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Characterization of aryl hydrocarbon receptor agonists in sediments of Wenyu River, Beijing, China

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ABSTRACT

Aryl hydrocarbon receptor agonistic (Ah-agonistic) effects of 23 sediments from Wenyu River in Beijing, China were evaluated using the H4IIE cell bioassay. Five samples were selected for chemical analysis of most concerned Ah-agonists, i.e. polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). All raw sediment extracts induced significant Ah-agonistic effects, and the bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents of raw extracts (TEQ_{raw}s) ranged from 8.5 to 336.0 pg/g dry weight (dw). Chemical analysis-derived TEQs (TEQ_{chem}s) ranged from 20.5 to 64.8 pg/g dw. When raw extracts were purified by sulphuric acid silica gel column to derive acid stable fraction, the TCDD equivalents in this fraction (TEQ_{stable}s) ranged from 2.7 to 63.8 pg/g dw. PCBs, PCDDs and PCDFs contributed about 51.4–72.1%, 1.2–7.3%, and 16.4–34.8% of TEQ_{stable}s, respectively, and the sum was 69.1–108.6%. Therefore, about 65.0% of TEQ_{raw}s could be attributed to acid labile fraction and only 3.3–9.6% to PAHs. These observations suggested that acid stable fraction and labile fraction contributed together to total Ah-agonistic effects in the sediments, and PCBs and PCDFs might be the two main components in acid stable Ah-agonists. The proposed approach using both H4IIE cell bioassay and chemical analysis could be used for characterization and prioritization of Ah-agonists in river sediments and helpful to the following progression of ecological remediation.

1. Introduction

Aryl hydrocarbon receptor agonists (Ah-agonists), which include structurally diverse synthetic and naturally-occurring chemicals, are ubiquitous in the environment (Denison et al., 2002). Ah-agonists share a common mechanism of action in binding to the Ah receptor as a first step, and toxicities are produced as a result of changes in gene expression mediated Ah receptor or interference in its related signaling pathways (Safe, 1990). In the past decades, the major concerns on Ah-agonistic effects were associated with dioxins and dioxin-like compounds which mainly referred to polyhalogenated aromatic hydrocarbons (PHAHs) including polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), naphthalenes (PCNs) and others (reviewed by Behnisch et al., 2001). These compounds have been shown to produce a number of toxic effects upon wildlife and human health due to both the persistent behavior and ability to bioaccumulate (Van den Berg

Abbreviations: PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; PCB, biphenyl; PAH, polycyclic aromatic hydrocarbons.

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et al., 1998; Tillitt et al., 1996). Moreover, other classes of non-halogenated Ah-agonists (e.g. PAHs, heterocyclic oxygen- and sulfur-containing compounds, humic substances) could coexist with the dioxin-like compounds (reviewed by Behnisch et al., 2001; Bittner et al., 2006; Brack and Schirmer, 2003).

Ah-agonists enter the aquatic environment from a number of potential sources via different routes, such as effluent discharges, atmospheric deposition and runoff from roads (US EPA, 1998; Blanchard et al., 2001; Kakimoto et al., 2006). Since Ah-agonists are generally hydrophobic and have a strong affinity to sediments, the sediments are the eventual sink for these compounds. Moreover, every Ah-agonist source could have its own distinctive composition feature of Ah-agonists. Thus, the profiles of Ah-agonists in sediments may vary widely with different local discharge scenarios. Therefore, it is of great importance to characterize Ah-agonists and conduct causality analysis using appropriate approaches in risk assessments of contaminated sediments.

To characterize complex mixtures of Ah-agonists in the environmental samples, toxic equivalency factor (TEF) conception was developed (Van den Berg et al., 1998). However, for a lot of Ah-agonists their TEFs are not available or their structures and characteristics are unknown. Therefore, chemical analysis provides only part of the knowledge about the biological effects of contaminants and does not take into consideration the total effects. A variety of bioassays have been developed to estimate the total biological activity of all Ah-agonists in complex environmental matrices (Giesy et al., 2002). The H4IIE cell bioassay (Tillitt and Giesy, 1991) applied in this study are based on Ah receptor-dependent ethoxyresorufin-*O*-deethylase (EROD) induction. This enzymatic assay is a sensitive biomarker that has been successfully applied in characterizing and assessing the Ah-agonistic effects for the sediment and soil (Qiao et al., 2006; Shen et al., 2008; Gale et al., 2000).

Wenyu River in Beijing is a typical water system in northern China with a flow of about 5 billion m^3 per year which was mainly used for receiving industrial and municipal discharges in the past years. The suspended particulates in this river can be sufficiently sedimentated due to low flow velocity (about 3.0 cm/s). It is imperative to assess individual classes of Ah-agonists and their contribution to overall hazards in sediments from this river for evaluating the risk and following the progression of a remediation strategy. In this study, we employed a combination of fractionation-based H4IIE cell bioassay and chemical analysis of most concerned and ubiquitous Ah-agonists, i.e., polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). The objective of this study is to assess the Ah-agonistic effects in the sediments and identify the specific compounds or classes of compounds which might be responsible for the Ah-agonistic effects.

2. Materials and methods

2.1. Sample collection and preparation

Surface sediments (<10 cm depth) from 23 sites of Wenyu River, Beijing, China (Fig. 1), were collected in October, 2006 by a stainless steel grab and scooped into aluminum jars that had been prerinsed with dichloromethane (DCM). The samples were immediately transferred to the laboratory and kept at −20 °C until further analyses.

Total organic carbon (TOC) of the freeze-dried and sieved (425-μm sieve) sediments after removing the inorganic carbon with 21% phosphoric acid was analyzed by TOC analyzer (Appollo 9000, Tekmar Dohrmann Co., USA). The sediment samples were extracted with 200 mL DCM/acetone (1/1, v/v) with activated copper for desulfurization in a Soxhlet apparatus for 48 h. A set of raw extract aliquots were preconcentrated on a rotary evaporator, solvent-exchanged to dimethyl sulfoxide (DMSO; Sigma–Aldrich Chemical Co., USA) under a gentle stream of nitrogen gas and stored at −20 °C for the H4IIE cell bioassay. Another set of aliquots were further cleaned by a multilayer silica gel column containing: 2 g of anhydrous sodium sulfate; 2 g of deactivated silica (3.3% organic-free reagent water, w/w); 12 g of acidic silica (44% sulphuric acid, w/w); 1 g of deactivated silica (3.3% organic-free reagent water, w/w). The silica gel column was pre-eluted with 80 mL of hexane prior to adding the extract. The fraction eluted with *n*-hexane (100 mL) and DCM/hexane (100 mL, 1:9 v/v) was the acid stable fraction intended for collection of the dioxin-like compounds, such as PCDDs, PCDFs, PCBs (US EPA, 1994). The eluates were concentrated and solvent-exchanged to DMSO for detecting the TCDD equivalents of acid stable fraction (TEQ_{stable}s).

2.2. H4IIE cell bioassay

H4IIE cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, Germany) supplemented with 10% (v/v) fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. The routine maintenance of cells was as described elsewhere (Qiao et al., 2006; Gale et al., 2000). Cells were seeded into the 96-well culture plates and stored at 37 °C with 5% CO_2 in an incubator (Sanyo, Japan), and the initial cell density was about 3000 cells per well. After 24 h, when a monolayer was formed consisting of 70–80% cells cover, the original culture medium was removed and replaced by a culture medium containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) standard solutions or the test solutions. The standard solutions were prepared by serial dilution of TCDD stock solution that could yield a full dose-response curve of EROD induction (TCDD: 0–140 pg/mL in culture). Six concentrations of the raw extracts were prepared by three fold dilution, and four concentrations for the cleanup fraction. In each well, the final concentration of DMSO was 0.5%. Meantime, a 0.5% DMSO solution was also used as the solvent control. Each test solution was assayed in triplicate, and three solvent controls (negative control) were assayed simultaneously on each 96-well plate. The edges of the 96-well culture plates were wrapped with parafilm and were incubated for 72 h at 37 °C. After incubation, EROD enzyme activity was analyzed following the methods of Qiao et al. (2006). Protein concentrations were determined as described by Bradford, 1976. Resorufin standard curve was analyzed at the same time to convert fluorescent units to picomoles of resorufin. The bioassay-derived TCDD equivalents were determined according to Tillitt et al. (1991) by comparing the induction of