Chemosphere 79 (2010) 1–7

Contents lists available at ScienceDirect

Chemosphere

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

Exploiting lipid-free tubing passive samplers and embryonic zebrafish to link site specific contaminant mixtures to biological responses

Wendy E. Hillwalker, Sarah E. Allan, Robert L. Tanguay, Kim A. Anderson *

Environmental and Molecular Toxicology Department, Oregon State University, ALS 1007, Corvallis, OR 97331, USA

ARTICLE INFO

Article history:
Received 10 September 2009
Received in revised form 28 January 2010
Accepted 1 February 2010
Available online 20 February 2010

Keywords:
Passive sampling device
Zebrafish
Bioavailable
Mixture toxicity
Superfund

ABSTRACT

The Biological Response Indicator Devices Gauging Environmental Stressors (BRIDGES) bio-analytical tool was developed in response to the need for a quantitative technology for assessing the toxicity of environmentally relevant contaminant mixtures. This tool combines passive samplers with the embryonic zebrafish model. When applied in an urban river it effectively linked site specific, bioavailable contaminant mixtures to multiple biological responses. Embryonic zebrafish exposed to extracts from lipid-free passive samplers that were deployed at five locations, within and outside of the Portland Harbor Superfund Megasite, displayed different responses. Six of the eighteen biological responses observed in 941 exposed zebrafish were significantly different between sites. This demonstrates the sensitivity of the bio-analytical tool for detecting spatially distinct toxicity in aquatic systems; bridging environmental exposure to biological response.

1. Introduction

Human and ecosystem exposure to contaminants generally involves complex mixtures of chemicals. Determining the concentrations of a wide range of chemicals in an environmental matrix is limited to the detection of known compounds and may often exclude toxicologically relevant chemicals. Toxicological studies tend to focus on the effects of exposure to a pure chemical or specific class of chemicals. Mixture toxicity is not well understood but recent studies demonstrate non-additive toxic effects elicited by chemical mixtures (Incardona et al., 2004; Wassenberg and Di Giulio, 2004; Boobis et al., 2008; Duan et al., 2008). Present day risk assessment models are inadequate for predicting toxic effects of complex chemical mixtures because they do not take into account interactions between components that cause synergistic, potentiating or inhibiting effects (Dardenne et al., 2008).

There is a need for environmental assessment methods that address the issue of determining the toxicity of environmentally relevant complex mixtures (Eggen et al., 2004; Collins et al., 2008). In response to this need Biological Response Indicator Devices Gauging Environmental Stressors (BRIDGES) was developed to bridge the gap between real-life exposure scenarios and toxicity. We demonstrate the feasibility of conjoining two established technologies, passive sampling devices and the embryonic zebrafish model, to create a rapid throughput bio-analytical tool that assesses multiple biological responses to environmentally relevant contaminant mixtures in a whole organism vertebrate model.

Passive sampling devices (PSDs) are used extensively for the assessment of contamination in air, water and soil (Mayer et al., 2003). They sequester and concentrate the freely dissolved portion of a variety of hydrophobic organic contaminants (Adams et al., 2007). PSDs mimic bioconcentration mechanisms, such as diffusion through biomembranes and partitioning between an organism and it's medium (Huckins et al., 2006). They are thought to be adequate biological surrogates for the uptake of many organic contaminants and do not present the disadvantages inherent in using organisms for environmental monitoring, such as motility, growth and metabolism (Awata et al., 1999; Wells and Lanno, 2001; Zhang et al., 2006). PSDs provide a time integrated concentration of the freely dissolved, bioavailable, fraction of a wide range of analytes (Huckins et al., 2006). Lipid-free tubing (LFT) is a polyethylene membrane with demonstrated capacity for sequestering organic contaminants from waters. Unlike other PSDs, such as the semipermeable membrane devices (SPMDs), LFTs do not contain triolein or other lipids, which facilitates clean-up, analysis and modeling of results (Anderson et al., 2008).

Bioassays are experiments designed to evaluate the ability of contaminants to cause certain biological responses, their potency in doing so, and the nature of the dose–response relationship (Hill et al., 2005). The embryonic zebrafish has been identified as an ideal organism for *in vivo*, full organism bioassays (Hill et al., 2005; Usenko et al., 2007; Renner, 2008) and is widely used by researchers in a variety of fields. Zebrafish have many advantages over other vertebrate bioassay models with respect to their size,

* Corresponding author. Tel.: +1 541 737 8501; fax: +1 541 737 0497.
E-mail address: kim.anderson@oregonstate.edu (K.A. Anderson).

0045-6535/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2010.02.001

husbandry and early morphology. The small size of the fish reduces housing costs and allows for larger sample sizes. Zebrafish are very fecund, producing up to 200 eggs per adult every 5–7 d. Furthermore, the embryos are nearly transparent, allowing for clear non-invasive visualization of internal organs (Hill et al., 2005). A number of molecular tools are also in place to permit integrative studies of the mechanisms of action underlying observed non-specific biological responses (Vogel, 2000).

There is a recognized need to connect effective and efficient environmental sampling directly to toxicity evaluations and risk assessment (Eggen et al., 2004; Collins et al., 2008). Research to chemically characterize the Portland Harbor Superfund Megasite has been ongoing for many years (Sethajintanin et al., 2004; Sower and Anderson, 2008; Integral et al., 2009). This study does not seek to present additional chemical data but rather to demonstrate the potential advantage of utilizing a complementary bioassay tool in combination with a fit-for-purpose sampling methodology for environmental and risk assessment. A limited number of publications address the possibility of using environmental samples obtained from PSDs in toxicity bioassays (Parrott and Tillitt, 1997; Parrott et al., 1999; Sabaliunas et al., 2000; Heinis et al., 2004; Petty et al., 2004; Ma et al., 2005; Ke et al., 2007; Springman et al., 2008). However, the majority of these studies use *in vitro* assays or assess only a single biological effect. This present study is the first report of coupling passive sampler technology with the assessment of multiple developmental biological responses in a whole organism vertebrate model. The toxicity of environmentally relevant chemical mixtures was assessed using the embryonic zebrafish model and LFT passive samplers deployed in a model river system. Furthermore, we evaluate differences in the biological responses observed in the zebrafish model related to the spatial deployment of LFT in the river system; Superfund versus upriver or downriver sites, in an extract concentration-dependent manner.

2. Materials and methods

2.1. PSD deployment and processing

2.1.1. Study area

Like many urban rivers, the lower Willamette River, Portland, OR, has been the site of heavy industrial use. The area between river miles (RM) 3.5 and 9.2 was designated a Superfund Megasite in 2000 due to contamination with a number of urban and industrial contaminants including metals, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins and organochlorine pesticides (USEPA, 2000). Remediation within the Superfund Megasite is ongoing. A sediment cap was placed over 23 acres of creosote contaminated sediment at the McCormick and Baxter Superfund site at RM 7 east (E) in 2004. Over 11 500 m^3 of coal tar was removed from RM 6.3 west (W), the GASCO site within the Portland Harbor Megasite in 2005 (Sower and Anderson, 2008). The Willamette River is populated by resident and migratory fish populations and extensively used by sport and subsistence anglers and recreational boaters (Sethajintanin et al., 2004; Sower and Anderson, 2008). The Portland Harbor Superfund Megasite is a representative river system to investigate the availability and developmental health consequences of urban and industrial compounds to aquatic organisms and, ultimately, to humans.

The study area consists of five locations; upstream (RM 17E), within (RMs 3.5E, 7W, 7E) and downstream (RM 1E) of the Portland Harbor Superfund Megasite (Fig. 1). The site locations were selected to coincide with past studies that quantify freely dissolved fractions of PAHs (Anderson et al., 2008; Sower and Anderson, 2008), PCBs and organochlorine pesticides (Anderson et al., 2008) in the surface water using passive sampling devices.

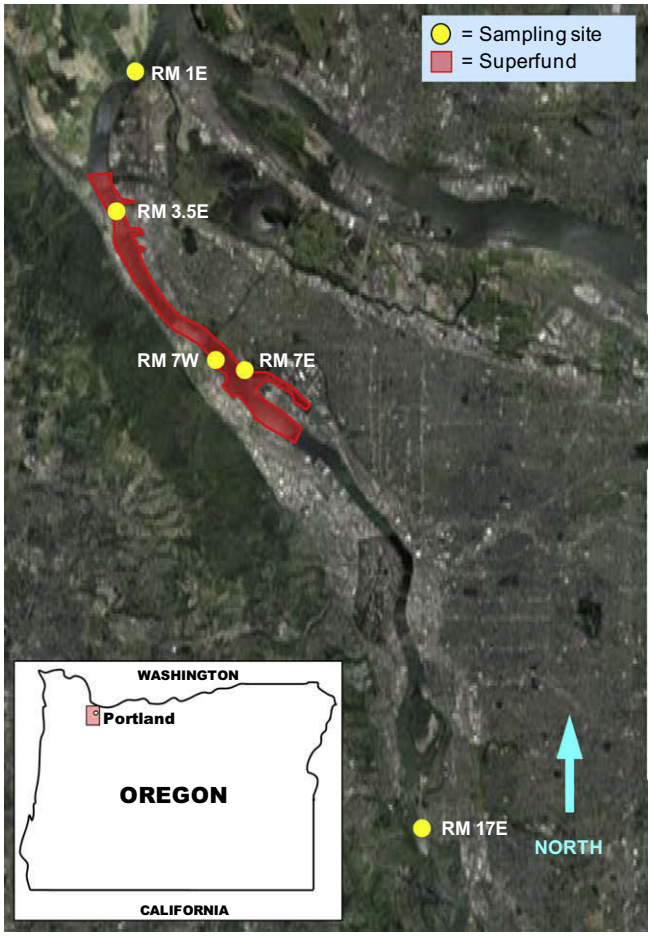

Fig. 1. The lower Willamette River, OR (north flowing). LFT passive samplers were deployed in the water column, 10 ft above the substrate, at the sites indicated by the yellow circles. The Portland Harbor Superfund Megasite is outlined in red. The McCormick and Baxter Superfund site is located on the east bank at river mile 7 (RM 7E).

2.1.2. Sample collection

The PSDs deployed in the lower Willamette River were lipid-free tubing (LFT). Details about LFT preparation, deployment and extraction can be found in Anderson et al. (2008). Briefly, additive-free low-density polyethylene membrane (lay-flat tubing) was cleaned with optima grade hexanes then heat sealed at both ends (final dimensions 2.7 × 100 cm). Unspiked LFT (not containing performance reference compounds) were deployed at 5 sites in the lower Willamette River for 21 d in May, 2006. Five LFT were co-deployed in a single stainless steel cage at each sampling site. Following exposure, LFTs were transported to the lab in coolers, extracted into hexanes and split. One part of the split LFT extract was solvent exchanged to dimethyl sulfoxide (DMSO) for the embryonic zebrafish exposures, while the other was kept in hexanes for chemical analysis.

2.2. Zebrafish rearing and preparation

Embryos were collected from the Tropical 5D strain of zebrafish (*Danio rerio*) reared in the Sinnhuber Aquatic Research Laboratory (SARL) at Oregon State University. Adults were kept at standard laboratory conditions of 28 °C on a 14 h light/10 h dark photoperiod. Fish water (FW) consisted of reverse osmosis water supplemented with a commercially available salt solution (0.6% Instant Ocean©). Zebrafish were group spawned and embryos were collected and staged as described by Kimmel et al. (1995).