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Semipermeable membrane devices link site-specific contaminants to effects: Part 1 – Induction of CYP1A in rainbow trout from contaminants in Prince William Sound, Alaska

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ABSTRACT

Extracts from semi-permeable membrane devices (SPMDs) deployed on beaches in Prince William Sound (PWS), Alaska, were used to evaluate if complex contaminant mixtures from different sources can be distinguished by the resulting cytochrome P450 1A (CYP1A) activity in exposed test animals. Deployment sites included canneries, salmon hatcheries, and beaches where lingering oil remains from discharges during the 1964 earthquake or the 1989 *Exxon Valdez* oil spill. Other sites were selected at random to evaluate region-wide contaminant inputs or were located in salmon streams to evaluate contaminants carried and released by migrating salmon carcasses following reproduction. Following standard deployments of approximately 28 d, an aliquot of the accumulated contaminants was intraperitoneally injected without cleanup into juvenile rainbow trout (*Oncorhynchus mykiss*). After 2 d and 7 d, the activity of CYP1A was measured by the ethoxyresorufin-*o*-deethylase (EROD) assay. Exposure to extracts from the oiled sites and one hatchery site with numerous creosote pilings elicited strong EROD responses, whereas fish exposed to salmon stream extracts elicited weak but significant responses during late summer compared to late spring. Responses from the other sites were not significant, indicating contaminants from these sources are unlikely to cause CYP1A induction in resident biota. Rather than simply assessing extant contaminants, this method evaluates the potency of the different sites for bringing about aryl hydrocarbon receptor responses in resident biota.

1. Introduction

Biochemical responses can be sensitive indicators of pollution exposure. Such responses, termed biomarkers, are indicators in biological fluids, cells or tissues signaling modifications in the biological system due to contaminants (NRC, 1987). Biomarker responses have been extensively investigated and provide a convenient and toxicologically relevant means of detecting exposure to low concentrations of contaminants (Dickerson et al., 1994). While biomarkers may indicate exposure to specific classes of contaminants, these responses may provide little information regarding the contaminant source, beyond what may be inferred from the geographic and temporal patterns of biomarker expression (Short and Springman, 2006). Even the location of contaminant sources may be ambiguous when biomarkers are assessed in mobile biota. Moreover, biomarkers usually do not account for chemical speciation or availability (Bucheli and Fent, 1995).

Biomarker expression can be linked more directly to contaminant sources via an intermediary contaminant sampler such as semipermeable membrane devices (SPMDs). These passive samplers may be deployed to concentrate lipophilic organic contaminants from acknowledged or probable sources. At equilibrium with the exposure medium, SPMDs accumulate contaminants according to their octanol–water partition coefficient (K_{ow}) values (Huckins et al., 2006). The contaminants retained in the SPMD are capable of membrane passage, and as such are potentially a greater risk to biota. Once retrieved by dialysis, these contaminants may be injected into test organisms such as fish, which can then be examined for responses. This relates the response expression to bioavailable contaminants present at the deployment site. Previous studies have linked the activity of cytochrome P450 1A (CYP1A) in fish liver cells (PLHC-1) to inducers absorbed by environmentally exposed SPMDs (Parrott et al., 1999; Villeneuve et al., 1997). Sundberg et al. (2005) injected trout eggs with fractionated SPMD

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extracts to evaluate the induction potency of different contaminant classes found in a region where a single point source was dominant. In all these studies, the contaminants recovered from the SPMDs were purified by silica gel chromatography or other means to facilitate chemical analysis prior to their use for the biological assays. Because purification may remove contaminants that affect the selected biomarker, none of these methods assess the induction potential of the environment *per se*, but only the target contaminants of the study. Our aim in this work is to extend these earlier methods to evaluate the CYP1A-induction potential of *all* non-polar, lipophilic contaminants absorbed by SPMDs deployed in the environment under study by injecting recovered contaminants into juvenile rainbow trout without clean-up.

In situations where multiple contaminant sources are plausible, the biomarker response of injected contaminants extracted from SPMDs provides a method of evaluating the relative potency of different sources. This approach quantifies the biochemical effect triggered by the mixture of non-polar compounds as a group, and does not require identification of the particular contaminants causing induction. This may be a considerable advantage in cases where a positive biomarker response is evident in resident biota, multiple potential sources are present, and the identity of the contaminants that are driving the biomarker response is not apparent.

Prince William Sound (PWS), Alaska provides an excellent setting to test the utility of this method. It is a sparsely populated region where relatively few candidate sources are present, one of which is oil from the 1989 *Exxon Valdez* oil spill that is still found on beaches in western PWS (Short et al., 2004, 2007). This oil is a CYP1A inducer (Woodin et al., 1997). Induction of CYP1A has been reported in resident sea otters (*Enhydra lutris*) and harlequin ducks (*Histrionicus histrionicus*) that frequent beaches where oil persists (Short et al., 2006; Bodkin et al., 2002; Trust et al., 2000). However, confounding potential sources of CYP1A inducers are possible. These include: (1) asphalt and heavy bunker fuels released from storage tanks damaged by the 1964 Alaska earthquake (Kvenvolden et al., 1995); (2) fuel spills from commercial fishing, marinas, salmon hatchery docks and other marine traffic (Page et al., 1999); (3) broad-scale background pollutants from atmospheric deposition of persistent organic pollutants (Stern et al., 1997); (4) polycyclic aromatic hydrocarbons (PAH) associated with oil seeps and erosion of hydrocarbon-rich source rocks and coal outcrops (Short et al., 2004; Short et al., 1999; Page et al., 1996); (5) PAH from forest fires (Page et al., 1999); (6) contaminants from marine vessels (Page et al., 1996); and (7) persistent organic pollutants associated with spawning migrations of adult salmon returning to their natal streams (Krümmel et al., 2003; Ewald et al., 1998). Concerns regarding these potential sources of CYP1A inducers in the region continue to be raised (Boehm et al., 2007; Huggett et al., 2006; Neff et al., 2006; Lee and Anderson, 2005; Page et al., 2004; Huggett et al., 2003; Jewett et al., 2002). Injecting fish with extracts from SPMDs and measuring their CYP1A activity provides a way of identifying which sources are capable of stimulating a response. Those that do not, can be eliminated as causes of the CYP1A induction observed in wildlife in that region.

Here, we extended the approach used by Sundberg et al. (2005) to evaluate which pollution sources in PWS were capable of stimulating CYP1A induction. In fish, the activity of this enzyme, as determined with the ethoxyresorufin-*o*-deethylase (EROD) assay, is an established biomarker of exposure to PAH and structurally related compounds. The activity of this enzyme is particularly suited to monitor discharges of petroleum or other PAH sources, co-planar polychlorinated biphenyls (PCBs), and dioxins (Whyte et al., 2000; Stegeman and Hahn, 1994). Our overall objectives are to identify which of these sources contained compounds that induced CYP1A in exposed biota, and to determine which of the bioavailable contaminants are responsible. We report our results in two parts. Here (Part I), our objectives are to describe and validate the method, which includes an assessment of the precision, sensitivity, and dynamic range. We present the results of our field deployments of SPMDs in PWS to evaluate the CYP1A induction potential of prospective contaminant sources. In Part II (Short et al., 2008), we relate the CYP1A induction results to the chemical composition of the contaminants accumulated by the SPMDs.

2. Methods

2.1. Sampling design

Prince William Sound is a complex, fjord-type ecosystem with a sea surface area of about 8800 km^2 (Schmidt, 1977). Most of its 7000 inhabitants reside in Cordova, Valdez or Whittier (Fig. 1), while fewer than 200 live in the vicinity of Chenega or the regional salmon hatcheries. The latter group is near the path followed by oil released by the T/V *Exxon Valdez* during the oil spill in 1989. Past industrial activities within the spill path were sporadic. These include two large mine sites at Latouche, six fish processing plants, and a number of smallholdings devoted to mineral prospecting, artisanal sawmills and fur-farming. All of these were abandoned during the mid-20th century (Wooley, 2002). Current industrial activities include the Alyeska oil terminal in Port Valdez, commercial fishing and oyster farming, five large salmon hatcheries, and seasonal tourism.

We deployed SPMDs to evaluate the CYP1A induction potential of six classes of contaminant sources: (1) lingering, subsurface, Alaska North Slope (ANS) oil from the *Exxon Valdez* oil spill ($N = 5$ sites, three of which were replicated); (2) human activity ($N = 6$) sites, including abandoned industrial sites; (3) salmon hatcheries ($N = 5$); (4) salmon streams ($N = 5$), because salmon carcasses can release accumulated CYP1A-inducing pollutants as they decompose in their natal streams following reproduction; (5) random sites ($N = 9$) to evaluate area-wide pollution sources including atmospherically transported contaminants; and (6) a site at Constantine Harbor where PAH associated with organic-rich rocks eroded from geologic sources east of PWS are incorporated into the intertidal sediments (Short and Babcock, 1996). At the ANS sites, we deployed additional SPMDs at adjacent paired control sites to account for local inducing agents other than ANS. We also deployed SPMDs at a remote site at Graves Harbor in southeast Alaska $\sim$300 km east of PWS and upcurrent of the geological hydrocarbon sources to serve as a negative regional field control site, and at Cordova Harbor to serve as a positive field control.

The SPMDs were deployed at most sites in spring from mid-May to mid-June 2004, prior to the annual spawning migration of salmon. At the salmon stream sites, they were again deployed in summer from mid-August to mid-September 2004, after adult salmon had returned to spawn. Five of the random site deployments were during spring, and the other four during summer to assess region-wide seasonal differences. The random selections for these sites were made within each of five sectors partitioning PWS (see Fig. 1 in Short et al., 2008). Each sector was allocated one or two sites to ensure dispersion, and each site was located on a randomly selected shore segment within a sector as described in Part II (Short et al., 2008).

2.2. Semipermeable membrane devices

The SPMDs were obtained from Environmental Sampling Technologies (St. Joseph, MO), and deployed in 30 cm long cylindrical cages, each loaded with five SPMDs woven on stainless steel carriers stacked within the cage. Each SPMD had dimensions of 91.4×2.5 cm and contained 1 ml of ultra-high purity (UHP)