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The Use of Mussels in Estimating Benzo(a)Pyrene Contamination of the Marine Environment (38971)

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In assessing the potential health hazards of a contaminated marine environment, particular attention should be directed to chemical carcinogens and mutagens. Such compounds may exert only a delayed effect, and remain undetected for a prolonged period, thus possibly accumulating to dangerous levels.

The presence of carcinogenic polycyclic aromatic hydrocarbons (PAH) in plankton, seaweeds, filter feeding organisms, and bottom samples has been reported (reviews, 1–4). Unsolved remains the question of their geographical distribution and source, and the problem of a simple yet reliable detection method that would be applicable in a large-scale monitoring system. In this paper we explore the possible use of mussels (*Mytilus edulis* and *Mytilus californianus*) in estimating the distribution of benzo(a)pyrene (B(a)P) in coastal waters, and attempt to assess their suitability in an economic, simple, and relevant monitoring system. Mussels were chosen because of their widespread distribution, their sedentary habits, and their capacity to accumulate foreign compounds, including PAH (5). Benzo(a)-pyrene was selected because of its potency as a carcinogen, and its presence in a great variety of contaminating discharges that may enter shallow coastal waters (1, 3).

Methods. The levels of benzo(a)pyrene in mussels and other samples were measured using a modification of a previously described procedure (6); 20–40 g (wet drained weight) of mussel tissue (generally 10–15 mussels) or 5–10 mg of creosoted wood were refluxed with ethanol and KOH, then water added and the PAH extracted into iso-octane. Interfering materials were removed by chromatography on a column of Florisil, followed by dimethylsulfoxide extraction. Benzo(a)pyrene was separated from other PAH by thin-layer chromatography on cellulose-acetate. The B(a)P was quantitated fluorimetrically in hexadecane, using a baseline technique (7) to estimate the height of one of the peaks in the B(a)P emission spectrum. Recoveries (generally 70–80%) were measured for each sample by adding an aliquot of radioactive benzo(a)pyrene (3,4 - Benz(3,6 - C14)pyrene, Amersham/Searle Corp.) to each sample before reflux with ethanol/KOH, and then measuring the amount of radioactivity in the final fluorimetry sample. The levels of B(a)P reported in this communication are corrected for losses during the extraction and measurement procedures.[2]

Results. To obtain information on the background levels of B(a)P, mussels from a wide range of locations were examined (Table I). Specimens taken from the west coast of Vancouver Island, remote from human activities such as industry, mines, or cities have very low levels of B(a)P, ranging from 0.0 to 0.2 μg/kg. In contrast, mussels collected from the outer harbour of Vancouver contained substantial amounts of B(a)P. This area is an open bay approximately 6 by 8 km, surrounded by residential districts and swimming beaches. It is traversed by approximately 25 million tons of shipping yearly, and is an anchorage for 10–20 deep-sea vessels. Even higher concentrations of B(a)P were found in mussels from rocks within wharf, marina, and dock areas in the Vancouver vicinity (mean of 18 μg/kg) while mussels collected from the inner segment of a poorly flushed harbour inlet receiving a variety of discharges (from machine shops, shipping terminals, lumber yards, marinas, and storm drains) showed the highest levels of B(a)P (mean of 42 μg/kg).

An examination was made of the level of

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[2] The procedures used will be more fully described in a subsequent technical communication.

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TABLE I. CONCENTRATION OF BENZO(a)PYRENE IN MUSSELS ON ROCKS IN VARIOUS AREAS.

Sample Location and Description	n	B(a)P level, μg/kg wet wt[a] Mean ± SE	Range
Open Pacific coast, no human activity within 5 km (west coast of Vancouver Island)	4[b]	0.1 ± 0.1	0.0–0.2
Outer harbor (Vancouver)	44	2.0 ± 0.3	0.0–8.3
Wharf, marina, and dock areas (Vancouver vicinity)	15	18 ± 4.9	1.5–60
Poorly flushed inlet, heavy marina and industrial usage (Vancouver, False Creek)	6	42 ± 6.0	27–63

[a] Mussels were collected from the midintertidal zone. Mussels 3–6 cm in size were cleaned externally, and shucked into clean glass containers. Byssal threads were removed. The tissue was drained for 1 min by agitation over a screen of mesh size 1.5 mm. Samples were frozen at −10° for no more than 4 wk before analysis.

[b] *Mytilus californianus*, all other samples are *M. edulis*.

B(a)P in mussels taken at and near two locations that appeared to be point sources of PAH pollution. The first area, potentially contaminated by creosote and petroleum products, was a powerboat marine consisting of a network of untreated wood floats covering an area of approximately 75 by 150 m, having mooring facilities for approximately 200 pleasure motorboats, and containing approximately 100 creosoted pilings 7 yr old. The levels of B(a)P in samples of *M. edulis* collected from rocks outside the wharf area (25–550 m from the nearest dock structure), rocks or steel cables within the wharf area, and creosoted pilings within the wharf area are shown in Table II. Inside the wharf area, mussels growing on creosoted pilings had significantly more B(a)P than those growing on other surfaces. The levels of B(a)P in mussels taken from the wharf area in September were generally higher than those taken in May. When mussels taken from rocks at various distances from the wharf area are examined, a gradient of B(a)P contamination to the south of the marina can readily be seen (Fig. 1). Samples taken to the north of the marina had generally lower levels of B(a)P than those at similar distances to the south—this difference may be due to prevailing currents. At 550 m

TABLE II. CONCENTRATION OF BENZO(a)PYRENE IN MUSSELS IN THE VICINITY OF A POWERBOAT MARINA.

Location of mussels	B(a)P level, μg/kg wet wt[a] Mean ± SE (n) May 1974	September 1974
Rocks 25–550 m from wharf structures	1.9 ± 0.4 (11)	1.8 ± 0.1 (6)
Rocks or steel cables within wharf area	13 ± 5.3 (5)	39 ± 7.3 (6)
Creosoted pilings within wharf area	68 ± 13 (4)	133 ± 19 (5)

[a] Preparation of samples as described for Table I. All samples are *M. edulis*.

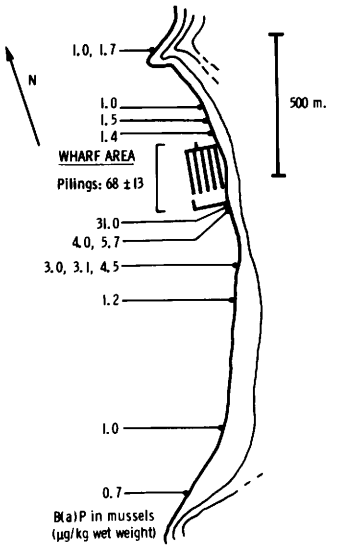

FIG. 1. Concentration of benzo(a)pyrene in *M. edulis* collected from rocks along the shoreline adjacent to a powerboat marina.

from the marina the B(a)P content of mussels was reduced to 0.7 μg/kg, which approximates the background level of contamination in this region.

The second site examined was a barricade of creosoted pilings and timbers at the base of a bridge which spanned tidal waters. It was selected as a location in which PAH contamination from creosote could be investigated in isolation from that due to petroleum pollution from moored boats. Mussels taken from the creosoted wood showed B(a)P levels of 49 ± 5.8 μg/kg ($n = 6$), while mussels taken from the same body of water but remote from the pilings showed a contamination of only 2.1 ± 0.3 μg/kg ($n = 12$). Samples of creosote treated wood from the pilings (outer 4 mm) showed B(a)P levels averaging 570,000 μg/kg. Thin-layer chromatograms of extracts of creosoted wood and of mussels growing on the pilings showed a very similar pattern of fluorescent bands.

Discussion. The results point to a correlation between the level of B(a)P in mussels and industrial, urban, and recreational (e.g., powerboat) activity. Apart from a general contamination of mussels toward and within the harbour area, there are numerous local "hot spots" such as wharfs, docks, and constructions utilizing pilings. Whether creosote from pilings or petroleum pollution is the main contributor to the higher B(a)P levels around marinas and wharfs is difficult to assess at present. However creosote does appear a major source of contamination of mussels located on pilings. Our results do not support the suggestion that large amounts of PAH in the marine environment originate by endogenous synthesis by marine flora (1). The lowest levels of B(a)P were recorded along the Pacific coastline of Vancouver Island—an area rich in algal growth, while the highest levels were found in mussels from a relatively barren inner harbour area.

The results indicate that the level of B(a)P in mussels, which are easy to collect and abound in the areas of greatest interest, may represent a simple indicator for the degree of contamination of the marine environment by polycyclic aromatic hydrocarbons.

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