Chemosphere 107 (2014) 257–264

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Occurrence of aryl hydrocarbon receptor agonists and genotoxic compounds in the river systems in Southern Taiwan

Pei-Hsin Chou [a,*], Tong-Cun Liu [a], Fung-Chi Ko [b,c], Mong-Wei Liao [a], Hsiao-Mei Yeh [a], Tse-Han Yang [a], Chun-Ting Wu [a], Chien-Hsun Chen [a], Tsung-Ya Tsai [a]

[a] Department of Environmental Engineering, National Cheng Kung University, Tainan, Taiwan
[b] National Museum of Marine Biology and Aquarium, Pingtung, Taiwan
[c] Institute of Marine Biodiversity and Evolutionary Biology, National Dong Hwa University, Pingtung, Taiwan

HIGHLIGHTS

- AhR agonist contaminants were frequently detected in rivers in Southern Taiwan.
- Genotoxicity was often found in dry-season samples collected from Erren River.
- AhR agonist activity and genotoxicity were caused by different contaminants.
- PAHs were minor contributors to the AhR agonist activity elicited by sediment.
- Bioassay analysis is useful in providing combined toxicity in environmental samples.

ARTICLE INFO

Article history:
Received 28 August 2013
Received in revised form 2 December 2013
Accepted 18 December 2013
Available online 8 January 2014

Keywords:
Aryl hydrocarbon receptor agonists
Genotoxicants
Bioassays
Sediment
Polycyclic aromatic hydrocarbons

ABSTRACT

Water and sediment samples from river systems located in Southern Taiwan were investigated for the presence of aryl hydrocarbon receptor (AhR) agonists and genotoxicants by a combination of recombinant cell assays and gas chromatography–mass spectrometry analysis. AhR agonist activity and genotoxic response were frequently detected in samples collected during different seasons. In particular, dry-season water and sediment samples from Erren River showed strong AhR agonist activity (201–1423 ng L^{-1} and 1374–5631 ng g^{-1} β-naphthoflavone equivalents) and high genotoxic potential. Although no significant correlation was found between AhR agonist activity and genotoxicity, potential genotoxicants in sample extracts were suggested to be causative agents for yeast growth inhibition in the AhR-responsive reporter gene assay. After high performance liquid chromatography fractionation, AhR agonist candidates were detected in several fractions of Erren River water and sediment extracts, while possible genotoxicants were only found in water extracts. In addition, polycyclic aromatic hydrocarbons, the typical contaminants showing high AhR binding affinity, were only minor contributors to the AhR agonist activity detected in Erren River sediment extracts. Our findings displayed the usefulness of bioassays in evaluating the extent of environmental contamination, which may be helpful in reducing the chances of false-negative results obtained from chemical analysis of conventional contaminants. Further research will be undertaken to identify major candidates for xenobiotic AhR agonists and genotoxicants to better protect the aquatic environments in Taiwan.

1. Introduction

A variety of anthropogenic pollutants are frequently discharged into the aquatic environment via human activities. Among them, hydrophobic organic contaminants receive much attention owing to their ubiquitous distribution and many adverse effects, such as persistence, bioaccumulation, carcinogenicity, or endocrine disruption (Muir and Howard, 2006; Bull et al., 2011; Söffker and Tyler, 2012). Though their environmentally relevant concentrations may be low, these contaminants pose problems because they may elicit additive or synergistic toxicity. Thus, it is important to assess the combined toxicity of multiple contaminants and to identify major toxicants in environmental samples to protect human health and the environment.

In recent years, *in vivo* animal testing and *in vitro* recombinant cell bioassays have been extensively used to evaluate the toxicity of various environmental samples. Invertebrates, fish, and bioluminescent bacteria *Vibrio fischeri* have been the major species used in aquatic toxicity tests since late 1980s (Brack, 2003; Eom et al.,

* Corresponding author. Tel.: +886 6 2757575x65840.
E-mail address: phchou@mail.ncku.edu.tw (P.-H. Chou).

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http://dx.doi.org/10.1016/j.chemosphere.2013.12.052

2007; Ocampo-Duque et al., 2008). Mutagenic, genotoxic, and endocrine disrupting activities of river and sediment samples have also been successfully detected by a variety of bioassays using engineered cells or microorganisms (Nukaya et al., 1997; Ono et al., 2000; Kawanishi et al., 2004; Macova et al., 2011). Compared to *in vivo* tests, the time- and cost-efficiency of *in vitro* assays make them convenient tools for preliminary screening of potential polluted sites. In addition, using *in vitro* assays in combination with chemical fractionation has been suggested to be a promising technique for isolating target toxicant candidates in environmental samples (Lukasewycz and Durhan, 1992; Reemtsma, 2001; Hewitt and Marvin, 2005; Chou et al., 2007).

In the present study, we aimed to investigate the occurrence and the combined toxicity of organic contaminants showing aryl hydrocarbon receptor (AhR) agonist activity or genotoxicity in several river systems located in Southern Taiwan. AhR is a ligand-activated transcription factor that mediates the toxic effects of numerous organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs) or polychlorinated dibenzo-*p*-dioxins (Denison and Heath-Pagliuso, 1998; Denison and Nagy, 2003; Bock and Köhle, 2006). AhR binding affinity of environmental pollutants may be considered as an indication of potential toxicity since many organic contaminants induce a broad spectrum of toxic responses via AhR activation. Genotoxic compounds are potential DNA-reactive agents that may damage DNA integrity. Several PAHs and their metabolites have been considered as possible genotoxicants, and one of the most famous examples is benzo[*a*]pyrene-7,8-diol-9,10 epoxide, a highly reactive electrophilic metabolite of benzo[*a*]pyrene that binds covalently to nucleic acids and forms DNA adducts (Bartsch, 1996; Alexandrov et al., 2010).

During the past decade, various organic contaminants have been detected in the aquatic environments in Taiwan, such as PAHs, halogenated compounds, and pharmaceuticals, while the combined toxicity remains unknown (Hung et al., 2006; Peng et al., 2007; Doong et al., 2008; Huang et al., 2008; Lin and Tsai, 2009). To examine the toxic effects elicited by various xenobiotic AhR agonists or genotoxic compounds, we collected water and sediment samples from three river systems in Southern Taiwan during different seasons, and analyzed the AhR agonist activity and genotoxicity of these samples using *in vitro* bioassays. In addition, samples were fractionated using high performance liquid chromatography (HPLC) to isolate active factions, and PAH concentrations were analyzed by gas chromatography–mass spectrometry (GC–MS) to evaluate their contribution to the combined toxicity. Our results revealed that AhR agonists and genotoxicants were frequently detected in the river systems in Southern Taiwan. However, PAH concentrations were unable to reflect the combined toxicity of sample extracts. Further monitoring and water quality improvement is necessary to protect the aquatic environments in Taiwan.

2. Materials and methods

2.1. Sample collection

River water, suspended solids (SS), and sediment (Sed) grab samples were taken at three river systems located in Southern Taiwan, including Yanshuei River (5 sites, YS1-YS5, sediment sample of YS4 was not available), Erren River (7 sites, ER1-ER7), and Agondian River (3 sites, AGD1-AGD3) (Fig. 1). The area is populated with around 1 million residents, and major pollution sources include domestic wastewater, swine wastewater, and industrial wastewater from electroplating factories, metal finishing industries, and etc. (Table S1, Supplementary material). Flow rates of the three river systems varied greatly during dry- and wet seasons (6.5–8.5-fold higher in wet seasons) owing to the difference in seasonal precipitation. To compare the AhR agonist activity and genotoxicity, dry- and wet-season samples were collected in December 2010 (dry season, ER), May 2011 (wet season, YS), July 2011 (wet season, AGD, ER), and December 2011 (dry season, YS, AGD). Sediment samples were also taken at ER River in September 2010 (wet season) and February 2011 (dry season) for PAH analysis.

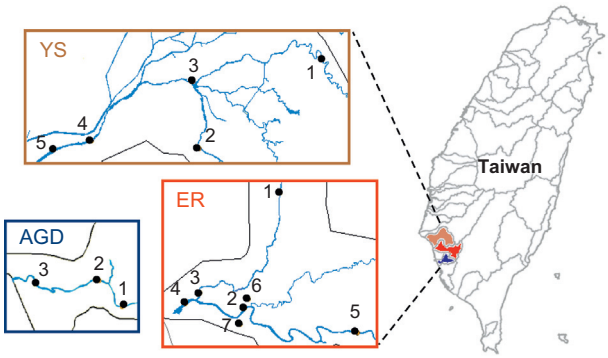

Fig. 1. Sampling locations in YS River, ER River, and AGD River.

2.2. Water and SS sample extraction

Water grab samples were filtered and extracted within 24 h after collection. Hydrophobic organic compounds were extracted from 1 L of each river water sample by solid phase extraction using Sep-Pak® Plus Environmental C18 Cartridge (Waters, USA) following filtration through 0.60 μm pre-weighed glass fiber filters (Advantec, Japan). Each cartridge was washed with water and eluted with 3 mL of methanol and 1 mL of dimethyl sulfoxide (DMSO) after extraction. The eluents were evaporated to dryness in a centrifugal vacuum concentrator (EYELA, Japan), and the extracted compounds were redissolved in 1 mL of DMSO to obtain a 1000-fold concentrated extract (concentration factor = 1000).

Filters containing SS of each river water sample were dried in a 105 °C oven before extraction. Hydrophobic organic compounds in each SS sample were extracted with 30 mL of anhydrous sodium sulfate-added hexane:acetone (1:1, v:v) solution by ultrasonic extraction for 60 min, and were further extracted by 15-min ultrasonic extraction for another three times with 5 mL of hexane:acetone (1:1, v:v) solution, 5 mL of hexane, and 5 mL of hexane. Supernatants of each extraction were combined, evaporated to dryness, and redissolved in 1 mL of DMSO to obtain a 1000-fold concentrated extract. The 1000-fold concentrated extract was further diluted using DMSO to obtain a dilution series for bioassay analysis.

2.3. Sediment extraction

Sediment grab samples were obtained with a stainless-steel Ekman dredge (Wildlife Supply Company, USA). After collection, 1 g of each sediment sample was dried in a hood for 48–72 h, and was ground and passed through a 20 mesh sieve. Hydrophobic organic compounds in each air-dried sediment sample were extracted with 10 mL of anhydrous sodium sulfate-added hexane:acetone (1:1, v:v) solution by shaking extraction at 200 rpm for 24 h. Supernatants were collected after centrifugation, evaporated to dryness, and redissolved in 1 mL of DMSO to obtain a concentrated sample extract (1000 mg Sed equivalent mL^{-1} DMSO). The concentrated extract was also diluted using DMSO to obtain a dilution series for bioassay analysis.

Sediment samples collected at ER River were also extracted using Soxhlet apparatus for GC–MS analysis. One gram of each