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Comparison of Techniques for Monitoring Water-Borne Polycyclic Mutagens: Efficiency of Blue Rayon, Sep-Pak C18, and a Biota, *Corbicula,* in Concentrating Benzo(a)pyrene in a Model Water System

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For monitoring water-borne mutagens, it is usual practice to collect samples of water, sediment or biota habitat, and subject them to chemical analysis and bioassays. Levels of mutagens or the mutagenic activity in a spot-sampled water represent only the state at the moment of sampling. Thus, many periodical samplings are required for assessing the water quality at a site. In our previous studies, we proposed a unique sampling technique for water-borne mutagens: hanging blue rayon, which is an adsorbent and a rayon bearing covalently linked copper phthalocyanine trisufonate, selective for polycyclic planar compounds with three or more fused rings (Hayatsu et al. 1983; Hayatsu 1992), in water sites for a period of 24 hr. Using this technique, we and other scientists detected mutagenicity in marine waters (Kira et al. 1989; Kira et al. 1994) and river waters (Hayatsu et al. 1983; Sakamoto and Hayatsu 1990: Sayato et al. 1990; Kusamuran et al. 1994). The mutagenicity detected in the samples was correlated well with their benzo(a)pyrene (BaP) contents (Kira et al. 1995). A level of mutagenic activity found by this technique is a reflection of a time weighted level of mutagens in the site of sampling. This technique is simple and qualitatively informative.

A factor that can affect the adsorption is the water movement surrounding the blue rayon. In the present study, we planned to investigate how the movement affects the adsorption. For this purpose, we constructed a laboratory aquarium system (Figure l), in which water movement can be varied and BaP can be continuously supplied from an external source. We measured the efficiency of blue-rayon adsorption of the BaP and evaluated the effect of water movement.

In this system, we also immersed into the water a Sep-Pak Cl8 cartridge and corbicula japonica. With the Sep-Pak, through which the water was continuously passed,

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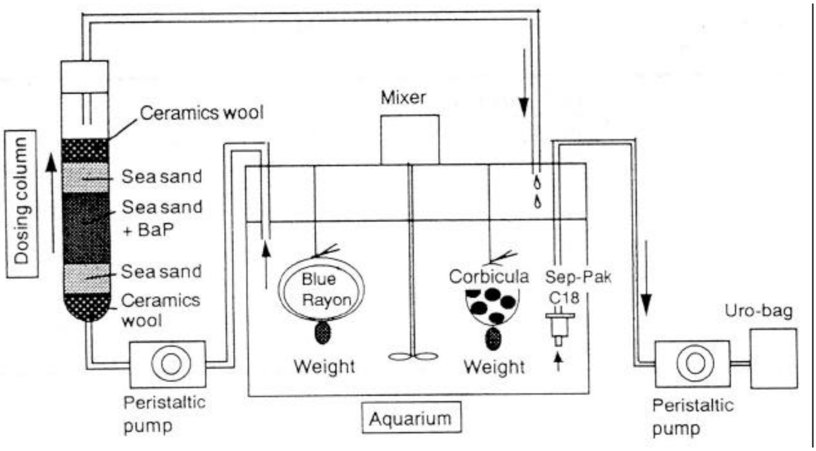

Figure 1. A schematic diagram of experimental system.

we expected to obtain a value of BaP content that may be designated as a time-weighted average (TWA), a term commonly used for air-borne pollutants. Corbicula japonica was used to evaluate the efficiency of bio-monitoring in this system.

MATERIALS AND METHODS

A laboratory glass aquarium, 65(w) x 30(d) x 35(h) cm in size containing 30 L water at the start of the experiment, was continuously supplied with BaP (Tokyo Kasei Co., Tokyo, Japan) from a dosing-column at a flow rate of 20-30 mL/min (Figure 1). The experimental apparatus used here was a modification of a system reported previously (Giam et al. 1980a). The water movement produced by vortexing with a mixer was set at 3 levels; rapid, moderate and none. Blue rayon was purchased from Funakoshi Chemicals (Tokyo, Japan). Blue rayon (l-g each in a net) was hung in the water for a desired period and the BaP adsorbed to it was recovered and quantified. Through the immersed Sep-Pak plus C18 Environmental Cartridge (Waters, Millipore Corp., Massachusetts, USA), the water was continuously passed by the use of a peristaltic pump at a rate of 50-100 mL/hr. The water that passed through the cartridge was collected in a uro-bag, a device commonly used in urological clinics. From the volume of water being passed through the cartridge during a sampling period of time (V liter) and the BaP recovered from the Sep-Pak (C ng), a TWA was determined: TWA of BaP =C/V ng/L, a formula conceptually identical to that of TWA for gases and vapors in the air (Ashton and Gill 1992).

Table 1. Concentrations of benzo(a)pyrene in the aquarium

Time of sampling (hr)	BaP concentration (ng/L)		
	Level of vortexing water		
	None	Moderate	Rapid
0	176	311	83
2	77	235	49
4	106	187	47
6	99	164	47
8	126	183	93
12	114	203	107
24	112	326	262

Bivalva, 40 to 44 corbicula (100-g total weight with shells a group in a net), was hung in the the water. These three treatments were done similutaneously.

BaP in the aquarium water was quantified from spot-samples at 0, 2, 4, 6, 8, 12, and 24 hr by extraction with petroleum ether (Giam et al. 1980b). Procedures for BaP extraction from blue rayon were described in a previous report (Kira et al. 1989). Corbicula were homogenized with acetone-acetonitrile (2:8) and the homogenate was worked up as described (Kira et al. 1989). BaP in the Sep-Pak Cl8 was eluted with tetrahydrofuran. The first 1-mL of tetrahydrofuran eluate was discarded and the following 2-mL eluate was found to recover 85-95% of BaP that had been spiked into the cartridge: hence this 2-mL eluate was used in analyzing the BaP from the Sep-Pak C18.

For quantifying BaP, a column of Nova-Pak C18, 60 A 4 µm, 3.9 x 150 mm (Waters, Millipore Corp., Massachusetts, USA) coupled with fluorometric detection was used. The column, which was maintained at 40 °C, was eluted with acetonitrile-water (65:35) at a flow rate of 1 mL/min. The fluorometer (Hitachi F1050) was set at 365 nm for excitation and at 405 nm for emission, and the peak areas of the spectra were recorded with an integrator (Hitachi D-2500). The HPLC equipment used was a Waters 600E system controller with a Roedyne 7125 injector. All solvents used for extraction and HPLC analysis were of HPLC grade purchased from Wako Pure Chemicals (Osaka, Japan).

RESULTS AND DISCUSSION

We manipulated the dosing of BaP into the aquarium water in a manner so that its concentration was maintained at around a 10^{-2} µg/L level (Table 1). With these settings, the recovery of BaP from the aquarium by use of the three different methods was determined.

Table 2. Levels of benzo(a)pyrene determined by three techniques: blue rayon (BR), Sep-Pak C18, and corbicula

Level of vortexing	Time of exposure (hr)	Geometric mean of BaP in aquarium[a] (ng/L)	BaP level found with: Blue rayon (ng/gBR)	Sep-Pak (TWA[b]), ng/L)	Corbicula[c] (ng/kg ww)
None	6	106	12	101	406
	12	113	15	116	1449
	24	113	23	128	799
Moderate	6	218	32	137	1923
	12	209	30	136	1973
	24	223	185	160	5549
Rapid	6	55	296	54	604
	12	67	759	67	2769
	24	81	2269	115	2981

a) Geometric mean was determined from the values in Table 1. For example, the "None, 6 hr" value (n6) was obtained as follows: log(n6) = [log(176) + log(77) + log(106) + log(99)]/4

b) Time-weighted average: see text for explanation.

c) Forty to 44 in a group of corbicula (100-g each) contained about 20 g wet weight (ww) of edible part, and the value represents levels in the edible part.

The results are shown in Table 2.

Blue rayon hung in the aquarium adsorbed BaP more efficiently as the water movement increased. During a 24-hr exposure with the rapid water movement, a 28-L equivalent BaP (2269 ng/gBR divided by 81 ng/L)) or a 20-L equivalent BaP (based on the Sep-Pak TWA value; 2269 ng/gBR divided by 115 ng/L)) was trapped in 1 g of blue rayon. These results suggest that the blue rayon procedure is highly efficient in adsorbing BaP when the water movement is rapid, and therefore it would be suitable for use in flowing river waters and in most sea waters.

The BaP concentrations found with Sep-Pak Cl8 showed a good correlation with the geometric means of BaP levels in the aquarium, regardless of the water movement. Therefore, our expectation that the Sep-Pak adsorption would give a value that can be regarded as TWA seemed to have been fulfilled. This result suggests that if in the future it becomes possible to provide in field studies a portable pumping system that enables sample-waters pass through Sep-Pak, estimation of TWAs of BaP will be automated, although the problem of turbidity removal is to be overcome first: since a tubid water plugged up the cartridge occasionally and the water flow is disturbed during sampling.

The *corbicula* accumulated BaP during a 24-hr exposure to increase its levels to 6-35 fold the TWA level (799 ng/kg divided by 128 ng/L, or 5549 ng/kg divided by 160 ng/L). The bivalve also showed a tendency to accumulate BaP in still water. The 24-hr BaP concentration in *corbicula* was not always higher than the short-exposure values. We speculate that the BaP could have been metabolized and excreted or eliminated in *corbicula* during the period.

We conclude that (1) blue rayon is a highly efficient monitor for BaP in moving waters, (2) the use of Sep-Pak Cl8 with continuous sampling gives a quantitatively accurate measure for BaP, but may not be applicable for water with turbidity, and (3) *corbicula* is useful for monitoring, but may have confounding biological factors such as bioconcentration and metabolism or excretion of the BaP.

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