Evaluation of Aquatic Environment by Polyaromatic Hydrocarbon Concentration and Induction of EROD Activity in HepG2 Cells

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Environmental contamination by polyaromatic hydrocarbons (PAHs) and dioxin-like compounds was evaluated by chemical analysis and biological tests. Though soils and road dust samples revealed more potent biological activities than incinerator ashes, the contribution of PAHs were low.

This study was designed to describe dioxin-like activity of various aquatic environmental samples, to estimate contribution of PAHs and, as a result, to propose simple, inexpensive and practical primary screening and monitoring of dioxin-like compounds.

Rain water, spring water, river water (middle stream and estuarine), coastal sea water, influent and effluent of the human excreta treatment plant (HETP) and the sewage treatment plant (STP), heating oil and gasoline were collected as liquid samples. River sediments, seashore sediments, forest soil, rice field soil, agricultural soil, fly and bottom ashes of municipal waste incinerator and road dust were also collected as solid samples between 1999 and 2002 at Okayama Prefecture, Japan.

The liquid samples (1–2 L) and the solid samples (5–10 g-dry, <2 mm) were extracted with *n*-hexane according to the method of Kenmotsu (1998).[1] An aliquot (50%) of the crude extracts was cleaned with a silica cartridge for liquid samples or a glass column filled with silica gel/5% H_2O for solid samples. Alkaline treatment with NaOH (pH 11) was also performed for the samples from the HETP and the STP, to evaluate the adsorption of PAHs to particulates. Benzophenone ($\log P_{OW}$, 3.18),[2] phenanthrene (4.46), anthracene (4.45), fluoranthene (5.16), pyrene (4.88), benzo[a]anthracene (5.76), chrysene (5.81), benzo[b]fluoranthene (5.78), benzo[k]fluoranthene [B[k]f] (6.11) and benzo[a]pyrene (6.13) were analyzed by GC/MS (QP5050, Shimadzu). After PAHs analysis, the crude and cleaned extracts were added in 50 μL DMSO. And these aliquots were used in the 7-ethoxyresorufin *O*-deethylase (EROD) activity test.

The determination of the EROD activities of cells that were cultured for 12 hrs with the test samples, was performed according to Donato et al. (1993)[3] except that MEM without phenol red and 70 units of β-glucuronidase (Type H-3) was used (Nakama et al. 1995).[4] Fluorescence was measured using HPLC (LC-10AD, Shimadzu) at 550 nm_{exc}/585 nm_{emis} (Hanioka et al. 2000).[5] Cellular protein was assayed in the plate with modified Lowry protein assay reagent kit (PIERCE Chemical Co.) and microplate reader (model550, Bio-Rad).

The sum of 10 PAHs concentration is indicated as ''Total PAHs''. Solid samples exhibited about 1000-fold higher concentration than liquid samples except mineral oil samples (Figure 1A). The highest concentration was detected in the heating oil, seashore sediment and the road dust. The seashore sediment contamination might be caused by oil from the ship. PAHs detected in road dust may not originate mainly from fuel oil but mobile exhaust gas. Because in the mineral oil samples, benzophenone and phenanthrene occupied over 94% of total PAHs, but in the road dust samples, the percentage was much lower. Total PAHs in the road dust tended to be higher in November than in July and correlated roughly to volume of traffic. In both of non-treated and alkaline treated samples from the HETP, total PAHs concentration of the influent decreased significantly to the level of the spring water after the treatment process. In the river sediments, each PAH concentration increased from several to 40 folds between middle stream and estuarine zone. Correlation analyses revealed that the magnifications were correlated significantly with the $\log P_{OW}$ ($r^2 = 0.78$–0.88). When the same types of analyses were performed for water samples from three rivers, the correlations were also found in the two rivers. ($r^2 = 0.88$, 0.69). Total PAHs of ashes were at the same range as river sediments. Total PAHs detected in the bottom ashes were five- to ten folds higher than the fly ashes.

EROD activity is defined as the ability of inducing CYP I A1 and, measured as resorufin production by using dose-response curves. It was estimated according to the concentration of samples producing a response equivalent to 50% of the maximal response of B[k]f (ECB[k]f50%) because the samples produced different levels of maximal induction of resorufin. The ECB[k]f50% of B[k]f was divided by the ECB[k]f50% of each samples to determine relative potency to B[k]f (induction equivalent factor : IEF) (Jones et al. 1999).[6]

IEFs were about 100- to 100000-fold higher in solid samples than in liquid one (Figure 1B). Although most of the samples indicate the same result as well total PAHs, more potent activities were obtained from the extracts of soil than expected from the total PAHs. The contribution of 10 PAHs to sample's EROD inducing potency was estimated by using each pure chemical's (Figure 1C).

IEFs were reduced to the level of negative control after the cleanup of extracts of the HETP samples and alkaline treated HETP samples. Also IEFs of crude extracts correlated with total PAHs. Therefore it can be assumed that organic compounds except 10 PAHs and dioxins induced EROD activities and had the same origin with 10 PAHs. Since the extracts from effluent samples cannot induce EROD activity, inducers might be removed or inactivated through the treatment processes. Effluent showed higher EROD activities than influent in case of STP, suggesting production or contamination of inducers through the treatment processes. In the river sediment, significant correlation was found between IEFs and total PAHs but 10 PAHs could not account for their EROD activity fully. It is suggesting that inducers are not 10 PAHs but organic compounds that may have similar chemical characteristics as well 10 PAHs. Moreover the fact that the increase of each PAH correlated well with $\log P_{OW}$ in

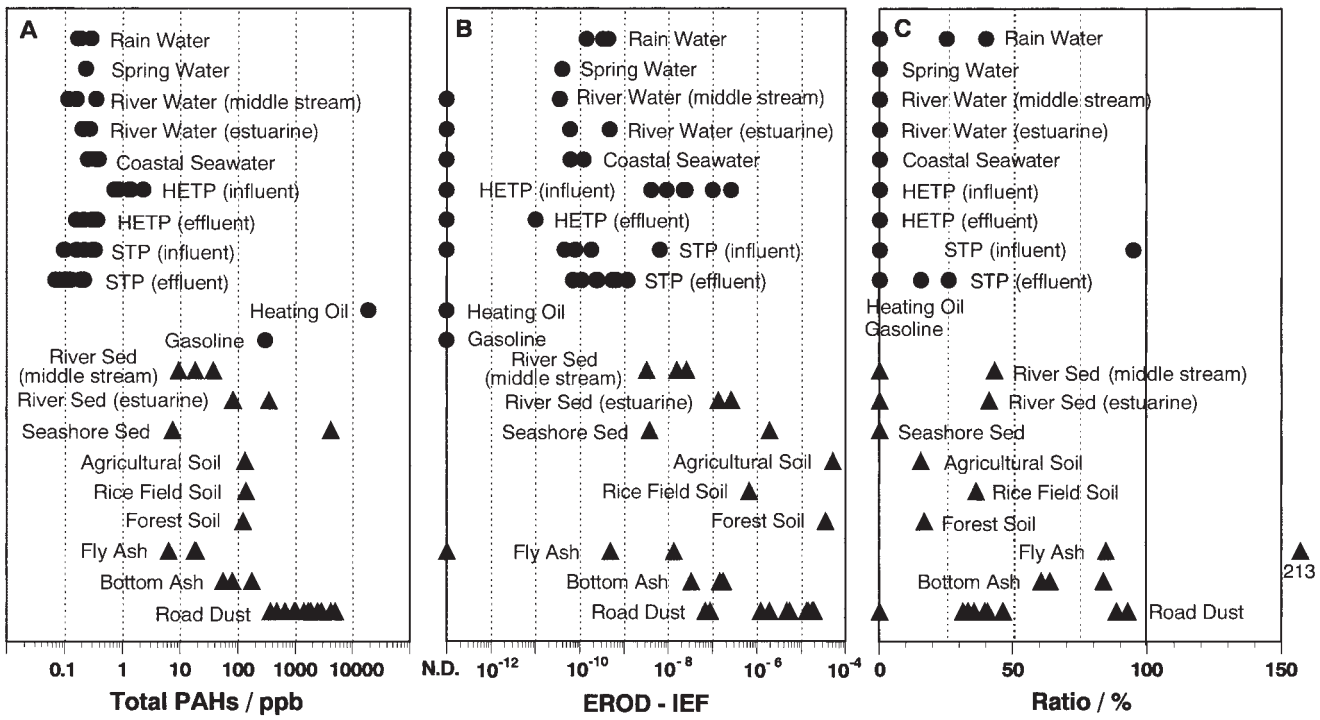

Figure 1. Levels of PAHs and EROD activity of various aquatic environmental sample extracts (●, liquid samples; ▲, solid samples) and contribution of 10 PAHs to EROD activity. (A) PAHs levels. "Total PAHs" indicate the sum of 10 PAHs. (B) Levels of EROD activity of crude extracts. IEF values represent the mean of four different wells. N.D., not detected. (C) Ratio of calculated EROD activity to observed one that obtained from crude extracts. Abbreviations: HETP, human excreta treatment plant; STP, sewage treatment plant; Sed, sediment; N.D., not detected.

river sediments and water might indicate that higher hydrophobic PAHs and inducers were more easily adsorbed and accumulated in the sediments and stirred up to the river water. As chemical analysis, IEFs of ashes were in the same range as in the river sediments and 10 PAHs accounted for a higher percentage of IEFs than in the other samples. This data may indicate that these ashes contain less dioxin-like organic compounds except 10 PAHs. The bottom ashes were more contaminated both total PAHs and EROD activity than the fly ashes. This fact might depends on the temperature and oxygen supply of incineration process. Potent induction was observed for the extracts of the road dust. Many crude extracts showed higher activity than cleaned ones. The contribution of 10 PAHs to EROD activity varied considerably between samples and no correlation was found between IEFs and total PAHs. These findings indicate that in the road dust, synergistic interactions of PAHs and other dioxin-like compounds may make a major contribution to induce EROD activity. IEFs similar to those in strongly contaminated road dust were found in agricultural soil, forest soil and rice field soil. Furthermore their maximum levels of the dose-response curves were higher than any other environmental samples' and each PAH's. The contribution of 10 PAHs was lower than in the case of many road dust samples. These findings might indicate that EROD activities observed in the soil samples were induced not by PAHs but by pesticide, herbicide and their by-products. However there may be natural inducers in the humus soil, as significant activity was found in the extract of spring water.

In this study, we could rank various environmental samples with PAHs concentration and EROD activity. As a result, municipal incinerator ashes have low dioxin-like activity. Furthermore their activities can be almost accounted for 10 PAHs. On the other hand, road dust and, unexpectedly, soils induced potent EROD activity that could not be accounted for 10 PAHs. For these samples, further identification and toxicological characterization of dioxin-like compounds are needed.

References and Notes

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