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Bioaccumulation of Polynuclear Aromatic Hydrocarbons by the Clam, *Rangia cuneata,* in the Vicinity of a Creosote Spill

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Polynuclear aromatic hydrocarbons (PAHs) are a major class of environmentally signficant organic chemicals. Their presence in the environment has been attributed primarily to fossil fuel usage. Sources for these chemicals include petroleum, creosote and coal-tar products, atmospheric deposition of combustion products, natural oil seepages, stormwater runoffs, and petroleum spills (Politzer et al. 1985; Stegeman 1981). Many compounds which comprise this class are known or suspected carcinogens. Others are known to produce serious cytotoxic and mutagenic effects in many different tissues from a variety of species (Sims and Grover 1974; Sexton 1980). In the aquatic habitat, many organisms, such as fish, shellfish, and crustaceans, readily accumulate PAHs from the environment, and store them at relatively high levels in their tissues (Stegeman and Teal 1973; Fossato and Canzonier 1976; Varanasi et al. 1978). Numerous studies with various species have shown that the metabolites are frequently more toxic to the organism than the parent PAHs (Sims and Grover 1974). Consequently, it is of interest to determine the bioavailability of PAHs, and to monitor the uptake and disposition of PAHs by biota in waters known or suspected to be contaminated with PAHs residues.

During 1980-81, as part of a NOAA/U.S. Coast Guard initiative, we participated in an environmental study of a creosote spill into Bayou Bonfouca at the American Creosote Works Plant (ACWP) site at Slidell, Louisiana (DeLeon 1981; Michel et al. 1982). The objectives for the study were: (1) to determine the nature and extent of creosote contamination at the site and in the bayou, and (2) to evaluate through biomonitoring the bioavailability and human health implications of creosote derived PAHs in the bayou and the estuarine system into which Bayou Bonfouca flows.

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So dramatic were our findings that our data were used in part by state and federal agencies to bring about in 1982, the inclusion of the Bayou Bonfouca site on the National Priorities List of hazardous waste sites that pose a threat to public health and the environment. What follows is a report of our findings on the biomonitoring segment of our study.

MATERIALS AND METHODS

Clams (Rangia cuneata) with an average shell length of 4.9 cm were collected from the southeastern shore of Lake Pontchartrain, Louisiana, and were tested for background PAHs content. They were subsequently transplanted to the study area in groups of 60 clams each in 15x15x17 cm aluminum cages which were placed on the bottom surface of the bayou system at three monitoring station sites (see Figure 1). Two stations, MS8 and MS9, were located in Bayou Bonfouca at 1.8 miles and 3.0 miles, respectively, downstream from the ACWP site. The third station, MS10, which served as a control site, was located in Bayou Liberty at 0.5 miles upstream from the point of confluence with Bayou Bonfouca. Bayou Liberty is a pristine bayou which merges with Bayou Bonfouca at approximately 7.0 miles downstream from the ACWP site. All test organisms were inspected at one-week intervals and sampled for chemical analysis at two-week intervals during the term of the study.

A representative sample consisting of 3-4 clams from each sample set was analyzed for PAHs. The clams were shucked and 12 grams of tissue were homogenized. The homogenate was digested at 100°C for 16 hours in sealed glass ampules each containing 5 mL of 3N lithium hydroxide solution. The digested homogenate was extracted three times with 30 mL of diethyl ether. The extracts were combined, dried over granular anhydrous sodium sulfate, and concentrated on a rotary evaporator. The concentrated extracts were fractionated on 30 x 1 cm glass columns packed with silica gel activated overnight at 150°C. The saturated hydrocarbons were eluted with 35 mL of n-hexane. Aromatic hydrocarbons were eluted with 60 mL of 20% dichloromethane in hexane. Each fraction was reduced in volume and analyzed by capillary gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

GC analyses were performed on a Hewlett-Packard (HP) 5710A gas chromatograph equipped with a flame ionization detector (FID) and a 0.3 mm x 30 m glass capillary column coated with SE-52 methylphenyl silicone. The injection port temperature was kept at 250°C; the detector temperature, at 300°C.

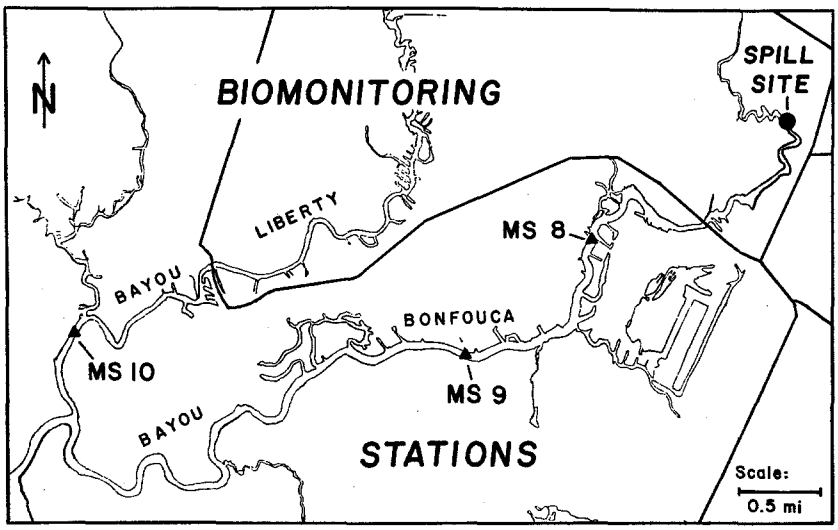

Figure 1. Map of the study area at Bayou Bonfouca, LA.

The separations were achieved by programming the column from 50°C to 260°C at 4°/min. All injections were splitless with a 35 sec delay in venting.

GC/MS analyses were performed on an HP5985A GC/MS system. The separations were performed on a 0.3 mm x 50 m glass capillary column coated with SE-52 methyl-phenyl silicone. The GC effluent was directed into the ion source through a glass-lined stainless steel transfer line maintained at 250°C. The injection port temperature was kept at 250°C. The column temperature was programmed from 50° to 260°C at 4°/min. All injections were splitless and used a 30 sec septum purge delay. The mass spectrometer was operated under standard conditions and the mass range from 40 to 400 amu was scanned at a rate of 267 amu/sec. The ionization voltage was 70 eV. The ion source was kept at 200°C. Full range mass spectra were measured and stored on a magnetic disc. Identifications were made by comparison of mass spectra, selected ion plots, and GC/MS retention times against those of authentic standards. Quantifications were made by the use of ion current profiles for selected masses in the molecular ion cluster of the compound of interest.

RESULTS AND DISCUSSION

The clams which served as biomonitors in Bayou Bonfouca accumulated substantial concentrations of PAHs in their tissues as shown in Table 1. A gradual rise in the levels of several PAHs components was observed after

Table 1. Biomonitoring levels of PAHs in transplanted clams from Bayou Bonfouca (Stations MS8 and MS9) and Bayou Liberty (Station MS10)

	AMOUNT DETECTED, nanogram/gram (ppb) wet weight		
	PRE-EXPOSURE	STATION MS8	
COMPONENT	WK 0	WK 2	WK 4
Naphthalene	43	60	120
C_1-isomers	12	4	19
C_2-isomers	10	7	15
C_3-isomers	14	14	30
Biphenyl/Acenaphthylene	17	13	42
C_1-isomers	13	5	29
C_2-isomers	7	8	13
C_3-isomers	13	42	70
Fluorene	7	5	11
C_1-isomers	6	5	27
C_2-isomers	14	18	48
C_3-isomers	16	32	63
Phenanthrene	34	10	28
Anthracene	12	18	39
C_1-isomers	27	15	36
C_2-isomers	35	30	74
C_3-isoemrs	24	57	100
Fluoranthrene	120	88	130
Pyrene	87	75	120
C_1-isomers	39	150	250
C_2-isomers	18	96	180
C_3-isomers	8	45	100
Benz(a)anthrancene	41	81	190
Chrysene	42	109	200
C_1-isomers	36	54	260
C_2-isomers	20	18	180
C_3-isomers	7	7	110
Benzopyrenes	87	132	600
C_1-isomers	14	36	110
C_2-isomers	-	-	40
C_3-isomers	-	-	3

two weeks. The most pronounced overall increase occurred after four weeks for biomonitor organisms at station MS8, the station closest to the spill site. The biomonitors at station MS9, which is further downstream, exhibited a slight increase in PAHs levels at two weeks, with a decrease at four weeks. The biomonitors at

Table 1 (continued)

COMPONENT	AMOUNT DETECTED, nanogram/gram (ppb) wet weight STATION MS9 WK 2	STATION MS9 WK 4	STATION MS10 WK 2	STATION MS10 WK 4
Naphthalene	89	39	5	57
C_1-isomers	18	10	5	14
C_2-isomers	14	8	3	10
C_3-isomers	24	11	2	18
Biphenyl/Acenaphthylene	50	24	8	26
C_1-isomers	26	17	7	17
C_2-isomers	19	5	-	7
C_3-isomers	28	25	2	12
Fluorene	11	7	2	6
C_1-isomers	8	9	-	6
C_2-isomers	8	19	-	12
C_3-isomers	14	22	2	11
Phenanthrene	36	21	8	16
Anthracene	26	27	3	9
C_1-isomers	18	17	2	10
C_2-isomers	32	35	2	17
C_3-isoemrs	45	36	1	13
Fluoranthrene	83	68	6	33
Pyrene	83	60	7	32
C_1-isomers	110	83	4	14
C_2-isomers	65	48	1	10
C_3-isomers	31	24	-	5
Benz(a)anthrancene	89	51	2	8
Chrysene	80	57	2	8
C_1-isomers	97	65	-	16
C_2-isomers	60	41	-	7
C_3-isomers	29	26	-	1
Benzopyrenes	190	120	2	22
C_1-isomers	28	20	-	-
C_2-isomers	1	1	-	-
C_3-isomers	-	-	-	-

station MS 10, the control station in Bayou Liberty, showed evidence of depuration after two weeks, and apparent equilibration at four weeks, reflecting the pristine conditions present at Bayou Liberty. It is interesting to note that the total creosote levels measured in the water column by UV-fluorescence spectrophotometry at weeks 2 and 4 of the study were:

MS8, 13 and 26 ppb; MS9, 14 and 22 ppb; and MS10, 9 and 10 ppb; respectively.

The most interesting and most significant observation was the accumulation of benzopyrenes by the biomonitors during the term of the study. A comparison of benzopyrenes levels at all stations is shown in Figure 2. The highest level measured was 600 ppb at station MS8 after four weeks, having gone from a background level of 87 ppb in the clams to 132 ppb after two weeks. By comparison, the benzopyrenes levels at station MS9 initially rose to 190 ppb after two weeks; then leveled off to 120 ppb after four weeks. The levels at station MS10, however, initially dropped to 2 ppb after two weeks; then rose to 22 ppb after four weeks. These differences are most probably due to concentration variations of the contaminants in the water column due to natural tidal fluctuations occurring during the study period. However, the accumulation of benzopyrene and the other PAHs are consistent with those derived from bioaccumulation studies using Rangia exposed to various PAHs in the laboratory.

The accumulation of the creosote-derived PAHs by the clam conclusively demonstrates the bioavailability of these compounds even when present in the water at only trace levels. Typical depuration rates for PAHs in bivalves range from a couple of weeks to several months depending on the organism and the contaminant (Politzer et al. 1985; Neff et al. 1976). Once accumulated by the biota these compounds are made available to the other components of the food chain through trophic transfer. The transfer of contaminants from a food source, the marsh clam, R. cuneata, to the blue crab, Callinectes sapidus, has been clearly demonstrated experimentally (Petrocelli et al. 1973).

These findings are also significant in that this bayou flows into Lake Ponchartrain, a shallow, oligohaline, 1631 km^2 estuary located in the deltaic plain of the Mississippi River. This estuary serves as an important nursing ground and fishery for commercially valuable crabs, shrimp and fish species. R. cuneata are abundant in the lake where they are the dominant benthic organism. These clams are a key life form in this estuarine community because they are responsible for the conversion of detritus and phytoplankton into tissue that feeds fish, shelfish, and crustaceans (Hopkins et al. 1973). Undoubtedly many of the contaminants accumulated by the R. cuneata will subsequently be transferred to these commmercially valuable species and ultimately to man.

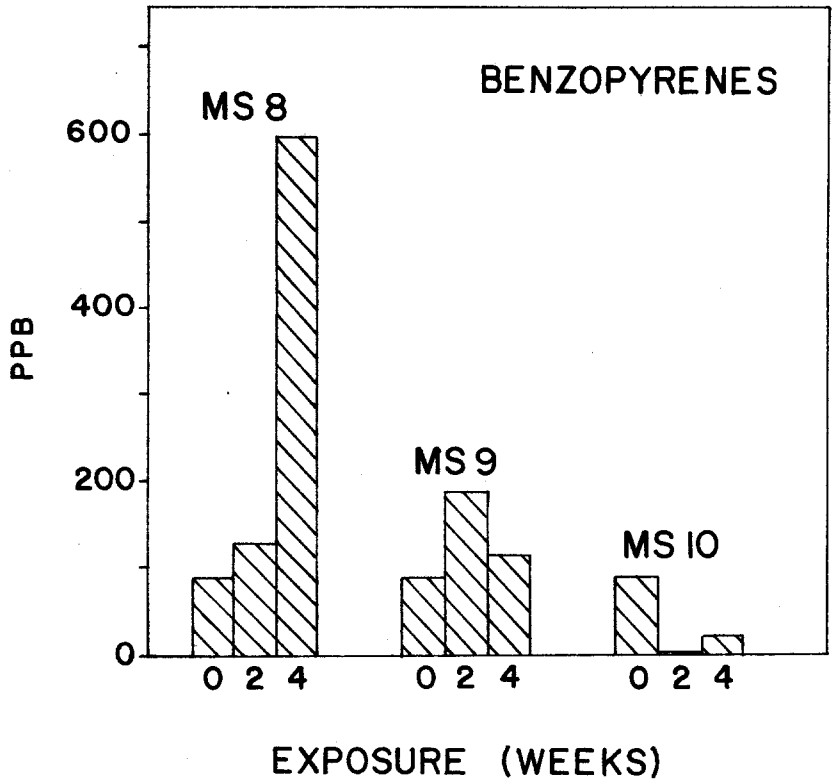

Figure 2. Comparison of benzopyrenes levels in the clams during the study period.

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REFERENCES

DeLeon IR (1981) The Bayou Bonfouca creosote spill--analytical results and chemistry support. Regional Response Team Meeting-Coastal Region VI, Dallas, Texas, February 4-5

Fossato VW, Canzonier WJ (1976) Hydrocarbon uptake and loss by the mussel Mytilus edulis. Mar Biol 36:243-250

Hopkins SW, Anderson JW, Horvath K (1973) The brackish water clam Rangia cuneata as indicator of ecological effects of salinity changes in coastal waters. U.S. Army Corps of Engineers, Report H-73-1

Michel J, Domeracki DD, Sexton WJ (1982) Site evaluation methodology and results, American Creosote Plant Site, Louisiana. Proc. 1982 Hazardous Materials Spill Conference, Milwaukee, WI, pp. 50-55
Neff JM, Cox BA, Dixit D, Anderson JW (1976) Accumulation and release of petroleum-derived aromatic hydrocarbons by four species of marine animals. Mar Biol 38:270-289
Petrocelli SR, Anderson JW (1975) Biomagnification of dieldrin by food-chain transfer from clams to blue crabs under controlled conditions. Bull Environ Contamin Toxicol 13:108
Politzer IR, DeLeon IR, Laseter JL (1985) Impact on human health of petroleum in the marine environment. American Petroleum Institute, Washington, D.C.
Sexton RJ (1980) The hazards to health in hydrogenation of coal. Arch Environ Health 1:208-231
Sims P, Grover PL (1974) Polycyclic aromatic hydrocarbons. In: Klein G, Weihouse S, Haddow A (eds) Advances in Cancer Research, vol 20. Academic Press, New York, pp 165-274
Stegeman JJ (1981) Polynuclear aromatic hydrocarbons and their metabolism in the marine environment. In: Gelboin HV, Ts'o POP (eds) Polycyclic Hydrocarbons and Cancer, vol 3. Academic Press, New York, pp 1-60
Stegeman JJ, Teal JM (1973) Accumulation, release and retention of petroleum hydrocarbons by the oyster Crassostrea virginica. Mar Biol 22:37-44
Varanasi U, Uhler M, Stranahan SI (1978) Uptake and release of naphthalene and its metabolites in skin and epidermal mucus of salmonids. Toxicol Appl Pharmacol 44:277-289
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