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Determination of Benzo(a)pyrene, Hexachlorobenzene and Pentachlorophenol in Oysters from Galveston Bay, Texas

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Intensive development of industrial plants located along the Houston Ship Channel is a major potential source of refractory organic contaminants to the Galveston Bay estuarine system. Petroleum production and shipping also contribute extensively to the pollutant load of the Bay. For example, previous workers (EPA 1970, EHRHARDT 1972, ANDERSON 1975) have reported that oyster samples collected at the lower end of the Houston Ship Channel, particularly Morgan's Point, consistently revealed high levels (130-240 ppm) of petroleum hydrocarbons. As bivalves have been suggested as potentially valuable sentinel organisms for indicating levels of pollutants in coastal marine waters (GOLDBERG 1978), this study was undertaken to analyze oysters from Galveston Bay for selected pollutants.

Three compounds, each representing a particular class of organic pollutant, were selected for determination in oysters (Crassostrea virginica) collected near Morgan's Point. These were benzo(a)pyrene (polycyclic aromatic hydrocarbon), hexachlorobenzene (polychloro-aromatic hydrocarbon); and pentachlrorophenol (chlorinated phenol). These compounds were selected because of their large annual production, patterns of use and disposal which favor their entry into the oceans, high toxicity, and persistence in the environment.

MATERIALS AND METHODS

Reagents. Solvents used were glass distilled (Burdick and Jackson). Silica gel (Woelm, 70-230 mesh) was activated at 150°C for at least 24 h before use. Water was purified by liquid-liquid extraction in a 15-L extractor with petroleum ether. The petroleum ether was changed at 24 h intervals until a 50-fold concentrate demonstrated no impurities by gas chromatography.

Instrumentation. A gas chromatograph equipped with a ^{63}Ni detector and a 1.8 m x 4 mm i.d. borosilicate column packed with 5% SP 2401 was used for HCB and PCP analyses. Injector, detector, and column temperatures were 250, 300, and 160°C, respectively.

A liquid chromatograph with a 4 x 300 mm C_{18} Micro Bondapak column was used for BaP analysis. The solvent system was 10%

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water in methanol at a flow rate of 1.5 mL/min. A spectrophotometer (excitation 365 nm, emission 403 nm) was used for quantitation.

Samples. Oysters were collected during May 1979 in Galveston Bay near Morgan's Point and were transported in dry ice back to the laboratory where they were maintained at -10°C until analysis.

Analysis. Residue analyses were conducted using methods based on our techniques for the careful exclusion or minimization of contamination during ultra-trace analyses (GIAM & WONG 1972, GIAM et al. 1975a,b, 1978; GIAM 1976). Prior to analyses, all glassware and equipment were washed with micro cleaning solution (International Products Corp.), rinsed with distilled tap water and heated at 320°C overnight. Just prior to use, glassware was rinsed with pesticide grade petroleum ether. The petroleum ether washing (100 mL) was concentrated to about 0.1 mL and checked for contaminants. If any impurities were present, rinsing was repeated as needed to obtain an acceptable blank. Procedure blanks were also performed at intervals to insure absence of contamination from reagents and solvents.

Oysters, after defrosting (2-3 h), were transferred to tared 200-mL square beakers. After weighing, samples were homogenized with a polytron homogenizer (Brinkman Instruments, Inc.). Procedures for analyses are outlined as follows:

HCB analysis. Tissue was homogenized twice using 30 mL 20% acetone/acetonitrile for each extraction. Extracts were filtered and placed into a 1-L separatory funnel containing 150 mL 5% NaCl solution. Combined extracts were then partitioned into petroleum ether (PE) (3 extractions x 25 mL). The sample was concentrated using a Kuderna-Danish (K-D) evaporative flask. Cleanup was by placing the sample on a column containing 10 g fully activated silica gel. The column was eluted with 50 mL PE. The first 15 mL of eluate were discarded and the remainder was saved for analysis.

BaP analysis. Tissue was homogenized twice using 50 mL acetonitrile for each extraction. Extracts were filtered and placed into a 1-L separatory funnel containing 350 mL 5% NaCl solution. Combined extracts were then partitioned into PE (3 extractions x 50 mL). The sample was concentrated using a K-D. Cleanup was by placing the sample on 10 g of 5% water-deactivated silica gel. The column was first eluted with 50 mL hexane which was discarded. This was followed by 200 mL 2% acetonitrile/PE. The eluate was concentrated to about 5 mL in a K-D and then transferred to a 3-dram vial. The sample was evaporated to dryness with a gentle stream of nitrogen, then redissolved in 200 µL methanol for analysis.

PCP analysis. Tissue was homogenized twice using 60 mL 20% acetone/acetonitrile each time. Extracts were filtered and placed into a 1-L separatory funnel containing 350 mL 5% NaCl solution. The solution was adjusted to pH > 11 with NaOH pellets. The sample was then partitioned into hexane (3 extractions x 50 mL). The hexane fraction was discarded and the aqueous fraction was adjusted to pH < 2

with concentrated H_2SO_4. The aqueous fraction was then extracted thrice using 50 mL hexane for each extraction. Combined extracts were concentrated with a K-D; derivatized with CH_2N_2 and; placed over 4 g acid alumina. The column was then eluted with 5 mL hexane, which was discarded, followed by 20 mL 10% benzene/hexane which was analyzed.

RESULTS AND DISCUSSION

Benzo(a)pyrene (BaP) concentrations in the oysters ranged from 0.07 to 0.14 ppb with a mean of 0.12±0.03 ppb (Table 1). These values appear to be somewhat low considering an earlier report of 33 ppm of polycyclic aromatic hydrocarbons (PAH) in Galveston Bay oysters (EHRHARDT 1972). However, they are in fair agreement with values found for other areas. For example, no BaP was found in mussels from Lake George, NY at the detection limit of 10 ppb (HEIT et al. 1979). Mussels from Vancouver Harbor had BaP concentrations from 0.6 to 49 ppb (DUNN 1976) and those from Yaquina Bay, Oregon ranged from 0.4 to 11 ppb (MIX & SCHAFFER 1979). Oyster from Norfolk, VA were found to have BaP in the range of 10 to 20 ppb (CAHNMANN & KURATSUNE 1957). The high levels of PAH found in the 1972 study may have been from an oil spill or other active input whereas the present levels appear to reflect a relatively low input of BaP into the Bay.

Concentration of hexachlorobenzene (HCB) in the oysters ranged from 0.31 to 1.4 ppb with a mean of 0.63±0.39 ppb (Table 1). HCB levels have apparently not been surveyed previously in oysters, but have been found to be relatively high in fish and crayfish from the Mississippi. Mosquitofish caught between Baton Rouge and New Orleans had HCB levels from 72 to 380 ppb (LASKA et al. 1976). Fish from Lake Ontario were also found to have higher levels of HCB (25 to 100 ppb) than oysters from this study (NIIMI 1979). Marine fish have been found to have a wide range, from 1 to 560 ppb, of HCB concentrations (GOLDBERG 1976). Values found in this study indicate that there is little active input of HCB into Galveston Bay.

Pentachlorophenol (PCP) concentrations in the oysters varied from 3.4 to 8.3 ppb with a mean of 5.3±1.9 ppb (Table 1). These values are somewhat higher than the 0.1 - 1.0 ppb found in jellyfish from the Gulf of Mexico (KUEHL & DOUGHERTY 1980) or the <0.5 to 4.0 ppb found in fish from St. Croix and St. John's estuaries in Canada (ZITKO et al. 1974). Thus, overall, oysters analyzed for this study denote a relatively high input of PCP into Galveston Bay.

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TABLE 1. BaP, HCB, and PCP Concentrations in Crassostrea virginica from a Reef Near Morgan's Point

Compound	Specimen No.	Weight (g)	Concentration (ng/g)
BaP	1	27.3	0.14
	2	18.3	0.14
	3	27.3	0.07
	4	29.4	0.13
	5	36.0	0.12
	Mean ($\bar{x}$) Concentration		0.12
	Standard Deviation (S.D.)		±0.03
HCB	6	24.5	0.31
	7	62.4	0.36
	8	21.5	0.62
	9	24.2	1.37
	10	35.3	0.39
	11	48.5	0.70
	Mean ($\bar{x}$) Concentration		0.63
	Standard Deviation (S.D.)		±0.39
PCP	12	4.0	3.60
	13	21.5	6.20
	14	6.3	8.30
	15	20.0	4.60
	16	15.5	3.40
	17	8.4	5.50
	Mean ($\bar{x}$) Concentration		5.30
	Standard Deviation (S.D.)		±1.90

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