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AhR-mediated potency of sediments and soils in estuarine and coastal areas of the Yellow Sea region: A comparison between Korea and China

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ABSTRACT

Extracts of sediments ($n = 45$) and soils ($n = 37$) collected from the coast of the Yellow Sea, in Korea and China, were screened for their ability to induce dioxin-like gene expression *in vitro* using the H4IIE-*luc*, transactivation bioassay. Significant dioxin-like potency was observed except for a few soils from Korea. Concentrations of TCDD-EQ (2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents) in sediments were comparable between Korea and China, but concentrations of TCDD-EQ in soil were 2-fold greater from Korea. Mass balance analysis indicated that concentrations of TCDD-EQ were to some degree chemical- and/or matrix-dependent, but were much more site-specific. For example, the proportion of the TCDD-EQ that could be identified varied among locations, which suggests different sources. Unidentified AhR-active compounds represented a greater proportion of the TCDD-EQ in samples from Korea, which suggests that sources in Korea were more complex than those in China. Potential sources of TCDD-EQ were investigated by considering land-uses and local activities.

1. Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor which can be activated by numerous chemicals that have structures similar to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Chemicals that have, or can attain, a planar configuration of approximately 3 × 10 å can bind to the AhR and result in expression of AhR-mediated responses (Giesy et al., 2002). These "dioxin-like" chemicals, among others, include polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) (Giesy and Kannan, 1998; Hilscherova et al., 2000; Palermo et al., 2005). It has been reported that the affinity with which chemicals bind to the AhR is directly proportional to the toxicity, including enhanced gene transcription and enzyme activities (Behnisch et al., 2001).

AhR agonists are of natural and human origin and include products and/or unwanted byproducts of combustion and due to their characteristics are widely distributed in environmental media and can be bioaccumulated and biomagnified (Tillitt et al., 1995; Van den Berg et al., 2006; Bittner et al., 2009). Risk assessments for dioxin-like compounds in sediments and soils are difficult due to the presence of complex mixtures of AhR agonists of differing persistence and accumulation and metabolic susceptibility (Giesy et al., 2002; Dévier et al., 2011).

There are several ways to characterize the overall potency of complex mixtures of AhR agonists of differential potency. The first is to measure all of the AhR agonists and then using relative potency factors to determine the overall potency of the mixture (Mousa et al., 1998). In this approach adopted by the World Health Organization (WHO) 2,3,7,8-TCDD equivalency factors are multiplied by the concentration of each AhR-active chemical in a mixture and reported as 2,3,7,8-TCDD equivalents (TEQ) (Van den Berg et al., 1998). Concentrations of TEQs in an extract are calculated as the sum of the product of the congener-specific toxic equivalency factor (TEF) and the concentration of the respective congener (Van den

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Berg et al., 1998; Villeneuve et al., 2000). Values for TEFs are consensus values to be used in risk assessments and are based on a range of endpoints. While TEFs correct for the relative potency of AhR agonists at the receptor, they do not correct for differential solubility, sorption, bioaccumulation, and biotransformation (Sanderson et al., 1996; Sanderson and Giesy, 1998). The TEFs used by the WHO were developed for use in risk assessments and are meant to be protective and not predictive (Van den Berg et al., 1998). The greatest limitation of the TEQ approach is the fact that all of the AhR agonists in a mixture need to be identified and quantified (Newsted et al., 1995). This can be highly challenging without comprehensive *a priori* knowledge concerning what AhR agonists might be present and/or without established analytical methods and standards for measuring them (Giesy et al., 2002). An additional limitation is the fact that TEFs have not been developed for many of the potential AhR agonists.

Because of the inherent limitations of the TEQ approach, a bioassay-based approach has been developed to measure the entire AhR-mediated potency of mixtures (Denison et al., 1993; Garrison et al., 1996). The H4IIE-*luc* transactivation bioassay measures the total potency of responses mediated by the AhR (Giesy et al., 1994a, 1994b). The H4IIE-*luc* assay is based on stably transfected cells in which the luciferase reporter gene has been inserted into the genome (El-Fouly et al., 1994). This transactivation assay uses the endogenous AhR of cells and the amount of light produced by the luciferase enzyme is directly proportional to binding of AhR agonists to the AhR. Results of the assay are expressed as 2,3,7,8-TCDD equivalents (TCDD-EQ). The emission of light, measured by use of a luminometer, is dependent on time, dose, persistence, and the potency of the AhR-active chemicals found in the sample/extract (Murk et al., 1996). The H4IIE-*luc* assay has been widely used as a screening tool for AhR-mediated potency of various complex mixtures in contaminated sediments, soils, and biological samples, such as human serum and plasma, and breast milk (Murk et al., 1996; Brown et al., 2000; Schroijen et al., 2006; Hasegawa et al., 2007; Hui et al., 2007; Kaisarevic et al., 2011). The results of the H4IIE-*luc* bioassay have been shown to be correlated with adverse outcomes caused by dioxin-like chemicals (Tillitt et al., 1991). Such a bioanalytical screening tool is a suitable and powerful alternative, because it is a relatively simple, rapid, integrative, and inexpensive, and reads out directly in total AhR-mediated potency expressed as concentrations of TCDD-EQ. Thus, the H4IIE-*luc* bioassay has been successfully applied in ecological risk assessment during the last few decades (Khim et al., 1999a; Hilscherova et al., 2001; Behnisch et al., 2003; Song et al., 2006).

The Yellow Sea, together with nearby coastal and riverine areas, is a major commercial artery of East Asia and has been significantly urbanized and industrialized (Luo et al., 2010; Naile et al., 2010). Rapid social and economic development in surrounding countries has brought economic development, but has also contributed to local contamination with persistent organic pollutants, metals and metalloids (Kim et al., 2007; Hu et al., 2010; Luo et al., 2010). Several major rivers including the Yellow, Liaohe, Haihe, Luanhe, and Dalinhe from China and Han, Geum, and Yeongsan from South Korea discharge directly into the Yellow Sea. The drainage areas of these rivers in both countries are used for both agricultural and chemical production, which can release inorganic and organic contaminants from both point- and nonpoint-sources. Because the Yellow Sea is a semi-enclosed system, water exchange with the Pacific Ocean is relatively slow; and as a result, such persistent pollutants tend to sediment and accumulate.

There have been studies of well-known AhR agonists such as PCDD/Fs, PCBs, and PAHs in sediments and soils of estuarine and riverine areas of Korea and China (Khim et al., 1999a; Lee et al., 2001; Naile et al., 2011). However, few studies have reported total concentrations of AhR-mediated potency by these compounds and/or other compounds, including unidentified natural and synthetic chemicals in sediments and soils from north coastal and riverine regions of the Yellow Sea. In particular, this information was not previously available for the Bohai Sea area of China. Therefore, baseline information on AhR-mediated potency of sediments and soils was needed to assess current environmental conditions and associated risk in aquatic and coastal environments of the region. In addition, in support of management decisions, it was necessary to know the nature of the compounds, including novel chemicals, that comprise the TCDD-EQ and if possible to identify their sources.

The present study was designated primarily for the purpose of investigating 1) the AhR-mediated activities of sediments and soils by use of H4IIE-*luc* bioassay, 2) the concentrations and sources of AhR-agonists using chemical analysis, and 3) the contributions of each AhR agonists to the total induced AhR-mediated potency (viz., mass balance analysis). Locations were chosen to detect possible point sources which were major freshwaters along the coasts of the West Sea of Korea and North Bohai Sea of China discharging directly into the Yellow Sea system. The results of this study will provide baseline information on AhR-mediated potency of sediments and soils in Yellow Sea regions and useful for regulation of chemicals of concern and predictive ecological risk assessment.

2. Materials and methods

2.1. Sampling

Sediments and soils were collected from 47 and 41 locations in Korea and China, respectively. Samples were collected from areas with different land uses, along the estuarine and coastal areas of the Yellow Sea during April and May, 2008 (Fig. 1 and Table S1 of Supplemental Materials). Surface sediments (0–10 cm) were collected from 12 sites in Korea and 35 sites in China. Soils were collected from 11 sites in Korea and 30 sites in China. Samples of individual soils consisted of approximately 15 cm of top soil from a central point and 15 cm of top soil from each of four additional points located 10–20 m in the four primary directions (N, E, S, and W) from the central point. Composite soil samples were then prepared after thoroughly mixing the sub-samples. All samples were immediately transferred to the laboratory and stored at −20 °C until analyses.

2.2. Sample preparation

Detailed descriptions of sample preparation have been published previously (Khim et al., 1999a; Koh et al., 2006). In brief, a 10 g sample of freeze-dried sediment or soil was extracted for 24 h by use of 400 mL of dichloromethane (DCM, Burdick and Jackson, Muskegon, MI, USA) in a Soxhlet extractor. Elemental sulfur was removed by reaction with activated copper (Merck, Darmstadt, Germany) and the extracts were concentrated to 1 mL. The extract was divided into two aliquots for use in the bioassay or identification and quantification of individual compounds. The portion of the extract to be used in the bioassay was transferred into dimethyl sulfoxide (DMSO, Burdick and Jackson) by use of differential volatilization.

2.3. In vitro bioassay

The H4IIE-*luc*, transactivation, reporter bioassay was performed by use of a slight modification of the methods of Khim et al. (1999b). Trypsinized cells from a culture plate were diluted to a concentration of approximately 8.0×10^4 cells mL^{-1} and seeded into the 60 interior wells of 96 well micro plates by adding 250 μL per well. After overnight incubation, test and control wells were dosed with 2.5 μL per well (1% dose) of the appropriate standards, sample extracts, or solvent controls. For sample dose–response characterization, extracts were prepared at six concentrations by 5-fold serial dilution (100, 20, 4.0, 0.8, 0.16, or 0.03%). All samples were tested in triplicate wells in the same assay. Emitted light was measured after 72 h of exposure by use of a ML3000 microplate reading luminometer (Dynatech Laboratories, Chantilly, U.S.). Cell viability and overall cytotoxicity of all samples were determined by use of the MTT assay as described in detail elsewhere (Yoo et al., 2006). No cytotoxic effects were observed in H4IIE-*luc* cells during exposure to sediment or soil extracts of any samples.

2.4. Determination of TCDD-EQ

Responses of the bioassay, expressed as mean relative luminescence units were converted to a percentage of the maximum response (%2,3,7,8-$TCDD_{max}$) for a standard containing 75 nM (=100 %$TCDD_{max}$) 2,3,7,8-TCDD (Wellington