APPLICATION OF A PANEL OF NUCLEAR RECEPTOR/REPORTER GENE BIOASSAYS TO MARINE HARBOR SEDIMENTS IN ASIA

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Abstract

The content of persistent organic pollutants (POPs) in sediment gives a significant indication of the general exposure to aquatic organisms and use of these compounds on land. In this study, eleven surface sediments were collected from commercial and industrial marine harbors in Asia (mainly in Japan). A panel of cell based CALUX reporter gene bioassays was utilized to test stable fractions from sediments for aryl hydrocarbon (AhR), androgen (AR), estrogen (ER), thyroid hormone (TR), glucocorticoid (GR) and progesterone (PR) receptor mediated agonistic/antagonistic activities. Chemical analysis was also conducted to clarify the POPs profile of the sediments. The results of the bioassays indicated that all the sediments have AR antagonism and some sediments have AR agonism, ER agonism, TR agonism, and PR agonism/antagonism. In terms of AhR agonistic activity for the tested samples, the bioassay TCDD-equivalents were in good agreements with toxicity equivalent concentrations (TEQ) calculated using WHO-TEF values. Our findings support further investigation of the real endocrine-disrupting (ED) effects by POPs and identification of responsible compounds.

Introduction

The content of persistent organic pollutants (POPs) in sediment gives a significant indication of the general exposure to aquatic organisms and use of these compounds on land. In this study, eleven surface sediments were collected from major commercial and industrial marine harbors in Asia (mainly in Japan). A panel of cell based CALUX reporter gene bioassays was utilized for the screening of endocrine-disrupting (ED) effects in stable fractions from sediments for aryl hydrocarbon (AhR), androgen (AR), estrogen (ER), thyroid hormone (TR), glucocorticoid (GR) and progesterone (PR) receptor mediated agonistic/antagonistic activities. In addition, chemical analysis was also conducted to clarify the POPs profile of the sediments and compare the analytical results with the bioassay results.

Materials and Methods

Sediment sampling. Surface sediment samples were taken at eight harbors (Tokyo, Ichihara, Yokohama, Nagoya, Osaka, Kure, Ube, and Sasebo) in Japan and three harbors in Asian countries (Hai Phong in Vietnam, Shihanoukville in Cambodia, and Subic in The Philippines) in 2004-2005.

Sample extraction and clean-up for bioassays. For the bioassays, dry sediment was soxhlet-extracted with toluene. The portion of the extract (corresponding to 5 g dry sediment) was used for the clean-up. Copper treatment was necessary to remove sulfur which was cytotoxic. The clean-up was conducted firstly by multilayer silica gel chromatography. The eluate (dichloromethane (DCM)/hexane (1:4)) was further processed by an activated carbon dispersed silica gel column. The elution was conducted with DCM/hexane (1:3) and toluene, respectively. The first and second fractions were carefully evaporated by nitrogen stream and replaced with 50 μL of dimethylsulfoxide (DMSO).

Bioassays. Stable sediment extracts were tested for AhR agonists by DR CALUX[1] and for each of AR, ERα, TRβ, GR, and PR agonists/antagonists by AR, ERα, TRβ, GR, and PR CALUX bioassays using the recombinant U2OS human cell line (the CALUX bioassays are developed by BioDetection Systems b.v., The Netherlands)[2]. The CALUX bioassays were performed in duplicate or triplicate as described previously[1,2]. For agonistic activity

testing, the amount of resulting luciferase activity was measured and compared with a dose-response curve of the reference compound (TCDD, estradiol, triiodothyronine, dihydrotestosterone, dexamethasone, or Org 2058 for the AhR, AR, ERα, TRβ, GR, and PR CALUX, respectively) for quantification of the response. For antagonistic activity testing, the exposure medium was supplemented with the reference compound at EC50 level. The decrease in response was interpolated in an dose-response curve by an antagonist reference compound (flutamide, tamoxifen, and RU486 for AR, ERα, and GR/PR CALUX, respectively). Cytotoxic effect by samples was checked by coexposure to 1,000×EC50 level of reference agonist and samples. No cytotoxic decrease in response was observed for the tested samples.

Chemical analysis. The POPs in the identical soxhlet-extracts were cleaned-up using column chromatography and determined in terms of polychlorinated dibenzo-*p*-dioxins/furans (PCDD/DFs), polybrominated dibenzo-*p*-dioxins/furans (PBDD/DFs), monobrominated/polychlorinated dibenzo-*p*-dioxins/furans (MoBPCDD/DFs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs: polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA), and hexabromocyclododecanes (HBCDs)), chlorobenzens (CBzs), and chlorophenols (CPhs) by GC/HRMS and polyaromatic hydrocarbons (PAHs: USEPA 16 priority pollutants) by GC/LRMS.

Results and Discussion

Chemical profile of sediment samples. The analytical results of sediment samples are summarized in Table 1. The chemical profile of the POPs is clearly different depending on the locations. For example, highest concentration of PCDD/DFs (WHO-TEQ: 2,400 pg/g) was detected from Ichihara. Osaka sediment showed highest concentrations of PCBs (8,300 ng/g) and PBDEs (790 ng/g). Elevated PAH concentration (280 μg/g) was detected from Subic sediment, which was oily in appearance.

Toxicological profile of sediment samples. *In vitro* ED potencies of the stable sediment extracts (i.e., fractions 1 and 2) were classified into four categories as shown in Fig. 1. Classification was based on limit-of-quantification (LOQ), EC/IC20 and EC/IC50 values of reference compounds. The assay results for all the sediment extracts can be compared in a common scale within each CALUX endpoint, because the original sample amount (5 g) and final DMSO volume of the assay fractions (50 μL) are the same among sediments.

Anti-androgenic activity was detected in fraction 1 of all sediment samples, which is the most striking finding of this study. The highest level of AR antagonism was detected in sediments in Osaka and Subic. In the same fraction 1 of the two samples, AR agonism, ERα agonism, TRβ agonism, and PR agonism/antagonism were also accompanied.

On the other hand, significant AhR agonism (i.e., dioxin-like activity) was detected in fraction 2 of all the samples. Fraction 2 collected planar stable AhR ligands such as PCDD/DFs and dioxin-like PCBs. The DR CALUX results (CALUX-TCDD-EQ) are in good agreement with WHO-TEQ values (Fig. 2). The slope factor of the observed linear correlation approaches to one, which suggests PCDD/DFs and dioxin-like PCBs are major responsible compounds for the DR CALUX results.

In the present study, our primary target compounds are stable ones which pass through multilayer silica gel chromatography. Four steroid hormone receptor bioassays applied showed significant or quantifiable responses for the stable fractions from sediments, especially for fraction 1 collecting slightly polar compounds, which are different from AhR-active planar compounds such as PCDD/DFs. It remains unknown whether identical (group of) compounds exhibit multiple hormonal activities. Our findings support further investigation of the real ED effects by the fractionated stable compounds and identification of responsible compounds in sediments. It has been reported that a group of BFRs has ED potency to induce AR antagonism and PR antagonism[3], which are similarly observed in sediments dealt in this study. BFRs may be causative compounds and further investigation is necessary to evaluate their contribution to the total activity.

Table 1 Organohalogen compounds determined in sediment samples.

Compounds	Unit	Tokyo	Ichihara	Yokohama	Nagoya	Osaka	Kure	Ube	Sasebo	Hai Phong (Vietnam)	Shihanoukville (Cambodia)	Subic (The Philippines)
PCDD/DFs	pg-TEQ/g	23	2,400	9.9	6.0	110	8.2	12	23	0.066	10	35
DL-PCBs	pg-TEQ/g	3.9	0.10	2.2	0.67	120	0.43	0.80	12	0.021	0.13	3.6
Total TEQ	pg-TEQ/g	26	**2,400**	12	6.7	**230**	8.6	13	36	0.087	10	39
PBDD/DFs	pg/g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MoBPCDD/DFs	pg/g	ND	3,800	ND	ND	13	ND	18	ND	ND	ND	ND
PCBs	ng/g	120	52	54	21	**8,300**	12	25	460	5.1	6.3	59
PBDEs	ng/g	17	6.2	51	120	**790**	1.9	27	41	0.52	0.32	4.1
TBBPA	ng/g	0.14	2.8	9.4	0.36	27	0.09	5.8	0.31	<0.01	<0.01	0.035
HBCDs	ng/g	0.38	0.58	0.047	1.7	4.9	0.19	0.10	1.1	<0.05	0.066	0.62
CBzs	ng/g	84	25	34	20	290	5.3	12	110	2.2	4.4	3.5
CPhs	ng/g	1.5	82	2.3	2.1	6.9	1.1	1.3	2.3	1.4	1.4	7.7
PAHs	ng/g	4,000	1,100	2,200	4,100	24,000	14,000	2,000	5,500	23,000	28,000	**280,000**

Fig. 1 CALUX panel response for stable fractions from sediment samples.

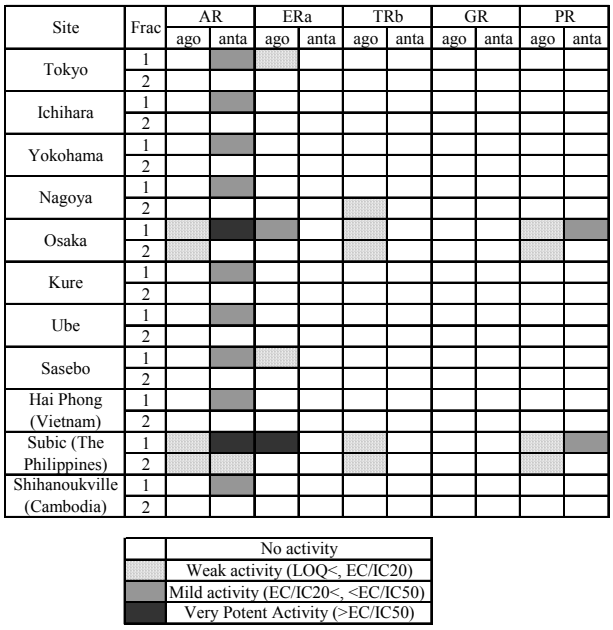
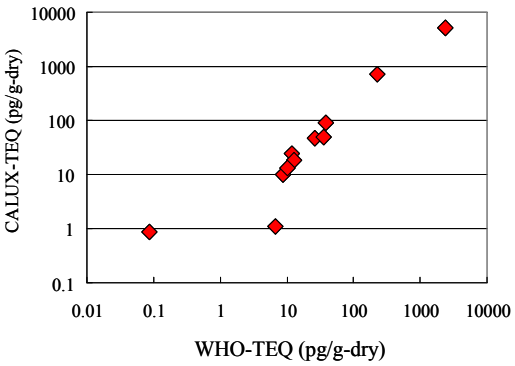


Fig. 2 Relationship between WHO-TEQ (pg/g, based on WHO-TEF (1998)) and CALUX-TCDD-EQ (pg/g, based on DR-CALUX results for fraction 2)



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