varanasi, V. *Mar. Environ. Res.* **1984**, *13*, 97–119.
(27) Fowler, S. W. In *Pollutant Transfer and Transport in the Sea*; Kullenberg, G., Ed.; CRC Press: Boca Raton, FL, 1982; Vol. 2, pp 1–66.
(28) Neff, J. M.; Anderson, J. W. *Response of Marine Animals to Petroleum and Specific Petroleum Hydrocarbons*; John Wiley and Sons, Inc.: New York, 1981; pp 177.
(29) Pruell, R. J.; Hoffman, E. J.; Quinn, J. G. *Mar. Environ. Res.* **1984**, *11*, 163–181.
(30) Teal, J. M.; Howarth, R. W. *J. Environ. Manage.* **1984**, *8*, 27–44.
(31) Dunn, B. P.; Stitch, H. F. *Bull. Environ. Contam. Toxicol.* **1976**, *15*, 398–401.
(32) Hoffman, E. J.; Mills, G. L.; Latimer, J. S.; Quinn, J. G. *Environ. Sci. Technol.* **1984**, *18*, 580–587.
(33) Bates, T. S.; Murphy, P. P.; Curl, Jr.; H. C., Feely, R. A. *Environ. Sci. Technol.* **1987**, *21*, 193–198.
(34) Jackim, E.; Wilson, L. In *Proceedings, 10th Annual National Shellfish Sanitation Meeting*; 1977; p 27.
(35) Fong, W. C. *J. Fish. Res. Board Canada* **1976**, *33*, 2774–2780.
(36) Burns, K. A.; Smith, J. L. *Estuarine Coastal, Shelf Sci.* **1981**, *13*, 433–443.
(37) Farrington, J. W.; Goldberg, E. D.; Risebrough, R. W.; Martin, J. H.; Bowen, V. T. *Environ. Sci. Technol.* **1983**, *17*, 490–496.

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