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Improved Blue Rayon Hanging Technique That Can Measure a Time-Weighted Average Concentration of Benzo(a)pyrene in Sea Water

S. Kira,[1] H. Horiguchi,[2] Y. Nogami,[2] T. Komatsu,[3] K. Fujisawa,[4] T. Ito,[5] H. Hayatsu[5]

[1]Department of Public Health, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama City, 700 Japan
[2]Department of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama City, 700 Japan
[3]Ocean Research Institute, University of Tokyo, 1-15-1 Minamidai, Nakano-ku, Tokyo, 164 Japan
[4]Fisheries Experiment Station of Okayama Prefecture, 35 Kasino, Ushimado Town, Okayama, 701-43 Japan
[5]Faculty of Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama City, 700 Japan

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Polycyclic aromatic hydrocarbons (PAHs) are present in the water environment at a low level. Some of them have been reported as mutagens. We have proposed a simple technique for monitoring waterborne PAHs using blue rayon (BR), which selectively adsorbs compounds having three or more fused rings (Hayatsu et al. 1983; Hayatsu 1992). A compact apparatus for this technique allows easy on-site concentration of PAHs, with BR hung in water for 24 h. For these reasons, the technique has been utilized to monitor mutagenicity and PAHs in the water environment by many researchers (Kira et al. 1989; Sakamoto and Hayatsu 1990; Sayato et al. 1990; Kusamuran et al. 1994; Kira et al. 1995). We also reported that the amount of benzo(a)pyrene (BaP) and the mutagenicity determined by the BR hanging technique correlate with each other (Kira et al. 1995).

The amount of BaP and the mutagenicity must be a reflection of a time-weighted average (TWA) concentration of the substance in water. The amount of PAHs adsorbed to the BR primarily depends on the concentration of PAHs in water. However, our previous study revealed that the measurement might be affected by the flow of the surrounding water (Kira et al, 1996); i.e. the more rapid the flow becomes, the greater the PAHs are adsorbed. If we can measure a cumulative intensity of the water movement, and the amount is corrected by the intensity, a TWA concentration of waterborne PAHs may be measured by the BR hanging technique. Nevertheless, there have been few reports on TWA concentration of waterborne PAHs measured by any technique. For this purpose, we have used a simple procedure proposed by Komatsu and Kawai (1992) in which plaster balls are hung in the water as an indicator for the intensity of water movement. An electromagnetic current meter was used to validate the estimation.

This paper describes the measurement of TWA concentration of BaP, a typical mutagenic and/or carcinogenic PAH in field water, using a BR-plaster ball hanging technique. To evaluate the performance of this technique, the TWA concentration of BaP determined by this technique was compared with that measured by a portable sampler, which continuously quantifies waterborne PAHs (Kira et al. 1997).

Correspondence to: S. Kira

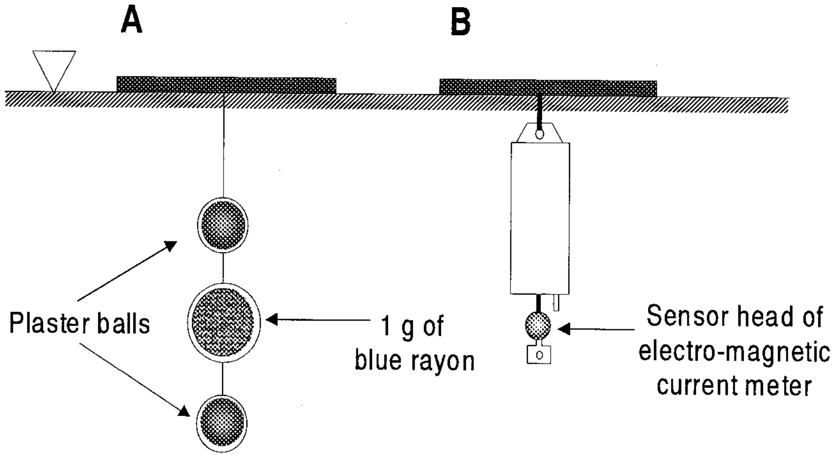

Figure 1. A scheme of the device for improved blue rayon hanging technique (A) and simultaneous measurement of an average water-flow intensity using an electro-magnetic current meter (B).

MATERIALS AND METHODS

A cumulative intensity of water movement at a sampling site was estimated by the plaster ball method that has been reported by Komatsu and Kawai (1992), with some modifications. To standardize the size and weight of the plaster ball, we used a ping-pong ball as a mold. Gypsum (anhydrous $CaSO_4$ for artists' use, Japan Sho-sekkou-kogyou-kumiai, Tokyo, Japan) and distilled water was mixed (w/w ratio 10:7), and the mixture was immediately injected into a ping-pong ball by use of a plastic syringe. The ball was allowed to stand for a day at room temperature. The ping-pong ball (mold) was then removed, and the ball obtained was allowed to stand at room temperature for two weeks or longer until its weight became constant (mean weight 37.6 g, coefficient of variance <1%). Before use, these balls were immersed in distilled water in a beaker for an hour, the balls were taken out and excess water on the surface was removed with a gentle application of a paper towel. Initial weight (W_0) of each ball was measured at this time. Two of these balls in a plastic net were tethered to blue rayon, also in the net, and the device was hung under a Styrofoam float (see Figure 1A). At the test site, the device was placed at a depth of 30-50 cm from the surface of the water for 24 hr. The site was in the Seto Inland Sea at the Fisheries Experiment Station of Okayama Prefecture, Ushimado Town, Okayama, Japan. The water flow was of natural nature; tidal currents and wind blowing were the main causes of water movement. The salinity of the water at these sampling sites ranged from 30 to 33 psu as measured by an electro-conductivity meter. The balls were recovered after 24 h, and the weight (W) of each ball was measured after wiping it to remove water on the surface. The decrease in weight of each ball (ΔW), i.e. $\Delta W = W_0 - W$ is a reflection of the water flow intensity surrounding the ball that is related to the dissolution of gypsum (Komatsu and

Kawai 1992). An average of the ΔW values of these two balls represents the flow speed at the position of BR (Fig.1). An electro-magnetic current meter with a temperature sensor (model ACM-1M, Alec Electronics Co, Kobe, Japan) was placed next to the plaster ball device by fixing it to a buoy. The sensor part of the flow-meter was set at the depth of the BR (in the middle of the two balls) (Figure 1B). The three dimensional movement of the water flow and the water temperature were monitored every 5 min. The average flow velocity (V) was calculated based on these data.

Extraction of BaP adsorbed on BR was performed according to the method previously reported (Kira et al. 1995). Briefly, the BR was rinsed with distilled water, and the water was removed by aspiration and blotting onto a paper towel. The BR was eluted with 150 ml of methanol/ concentrated ammonia solution (w/w 50:1), and the eluate was evaporated to dryness. The residue was dissolved in methanol, and submitted to high performance liquid chromatography (HPLC) analysis, for quantifying BaP.

We constructed a portable sampler with which we can continuously measure BaP and other PAHs in the water environment (Kira et al. 1997). This sampler is designed for field measurement of TWA concentrations of PAHs in water. The system consists of solid phase extraction cartridges (Sep-Pak plus C18 Environmental Cartridge, Waters, Millipore, MA, USA) , tubing, a peristaltic pump, portable batteries, and reservoir bags. Water is aspirated through the cartridges, and stored in the reservoir bags. The sampler was placed next to the apparatus described in Figure 1A. The extraction cartridges were recovered at the end of a 24 hr-sampling. After the analysis, the TWA concentration of BaP was calculated according to Ashton and Gill (1992) as reported in our previous report (Kira et al. 1996), dividing the amount of BaP by the volume of the sea water that was stored in the reservoir bags during the period of the hanging (24 hr). BaP that was adsorbed to Sep-Pak C18 was eluted from the cartridge with tetrahydrofurane; the first 1 mL was discarded and the following 2 mL were used for the analysis. The eluate was evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 0.2 ml of methanol and analyzed by HPLC.

BaP was assayed according to the previously reported methods (Kira et al. 1995). Briefly, we used a Waters 600E system with a Roedyne 7125 injector. A Nova-Pak C18 column (ϕ3.9 x 150 mm, mesh 4 µm, Waters, Millipore) coupled with a fluorometric detector was used. The column was maintained at 40°C and was eluted with acetonitrile/water (65/35) at a flow rate of 1.0 ml/mm. The fluoro-spectrophotometer (model Hitachi F-1080, Hitachi, Tokyo, Japan) was set at 365 nm for excitation and at 405 nm for emission. The peak area of the spectra was recorded with an integrator (model Hitachi D-2500, Hitachi, Tokyo, Japan).

All solvents used for elution and HPLC analysis were of HPLC grade purchased from Wako Pure Chemicals (Osaka, Japan).

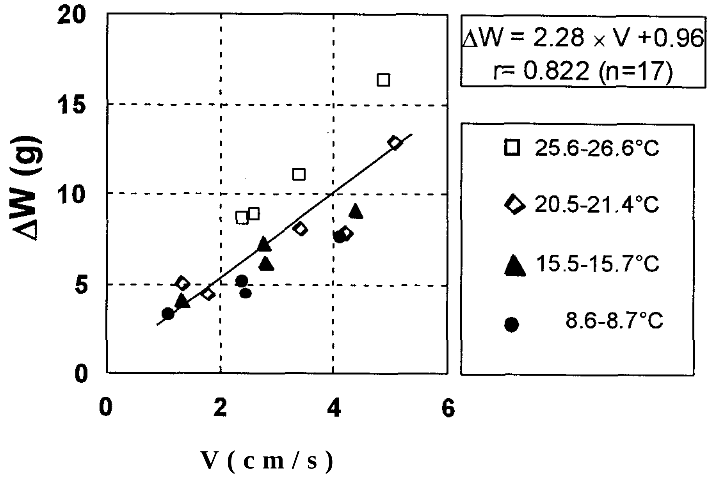

Figure 2. A linear correlation of average water-flow intensity and decrease in the weight of two plaster balls.

To compare the TWA concentration of BaP measured by the proposed technique and that measured by the portable sampler using Sep-Pak C18, we selected two sampling sites in the Seto Inland Sea of Japan where our previous studies were carried out (Kira et al. 1989, 1995). The Ushimado site was the same place where the tests for plaster balls were done and is far apart from obvious sources of pollution. Another site was in the Mizushima port (Okayama, Japan), which is surrounded by an industrial area with petrochemical factories and power plants.

RESULTS AND DISCUSSION

Figure 2 shows the typical linear correlation of V (average velocity of water flow measured by electro-magnetic current meter, which ranged from 1.31-5.05 cm/s) versus ΔW (decrease in weight of plaster ball in g). All the data were obtained at the Ushimado test site. As shown in Figure 2, ΔW correlates well with V. The figure also shows the effect of water temperature on the rate of weight reduction of a plaster ball. The average rate of decrease in weight of the plaster ball was 3.15, 1.95, 1.67, and 1.46 (g/cm/s) at 25.6~26.6, 20.5~21.4, 15.5~15.7, and 8.6~8.7°C, respectively. The correlation coefficients were 0.995, 0.919, 0.972, and 0.976, (n= 4 or 5 each) at 25.6~26.6, 20.5~21.4, 15.5~15.7, and 8.6~8.7°C, respectively. The overall correlation coefficient for V and ΔW (i.e. including all of the temperature ranges) was 0.822 (n=17). These results showed that V in the range of 1.31-5.05 cm/s can be estimated regardless of the water temperature within 8~27°C using this method.

Here we hypothesize that the BaP concentration obtained using the BR hanging technique [BR-BaP] (ng/gBR), and that obtained by the portable sampler [TWA-BaP], (ng/L) are correlated by the following equation (1):

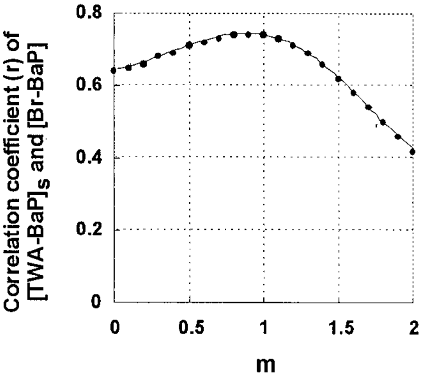

Figure 3. The relationship of “m” in equation (2) and the correlation coefficient between [BR-BaP]s and [TWA-BaP]

$[\text{TWA-BaP}]_s = k \times [\text{BR-BaP}]/V^m$ .....(1) where k, and m are constants.

By substituting V with ΔW, we obtain

$[\text{TWA-BaP}]_s = K \times [\text{BR-BaP}]/\Delta W^m$ . .....(2) in which K is a constant.

The “m” and “K” values were obtained by arbitrarily assigning values for a best fit. Thus, as Figure 3 shows, at m=1 a best fit can be obtained. When m=l, K was calculated to be 6.5 using equation (2) with a correlation coefficient(r) of 0.740. The temperature factor was added to the equation and curve-fitted, but without any significant improvement in the correlation (data not shown). Consequently, we use the following equation for converting the expression of the BaP level from ng/gBR to ng/L.

$[\text{TWA-BaP}] = 6.5 \times [\text{BR-BaP}]/\Delta W$ .....(3)

Figures 4A and B compare the correlations between the portable sampler BaP measurement $[\text{TWA-BaP}]_s$ vs. [BR-BaP] (A), and $[\text{TWA-BaP}]_s$ vs. [TWA-BaP] (B). [TWA-BaP], the corrected value, gave a higher correlation coefficient (r= 0.740, n=29) than [BR-BaP] which is the uncorrected value (r= 0.639, n=29).

The present results show that the measured average water-flow intensities by the electro-magnetic current meter and by the modified plaster ball method correlate well. Therefore, the average intensity of the water-flow that surrounds the BR could be estimated by measuring the decrease in the weight of plaster balls. Because the salinities of water may affect the dissolution rate of the gypsum (plaster) ball during immersion (James and Lupton 1978), in applying this method for the measurement in a fresh-water environment a calibration for the correction constant (K) may be necessary.

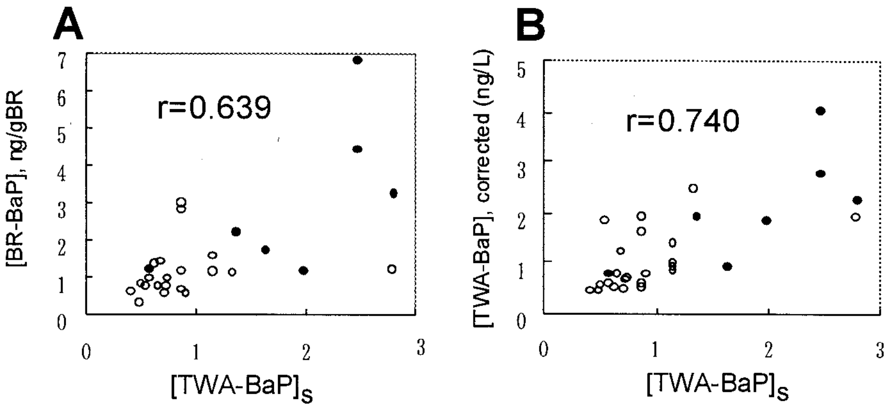

Figure 4. Correlation of $[\text{TWA-BaP}]_s$ and the amount measured by the blue rayon hanging technique. **A.** before correction [BR-BaP], **B.** after correction [TWA-BaP]. $[\text{TWA-BaP}]_s$; measured by the portable sampler. Open circles: Ushimado Port, filled circles: Mizushima Port.

In conclusion, we have reported an improved BR hanging technique, which could measure a time-weighted average concentration of BaP in the environmental water. Here, we propose this technique as a tool for environmental monitoring that would be suitable for international collaboration.

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