AN ABSTRACT OF THE DISSERTATION OF

Sarah E. Allan for the degree of Doctor of Philosophy in Toxicology presented on October 31, 2011.

Title: Development of Innovative Applications for Passive Sampling Devices: Monitoring Bioavailability, Risk from Exposure and Environmental Mixture Toxicity.

Abstract approved:_____
Kim A. Anderson

Chemicals must be bioavailable for there to be a potential for exposure and consequent risk to human or environmental health. Passive sampling devices (PSDs) are used to quantify the time-integrated concentration of bioavailable contaminants. We demonstrate that PSDs can be paired with the zebrafish developmental toxicity bioassay to produce site-specific, temporally resolved information about the toxicity of environmental samples. Furthermore, modeling associations between the chemical components of environmental mixtures and the toxic outcomes they elicit can link bioactive compounds to biological effects. This research also shows that PSDs can be used as direct biological surrogates in a risk assessment model. We were able to determine spatial and seasonal variations in exposure and risk from the consumption of polycyclic aromatic hydrocarbons (PAH) in organisms from the Portland Harbor Superfund that were not detected in the Public Health Assessment for the area. Additionally, PSDs are a tool that we were able to rapidly deploy after the Deepwater Horizon oil spill. We quantified biologically relevant PAH contamination on a large spatial scale, over a long period of time when the chemicals of concern were present at relatively low dissolved concentrations, their impact on certain areas was sporadic and their presence and toxicological significance were not easily visualized. The research presented here can be applied to improve environmental monitoring, mixture toxicity assessment and risk assessment.

Development of Innovative Applications for Passive Sampling Devices: Monitoring Bioavailability, Risk from Exposure and Environmental Mixture Toxicity

by
Sarah E. Allan

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Sarah E. Allan, Author

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CONTRIBUTION OF AUTHORS

Robert Tanguay directs the Sinhuber Aquatic Research Laboratory that was utilized for experimental work involving zebrafish and he contributed to the experimental designs described in Chapter 2. Gregory J. Sower was integral in the conception of the central idea in chapter 3 and participated in data collection and development of the manuscript. Brian Smith provided statistical modeling support for chapter 4.

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Chapter 1 – Introduction

The presence of toxic chemicals in the environment is not necessarily indicative of a risk to human and environmental health; the chemicals must be bioavailable in order for there to be an exposure, which is a necessary precursor to a toxic outcome. The bioavailable fraction of a chemical is the portion of the total bulk concentration that is capable of being taken up by organisms and is sometimes referred to as the external dose *(1)*. Bioavailability is therefore a more biologically relevant measure of contamination and potential exposure than total concentration. This understanding has lead to an interest in developing tools and methodologies for directly assessing bioavailability in environmental monitoring and risk assessment research.

Aquatic passive sampling devices (PSDs) include the widely employed semi-permeable membrane device (SPMD) and a more recently developed variant called lipid-free tubing (LFT) that does not contain triolein *(2-5)*. LFT were used in all of the studies presented in this dissertation because they are simpler, cheaper and require less clean-up prior to chemical analysis than SPMDs *(4)*. Like other PSDs, LFT sequester and concentrate the freely dissolved, and therefore bioavailable, fraction of hydrophobic organic contaminants from aqueous environments *(6)*. PSDs have been widely applied to environmental monitoring and continue to be a valuable tool for assessing bioavailability. This dissertation builds on a decade of monitoring bioavailable contaminants in the Portland Harbor Superfund Site in Portland, Oregon. The research detailed here significantly advances the science of environmental monitoring and exposure assessment by demonstrating novel, fit for purpose applications for passive sampling.

The BRIDGES (biological response indicator devices gauging environmental stressors) tool, which couples passive sampling with the zebrafish developmental toxicity assay, addresses three fundamental needs in toxicology research:

determining bioavailability in order to assess potential exposure *(7)*; directly connecting effective environmental sampling with toxicity evaluations *(8)*; and evaluating the toxicity of complex mixtures of contaminants in the environment *(9)*. Mixture toxicity is an area of research that has been identified as a priority for ecotoxicology *(8)* and risk assessment *(10)*. The study presented in Chapter 2 was the first report of coupling passive sampler technology with the assessment of multiple developmental biological responses in a whole organism vertebrate model. This study demonstrates the potential for directly assessing the toxicity of environmentally relevant chemical mixtures using a cost-effective, high-throughput sampling and bioassay techniques. Chapter 3 is the next step in the development of the BRIDGES tool. Here, spatial and temporal differences in the toxicity of samples obtained from Portland Harbor using PSDs are examined. Additionally, connections between specific toxic outcomes observed in zebrafish embryos exposed to PSD extracts and the chemicals identified in the environmental samples are identified and modeled.

PSDs mimic passive chemical uptake and accumulation by biomembranes and lipid tissues. Beyond sequestering only the freely dissolved, bioavailable fraction of chemicals, PSDs constructed from low-density polyethylene (LDPE) possess physical characteristics that are similar to biological membranes that control uptake in organisms. The transient cavities in the LDPE tubing are approximately the same size as the pores in cellular membranes, which prevents sequestration of chemicals that are too large to pass through a cellular membrane *(6)*. The similarities between how organisms and PSDs accumulate contaminants from the environment are the basis for the research presented in Chapter 4. This study is the first reported use of PSDs as direct biological surrogates for risk assessment and demonstrates the feasibility and utility of this approach. Using PSDs directly to estimate exposure from consumption

of resident organisms has the potential to make sampling for public health assessments cheaper, easier, site-specific and temporally refined.

PSDs provide a site-specific, time-integrated measure of bioavailable hydrophobic chemicals in the environment that they are deployed in. Furthermore, they have significantly lower detection limits than grab-water samples and are capable of capturing episodic event which could be missed with periodic sampling *(6)*. Freely dissolved water concentrations are calculated based on the quantities that were sequestered by the PSDs and the uptake rates of the analytes into the sampler. Adding performance reference compounds (PRCs) to the samplers prior to field deployment allows for the in situ determination of uptake rates based on the diffusion rates of the PRCs *(6, 11)*. This approach was used for the study presented in Chapter 5, which details spatial and temporal changes in bioavailable polycyclic aromatic hydrocarbons (PAHs) before, during and after the Deepwater Horizon oil spill in the Gulf of Mexico. This study demonstrates the importance of having a sampling tool that can be rapidly deployed to monitor biologically relevant contamination when the chemicals of concern are present at low dissolved concentrations, their influx into sensitive areas is sporadic and their presence and toxicological significance is not readily visible.

The research presented in this dissertations represents a significant advancement in the 'creation, dissemination and application of new knowledge to ensure the protection of environment and public health', which is a component of the mission statement of Oregon State University's department of Environmental and Molecular Toxicology. The work described here advances the science of bioavailability assessment, demonstrates new methods for determining the toxicity of complex environmental mixtures, provides techniques for modeling associations between mixtures and toxic effects and demonstrates a novel application of an established sampling tool to facilitate and improve risk assessments.

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Chapter 2 – Developmental toxicity of bioavailable contaminants from the Portland Harbor Superfund site: bridging environmental mixtures and toxic effects

2.1 Abstract

BRIDGES is a bioanalytical tool that combines passive sampling with the embryonic zebrafish developmental toxicity bioassay to provide a quantitative measure of the toxicity of bioavailable complex mixtures. Passive sampling devices (PSDs) were deployed in the Willamette and Columbia Rivers within and outside of the Portland Harbor Superfund site in Portland Oregon. Samplers were deployed in six sampling events in summer and fall of 2009 and 2010. PSD extracts were analyzed for PAH compounds and scanned for 1201 chemicals of concern using deconvolution reporting software. Additionally, extracts were applied to the embryonic zebrafish bioassay. Significant spatial and temporal differences in the concentration of contaminants at the sites were observed. Similarly, significant differences in the developmental toxicity of the samples were recorded. This demonstrates the importance of utilizing an environmental monitoring tool, such as BRIDGES, that can provide site-specific, temporally resolved information about environmental contaminants and directly link environmental samples to toxicity. Although this research highlights the complexity of discerning specific bioactive compounds in complex mixtures, it demonstrates methods for associating toxic effects with chemical characteristics of environmental samples.

2.2 Introduction

The BRIDGES (Biological Response Indicator Devices Gauging Environmental Stressors) bioanalytical tool provides a quantitative measure of the toxicity of environmentally relevant contaminant mixtures. It pairs passive sampling with the embryonic zebrafish developmental toxicity model to connect environmental chemical exposures to their biological effects. This tool responds to three fundamental needs in toxicology research: 1) determining bioavailability in order to

assess potential exposure *(1)*; 2) evaluating the toxicity of complex mixtures of contaminants in the environment *(2)*; and 3) directly connecting effective environmental sampling with toxicity evaluations *(3)*. Furthermore, it allows for the determination of the toxicity of environmentally relevant mixtures, even when all of the components of the mixture are not identified *(4, 5)* and can aid in the identification of bioactive chemicals *(5, 6)*.

The presence of chemicals in the environment is not necessarily indicative of bioavailability *(1, 7)*; chemicals can only be taken up by organisms and have a biological effect if they are bioavailable *(8)*. Developing methods for effectively assessing exposure, and integrating these into risk assessment frameworks, has been identified as a priority for ecotoxicology *(3)* and risk assessment *(9)*. Although humans and other organisms are exposed to complex mixtures of contaminants, toxicity testing is most often limited to determining the effects of exposure to individual chemicals or classes of chemicals. Models for predicting the effects of complex mixtures can be inadequate because they do not account for antagonistic and synergistic interactions between the components of the mixture *(10)*. Additionally, environmental exposure assessment is carried out by measuring known pollutants in environmental matrices using analytical chemistry. Chemicals that have not been previously identified, such as unknown substances and breakdown products of known contaminants, can escape detection during chemical analysis; however, these unidentified components of the mixture may be toxicologically significant *(5, 6)*.

Passive sampling devices (PSDs) sequester and concentrate the freely dissolved, and therefore bioavailable, fraction of hydrophobic organic contaminants from aquatic environments. They provide a time integrated measurement of these chemicals in the environment and are ideally suited for determining the

bioavailability of chemicals, quantifying chemicals that are present at low concentrations in the water and capturing episodic events *(11)*. Furthermore, samples obtained using PSDs can be applied to in-vitro and in-vivo bioassays *(4, 11-18)*.

Semi-permeable membrane devices (SPMDs) have been extensively utilized in environmental monitoring applications *(11)*. More recently, variants of SPMD samplers have been developed that do not contain triolein *(19-22)*. These lipid-free tubing (LFT) samplers are simpler, cheaper and require less clean-up prior to chemical analysis *(21)*. Furthermore, because samples obtained using LFT do not contain oleic acid impurities they do not require laboratory clean-up prior to use in bioassays and may be more suitable than SPMDs for this application *(4)*.

Bioassays are used to identify the potency and nature of toxic effects elicited by exposure to chemicals or other factors. The embryonic zebrafish is a widely utilized model vertebrate organism for bioassays *(23, 24)*. Its small size, fecundity, rapid development and early morphology are advantageous and allow for high throughput applications that most vertebrate organisms are not suited for *(24)*. The developmental morphology of zebrafish has been well documented *(25)* and genetic and molecular tools have been designed to elucidate mechanisms of action for biological outcomes *(26)*. A prior study, that laid the foundation for the development of the BRIDGES tool, demonstrated that pairing passive sampling with the embryonic zebrafish developmental model provided spatially and temporally resolved information about the toxicity of bioavailable contaminant mixtures in an industrialized river *(4)*.

The Portland Harbor Superfund site is an example of an area where the application of the BRIDGES tool can provide valuable insight to direct research and remediation goals. The range of chemical contaminants as well as the variety of point

and non-point source inputs leads to a situation where understanding the toxicity of environmentally relevant complex mixtures is a priority. Furthermore, significant differences in contaminant levels *(22, 27)* and toxicity *(4)* have been observed on reduced spatial and temporal scales. This necessitates the application of methods capable of providing highly resolved, site-specific information, which is a demonstrated advantage of the BRIDGES bioanalytical tool *(4)*.

This study uses the BRIDGES tool to examine spatial and temporal differences in the toxicity of bioavailable chemical mixtures obtained from sites within and outside of the Portland Harbor Superfund over the course of two years. The objective of this research is to associate differences in the toxic effects elicited by exposure to environmental samples with the chemicals identified in those samples.

2.3 Materials and methods

2.3.1 Study area. The Willamette River flows north through the Willamette Valley in Oregon before reaching its confluence with the Columbia River. Portland Harbor is a section of the River that has been heavily industrialized for over a century and continues to be impacted by urban and industrial activities. A 9 mile section of the harbor was designated a Superfund Site in 2000 due to high levels of PAHs, PCBs, dioxins, pesticides and metals in sediments and water *(27)*. Remediation efforts have been ongoing; most notably at the McCormick and Baxter Superfund site, located on the east bank of river mile (RM) 7 within the larger Portland Harbor Superfund, and the GASCO site at RM 6.3 west *(27)*. PSDs were deployed at nine sites in 2009-2010. Seven sites were located on the Willamette River: six were within the Portland Harbor Superfund site and one, RM 12, was upstream from the Superfund. Two sampling sites were located on the Columbia River; above and below the confluence with the Willamette River (Figure 2.1)

2.3.2 Sample collection. Lipid-free tubing PSDs were constructed from low-density polyethylene tubing using methods detailed elsewhere *(22)*. Briefly, additive free tubing was cleaned with hexanes then heat sealed at both ends, producing a 2.7 x 100 cm, two-layer membrane. PSDs designated for chemical analysis were fortified with deuterated performance reference compounds (PRCs) prior to sealing the tubing. PRCs are used for the determination of in situ uptake rates *(11)*. Samplers designated for bioassay applications were not fortified with PRCs.

Stainless steel cages that contained five PSDs were deployed in the water column, approximately 3 m above the ground using an anchored flotation system described elsewhere *(21)*. Two cages were deployed at each site; one containing samplers for chemical analysis and one with samplers for bioassay applications. Paired cages were deployed at nine sites (Figure 2.1) for six different 30 day deployments in 2009-2010. Deployments were carried out in September and October, 2009 and July, August, September and October, 2010. River flows in July-September were significantly lower than in October for both 2009 and 2010, which has been shown to affect the concentration of organic contaminants in the Portland Harbor Superfund area *(22)*. Samplers were lost at RM 6.5W in August and October, 2010 and at RM 7W and RM 12E in September 2010.

Following each 30 day deployment, samplers were retrieved from the field and transported to the laboratory where they were cleaned with hydrochloric acid and isopropanol to remove superficial fouling and water. The five PSDs from each cage were extracted together by dialysis in n-hexane; 40 mL per PSD for 4 hours, the dialysate was decanted then dialysis was repeated for 2 hours and the dialysates were combined. Samples were quantitatively concentrated to a final volume of 1 mL. Samples for bioassay applications were quantitatively solvent exchanged to dimethylsulfoxide.

2.3.3 Chemicals. Solvents used for pre-cleaning, clean-up, extraction and sample preparation were Optima® grade or better (Fisher Scientific, Pittsburgh, PA). The following 33 PAH analytes were included in analyses: naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 1,2-dimethylnaphthalene, 1,6-dimethylnaphthalene, acenaphthylene, acenaphthene, fluorene, dibenzothiophene, phenanthrene, 1-methylphenanthrene, 2-methylphenanthrene, 3,6-dimethylphenanthrene, anthracene, 2-methylanthracene, 9-methylanthracene, 2,3-dimethylanthracene, 9,10-dimethylanthracene, fluoranthene, pyrene, 1-methylpyrene, retene, benz(a)anthracene, chrysene, 6-methylchrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and dibenzo(a,l)pyrene. The deuterated PAH compounds used as PRCs were fluorene-D10, p,p'-DDE-D8 and benzo(b)fluoranthene-D10. The following deuterated PAHs were used as surrogate recovery standards: naphthalene-D8, acenaphthylene-D8, phenanthrene-D10, fluoranthene-D10, pyrene-D10, benzo(a)pyrene-D12 and benzo(g,h,i)perylene-D12; and perylene-D12 was the internal standard.

2.3.4 Chemical analysis. PSD extracts were analyzed for 33 PAH compounds, one of the principal chemical classes of concern in the Superfund site, and screened for 1201 chemicals of concern using Deconvolution Reporting Software (DRS) (Agilent Technologies).

The PAH analyses were carried out on an Agilent 5975B Gas Chromatograph-Mass Spectrometer (GC-MS); with a DB-5MS column (30 m x 0.25 mm x 0.25 um) in electron impact mode (70 eV) using selective ion monitoring (SIM). The GC parameters were as follows: injection port maintained at 300 °C, 1.0 mL min^{-1} helium flow, 70 °C initial temperature, 1 min hold, 10 °C min^{-1} ramp to 300 °C, 4 min hold, 10 °C min^{-1} ramp to 310 °C, 4 min hold. The MS temperatures were operated at 150, 230

and 280 °C for the quadrupole, source and transfer line respectively. Sample concentrations were determined by the relative response of the deuterated surrogate to the target analyte in a nine point calibration curve with a correlation coefficient greater than 0.98.

Screening for known chemicals of concern was carried out on the same Agilent GC-MS with the DB-5MS column used for PAH analyses. The instrument was operated in full scan acquisition mode (mass range 50-550) and absolute EMV mode. The GC parameters were as follows: injection port maintained at 265 °C, 2.3 ml min^{-1} helium nominal flow, 70 °C initial temperature, 2 minute hold, 25 °C min^{-1} ramp to 150 °C, 3 °C min^{-1} ramp to 200 °C, 8 °C min^{-1} ramp to 280 °C, 15 minute hold, 40 °C min^{-1} ramp to 310 °C, 3 minute hold. The MS temperatures were 150, 230 and 280 °C for the quadrupole, source and transfer line respectively. Chlorpyrifos was used for retention time locking at 19.23 min.

Identification of chemicals detected in the screening was carried out using DRS software with compiled mass spectral deconvolution and identification system (AMDIS) libraries *(28)* that included numerous classes of chemicals of concern; pesticides, polychlorinated biphenyls (PCBs), parent and substituted (methyl-, oxy-nitro-) PAHs, pharmaceuticals, phthalates and musks among others. A full list of the 1201 analytes that were screened for can be found in Appendix 1. Additional information about DRS software can be found on Agilent's webpage (www.chem.agilent.com).

2.3.5 Quality Assurance/Control. Quality control accounted for over 30% of the samples analyzed and included laboratory preparation blanks, field and trip blanks for each deployment/retrieval, laboratory clean-up blanks and reagent blanks. All target compounds were below the detection limit in all blank quality control samples.

Additional calibration verification standards for all analytes, PRCs and surrogates were analyzed at least every ten samples; limits for acceptable recovery were ±15%.

2.3.6 PAH acute toxicity. PAHs are known to have variable potencies in their ability to elicit acute effects in organisms. As a result, there may be a better association between the relative toxicities of the compounds in a mixture than their concentrations. The acute toxicity of PAH compounds in environmental samples was calculated using the equation from Neff et al *(29)*:

$$\text{Log } LC_{50} \text{ (mM/L)} = -1.162 \log K_{ow} + 2.496$$

Acute toxicity values were applied to analyses examining links between chemical components of mixtures and their observed toxic outcomes.

2.3.7 Zebrafish embryo-toxicity assay. 1 mL of PSD extract contains the chemicals sequestered by 5 PSDs during a 30 day deployment. The concentration of chemicals of concern in the samples was determined by analysis of the PSD samples that were co-deployed at the same site with the samples for bioassay applications. Water concentrations were calculated, for comparison to exposure solution concentrations, using the empirical uptake model with PRC derived sampling rates detailed elsewhere *(11)*. The highest exposure concentration that the zebrafish were exposed to was approximately 1000 times greater than the dissolved concentration in river water; the lowest exposure concentration was 8 times greater. This study did not intend to mimic environmental exposure, but rather identify differences in the toxicity of environmentally relevant mixtures in a high throughput bioassay, and link the observed toxic outcomes with the components of the mixture.

Zebrafish (*Danio rerio*), from the Tropical 5D strain, were reared in the Sinnhuber Aquatic Research Laboratory (SARL) at Oregon State University in accordance with approved Institutional Animal Care and Use Committee protocols.

Adults were kept at standard laboratory conditions: 14:10 h light:dark photoperiod in polycarbonate tanks on a recirculating system in which the water was maintained at 28 °C and a pH of 7.0. Zebrafish were group spawned and newly fertilized eggs were collected and staged according to previously described methods *(25)*.

Zebrafish embryos were exposed to PSD extract solution using a static waterborne method. PSD extract solutions were prepared in DMSO at 4 concentrations, corresponding to sequential 5-fold dilutions of the PSD extract: 100x (undiluted extract), 20x, 4x and 0.8x. Exposure solutions were made up by diluting the stock solutions 1:100 directly into embryo medium. The final concentration of DMSO was 1% for all exposure solutions. Embryo medium is made by adding sodium bicarbonate (buffer) and methylene blue (mold growth inhibitor) to reverse osmosis water, creating a solution with a pH of 7.3 that is ideal for rearing embryos until 5 days post fertilization (dpf). 1% DMSO was used as a vehicle control. Trimethyltin (TMT) was used as a positive control; the 5 uM concentration used elicits 100% morphological malformations and less than 20% mortality in exposed zebrafish embryos. Exposures were carried out in 96-well plates, with one embryo in 100 uL of exposure solution in each well. Each plate contained 8 embryos exposed to the vehicle control, 8 embryos exposed to the positive control and 20 embryos exposed to each of the 4 concentrations of a PSD extract. Every set-up was repeated twice meaning that 40 individual embryos were exposed to each concentration of each PSD extract sample. In cases where the vehicle or positive controls were outside of acceptable limits (TMT ≤ 2/16 zebrafish embryos dead or deformed ≤ 1% DMSO) or there were significant differences between the observed results for the two plates for the same sample, new exposure solutions were prepared and the set-up was repeated.

Preceding exposure, the chorion was removed by pronase treatment to minimize blockage of chemical uptake *(30)*. All embryos were assessed for viability prior to beginning the exposure and after transfer to the 96 well plates. After all of the embryos were transferred to the exposure solutions, the 96-well plates were sealed, protected from light and maintained at 28 °C for the duration of the exposure. Zebrafish embryo exposures began at approximately 6 hours post fertilization (hpf) and observations were carried out, using a stereo microscope, at 24 hpf and 5 dpf. At the 24 hpf observation period, each embryos was assessed for mortality, developmental progress and notochord malformations. After 5 days, the embryos were each assessed for mortality and 19 sublethal developmental endpoints: yolk sac edema, pericardial edema, touch response, and malformations of the body axis, eye, snout, jaw, otic vesicle, brain, somites, pectoral fins, caudal fin, pigmentation, circulatory system, trunk, swim bladder and/or notochord. A malformation, or embryotoxic outcome was considered mortality or any development that deviated from normal morphology described by Kimmel et al *(25)*.

2.3.8 Analysis of toxic outcome. Toxic outcomes were analyzed individually (mortality at 2 time points and 19 sub-lethal endpoints) for exposure-dose response and associations with specific chemicals. Given the number of toxic effects assessed in this study, a metric was applied to assess the overall toxicity of the exposure to each individual embryo. This methodology proved to be effective for assessing general toxicity in prior work, but can overlook subtle differences in toxicity, such as specific outcomes associated with certain samples *(4)*. Each embryo was assigned a score from 0-1; 0 indicates an embryo that had normal development, 1 indicates death at 24 hours, 0.95 is assigned to death at 5 dpf and each sub-lethal endpoint is 0.045 so that the sum of all 19 sub-lethal outcomes is 0.855. The scores of all of the embryos in a treatment can be used in spatial and temporal comparisons of total developmental toxicity.

2.3.9 Statists and data modeling. All statistical analyses and graphing were performed using Sigma Plot 11.0 (Systat Software Inc.). Non-parametric tests were used for data that did not pass the Shapiro-Wilk normality test ($p<0.05$). Mann-Whitney rank sum tests were used for two-way comparisons of chemical characterization and toxic effects. Kruskal-Wallis one way analysis of variance on ranks, followed by the Dunn's Test for pairwise multiple comparisons when there are unequal treatment group sizes, was used to determine differences in chemical profiles and toxic effects between multiple groups. Comparisons of the incidence of individual endpoints were carried out using maximum likelihood ratios, which allows for the analysis of binary data. Correlations between variables were tested using Spearman rank order.

2.4 Results and discussion

2.4.1 Chemical characterization of samples used in zebrafish exposures. PSDs deployed in the Willamette or Columbia River for 30 days sequestered significantly different amounts of PAHs. The values reported here are the concentrations in the exposure solutions after dilution of the stock PSD extract to 1% in embryo medium; they are not back-calculated to ambient water concentrations in the rivers. The highest concentrations of PAHs were obtained from the site at RM 6.5W during every sampling period except August and October, 2010, when the samplers were lost from this site. The highest recorded concentration of the sum of 33 PAH compounds (Σ_{33}PAH), 492 µM, was obtained at RM 6.5W in September 2010. Samples from sites located within the Superfund had significantly higher concentrations of Σ_{33}PAH that the sites located outside of the Superfund (CRU, CRD and RM 12E) ($p<0.05$). RM 7E, which underwent remediation in 2006, had significantly lower Σ_{33}PAH than the two nearest sites (RM 7W and 6.5W) ($p<0.05$) as well as the other Superfund sites as a group ($p<0.05$). It was not significantly different from the sites located outside of the

Superfund area ($p>0.05$) (Figure 2.2). Prior to remediation, samples from RM 7E had had elevated PAH concentrations and toxicity similar to RM 7W *(4)*.

Significant temporal differences in the Σ_{33}PAH were also observed in the samples obtained from the Harbor. Given the variability across sites within each sampling period it was not possible to detect differences between sampling events ($p=0.08$). Data were grouped into two seasons based on the flow of the Willamette River. September 2009 and July, August and September 2010 ($n=36$) were considered 'dry season' sampling events because the flow of the Willamette River was significantly lower than the 'wet season' ($p<0.05$; based on 4 weekly averages for each deployment period), which included samples from October 2009 and October 2010 ($n=18$). The Σ_{33}PAH was significantly greater during the dry season (80.8 uM median) than the wet season (27.6 uM) ($p=0.005$). This tendency towards higher concentrations of bioavailable PAHs in the Superfund area during the dry season has been previously document in Portland Harbor *(22, 31)*. There was no significant difference between seasons at the upriver site (RM 12E), indicating that lower concentrations of PAHs at sites within the Superfund are during the wet season are not a result of dilution due to higher flows. Suggested explanations for seasonal differences have been attributed to bank storage, elevated hydraulic pressure during high flows and temporal changes in water temperature *(22)*.

The acute toxicity of the PAHs in each sample was calculated in order to control for the possibility that similar Σ_{33}PAH may result from different chemical compositions. The toxicity of the samples follows the same pattern as the Σ_{33}PAH and is significantly correlated ($p<0.001$, $r^2=0.96$) (Figure 2.2). Additionally, spatial and temporal comparisons of acute toxicity produced the same outcomes as those described for Σ_{33}PAH. As a result, further analyses involving individual PAHs or Σ_{33}PAH

are based on the concentration of the analytes, which is directly related to the toxicity of the sample.

All samples were screened for 1201 chemicals of concern using DRS; fifteen compounds, not including the PAHs previously quantified, were identified in samples using DRS. These included p,p'-DDE, o,p'-DDD and p,p'-DDD, which are intermediate breakdown products of the pesticide DDT (1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane), three oxygenated PAH compounds (9-fluorenone, benzofluorenone and benzanthrone), a nitrogen-PAH (6-nitrobenzo(a)pyrene), hexachlorobenzene (fungicide; persistent organic pollutant), chlorfenapyr (pro-insecticide), pendimethalin, pyrazone (herbicides), tonalide (musk found in personal care products), rabeprazole (antiulcer pharmaceutical), phenothiazine (antipsychotic and antihistaminic pharmaceutical) and methyl-triclosan (breakdown product of antibacterial/antifungal agent triclosan).

Phenothiazine and chlorfenapyr were only found in samples obtained from the Columbia River. Benzofluorenone, tonalide and 6-nitrobenzo(a)pyrene were found in samples from the Columbia and Willamette Rivers. The rest of the compounds listed were found in samples from Portland Harbor. Samples from RMs 3E and 6.5W contained the highest number of compounds identified by DRS (not including previously quantified PAHs); however, a minimum of four chemicals of concern, in addition to the PAHs, were found in all samples from sites on the Willamette River. Due to uncertainties regarding the quantification of chemicals identified using DRS, the compounds were treated as present/absent in modeling and statistical analyses.

2.4.2 Developmental toxicity of PSD extracts. A total of 10,944 embryos were exposed to 4 concentrations of 50 different environmental samples, as well as positive and vehicle controls. This highlights the truly high throughput character of

the BRIDGES bioanalytical tool. Zebrafish embryos that were exposed to PSD extracts demonstrated a range of responses including normal development, mortality, and a variety of morphological deformities, some of which are illustrated in Figure 2.3. The average number of developmental endpoints observed in embryos that showed sub-lethal effects at the 5 day observation was 6.2 of the 17 assessed at that time point. The median number of sublethal endpoints was 3. Analysis of a frequency graph of number of toxic endpoints in embryos that expressed sub-lethal deformities demonstrates a non-normal distribution in which it is more likely for affected embryos to express a small number (2-3) or large number (16-17) of endpoints but significantly less likely that an embryo will show the average number (6-7) of deformities ($p=0.001$). This distribution of number of expressed sub-lethal endpoints is likely due to a biological threshold above which development is completely disrupted. The non-linearity of developmental toxicity observed in zebrafish embryos is accounted for in further analyses.

Initial comparisons of developmental toxicity were based on the toxicity scores of each sample. Significant exposure dose-response relationships were observed in some samples. However this was most often seen as a greater toxicity score for the highest exposure dose compared to the other three doses and no significant difference between the lower doses. (Figure 2.4). A significantly greater response at the highest exposure dose, compared to the lower doses was seen in samples from RM 6.5W and 7W in September 2009, all samples in July 2010, all samples except CRU in August 2010, CRU and RMs 3.5W and 6.5W in September 2010 and RMs 7E, 7W and 12E in October 2010 (Figure 2.4). Due to uncertainties inherit in establishing a dose response curve based on data with these characteristics, as well as a large number of samples that showed no effects at the highest dose, further comparisons between samples were based on only the highest exposure dose (100X).

The developmental toxicity of the samples showed significant spatial variability. All samples from September 2009, except from 3.5W and CRU, were significantly different from the 1% DMSO control ($p<0.05$). The samples from RMs 6.5W and 7W were significantly more toxic than the sample from CRU, 3.5W, 8W and 7E ($p<0.05$); CRD and RM 3E were significantly more toxic than CRU, 3.5W and 8W ($p<0.05$) and 12E was more toxic than CRU ($p<0.05$). Only the sample obtained from RM 7W was significantly more toxic than the control in October 2009 ($p<0.05$) and there were no differences between the sites. All samples from July, 2010 were significantly more toxic than the control; however there were no significant differences between the sites. All of the samples obtained in August, 2010, except from CRU, were significantly more toxic than the control ($p<0.05$) and samples from all other sites were more toxic than CRU. All samples from September, 2010 were more toxic than the control ($p<0.05$) and the sample from RM 3.5W was more toxic than RM 3E. Samples from RMs 3.5W, 7E, 7W, 8W and 12E from October 2010 showed significantly greater toxicity than the control as well as the samples from CRU, CRD and 3E ($p<0.05$).

Differences in toxicity between the sites, including those that are located very close to one another on the river, were observed in a prior study *(4)*. The sites at RMs 7W, 7E and 6.5W are all located within 0.5 km of each other. However, samples from RMs 6.5W and 7W had higher Σ_{33}PAHs in all comparable sampling events and were more toxic than RM 7E during two sampling events. This demonstrates the effects of remediation at 7E, the McCormick and Baxter site on the chemical contamination and toxicity of the area. Heterogeneity in the contaminant levels in a variety of environmental media in Portland Harbor has been well documented *(27)*. These results provide additional evidence that toxicity can vary significantly within a reduced spatial scale and highlight the importance of using analytical tools that provide highly resolves, site-specific information, such as the BRIDGES tool.

Significant temporal differences in toxicity were observed in this study. Similar to the Σ_{33}PAH chemical data, the toxicity scores of the samples obtained during the 'dry season' in 2009 and 2010 were significantly greater (n=1440; 0.72 median toxicity on a scale of 0-1) than those obtained during the 'wet season' (n=840; 0.0 median toxicity) ($p<0.001$). Samples from July, 2010 and August, 2010 (0.810 median score for both) were significantly more toxic than samples from all other months included in this study (0.23, 0.00, 0.36 and 0.18 median scores in September and October 2009 and 2010 respectively) ($p<0.001$). PSD extracts from September, 2009 and 2010 were more toxic than those from October 2009 and 2010 and samples from October 2010 had greater toxicity than those from October 2009 ($p<0.001$).

Temporal changes in the concentration of bioavailable contaminants in the Willamette River have been recorded in previous studies *(22)*. These results demonstrate that those seasonal differences in contamination translate into differences in the toxicity of PSD extracts obtained from the environment. This is not a foregone conclusion considering that PAHs are a class of compounds with widely variable solubility and toxicity and changes in dissolved Σ_{33}PAH levels is not necessarily indicative of greater toxicity. As with the results that demonstrate significant spatial differences in toxicity, these data provide further support of the importance of applying environmental monitoring tools that are capable of capturing temporal changes in chemical concentrations and toxicity.

Incidences of specific biological endpoints were correlated to overall toxicity and showed similar spatial and temporal trends. Otic vesicle malformations and notochord deformities at 5 days were the only endpoints that did not have a significant correlation with the toxicity score. The strength of the associations between specific outcomes and the toxicity score is likely due to the tendency for

observed effects, individually or overall, to be on one extreme or the other of the toxicity scale, with few intermediate sub-lethal outcomes.

2.4.3 Bridging chemistry and biological effects. Two characteristics of the data limited the possibilities for linking chemicals present in mixtures to the toxic outcomes that they elicited: 1) the 'all-or-nothing' tendency in the number of toxic effects seen in the zebrafish embryos exposed to PSD extracts and 2) significant correlation between the concentration of individual analytes in a sample and the total concentration of contaminants in the sample. The chemical profiles of all of the samples were similar and diagnostic ratios between pairs of PAHs were not significantly different in any of the samples obtained from Portland Harbor. As a result, the magnitude of the concentration of analytes in the samples was variable, but the composition was similar. Differences were seen in the chemical profiles of samples from Portland Harbor and the site on the Columbia River located upstream from the confluence (CRU). However, most samples from CRU did not elicit toxic effects in zebrafish embryos and it was therefore difficult to establish a more detailed link between differences in the chemistry of the samples and their effects in the bioassay.

The presence/absence of chemicals of concern that were identified using DRS did not show a significant correlation with observed toxic outcomes in exposed embryos. This may be due to the lack of quantitative concentration information or knowledge of relative potency/toxicity. The greatest number of compounds identified by DRS were detected in samples from RM 6.5W and this site consistently showed elevated toxicity; however, there was not a significant correlation. The ability to screen for a large number of compounds of concern in environmental samples could help explain observed toxicity *(5)*, but in this case the relative similarity in the

chemicals that were present at sites in the Superfund area made it difficult to define specific links to toxic outcomes.

A significant correlation exists between the Σ_{33}PAH in the samples and the developmental toxicity score, however the strength of this relationship is relatively low (r^2=0.7). This is likely due to the nature of the biological data, where extreme responses to the treatments predominate over intermediate effects. Tests of associations between incidences of specific developmental outcomes and Σ_{33}PAH produced similar results, with r^2 values between 0.58 and 0.98 when all exposure dose treatments were included and the data were left-skewed. Identical analyses, using only the data from the highest exposure dose, showed no significant association between analyte concentrations and the incidence of any developmental endpoint.

Due to the correlation of individual analytes with the sum of all compounds measured, it is difficult to parse out associations between specific biological endpoints and components of the mixture that the embryos were exposed to. However, certain endpoints show a nearly identical association with most of the PAH compounds, while others are more associated with a specific suite of chemicals. Mortality, yolk sac edema, pericardial edema, curved body axis, developmental progress and deformities of the snout, jaw, brain, circulatory system and swim bladder were equally associated with the Σ_{33}PAH and the individual analytes quantified in the samples. This suggests that those endpoints are non-specific and their expression could be a result of overall disrupted development. The other endpoints showed a greater association with certain analytes, especially 9-methylanthracene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene and dibenzo(a,h)anthracene, than the Σ_{33}PAH. Given the correlation that exists between the concentrations of the analytes in each sample, it

is not possible to discern more specific associations. Correlating chemical fingerprints to toxic outcomes has been demonstrated in the past *(32)*; however this approach may require additional refinement before it can be applied to a data set of this size, with a large number of analytes and samples with similar chemical fingerprints. Future research should focus on fractionating the samples to reduce the number of potential analytes *(5, 33)*, and testing individual analytes that showed significant associations with specific endpoints to further refine the understanding of chemical components and biological effects. Obtaining samples from sites with different chemical contaminants may also help elucidate patterns in the biological responses that are masked by the similarity in the chemical profiles of the samples obtained for this study.

2.5 Conclusions

BRIDGES proved to be a sensitive bioanalytical tool that was capable of detecting highly resolved spatial and temporal differences in bioavailable chemicals in the environment and the toxicity of environmental mixtures. The bioavailable concentration of PAHs in Portland Harbor varied significantly between sites, both within the Superfund area and outside of it. Additionally, the concentration of PAHs was significantly greater during the ‘dry season’ than the ‘wet season’. A similar trend was observed in the overall developmental toxicity of PSD extract samples. It was possible to discern a group of chemicals that show a significant association with specific endpoints. Future research should focus on refining the list of bioactive components in the environmental mixtures and discerning specific developmental outcomes associated with those compounds.

2.6 Acknowledgements

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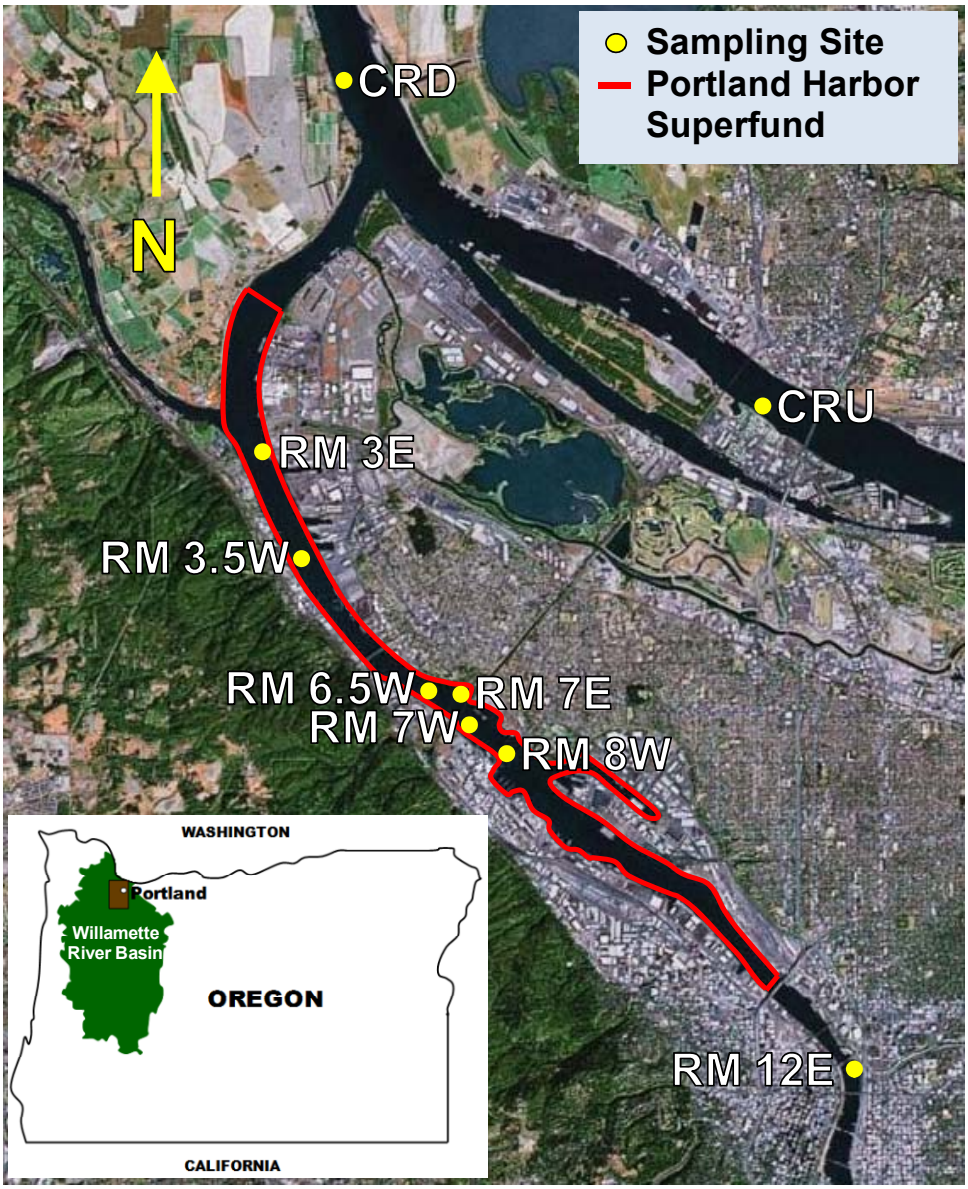


Figure 2.1 Study area and sampling sites

The Willamette River flows north, through metropolitan Portland, Oregon to its confluence with the Columbia River, which flows west along the border between Oregon and Washington State. The Portland Harbor Superfund area, on the Willamette River, is outlined in red. The sites where PSDs were deployed in 2009-2010 are indicated by yellow circles. Seven sites along the Willamette River are labeled with the river mile (RM) and the east (E) or west (W) bank that they are located near. Two sites on the Columbia River are located upstream (CRU) and downstream (CRD) of the Willamette River confluence.

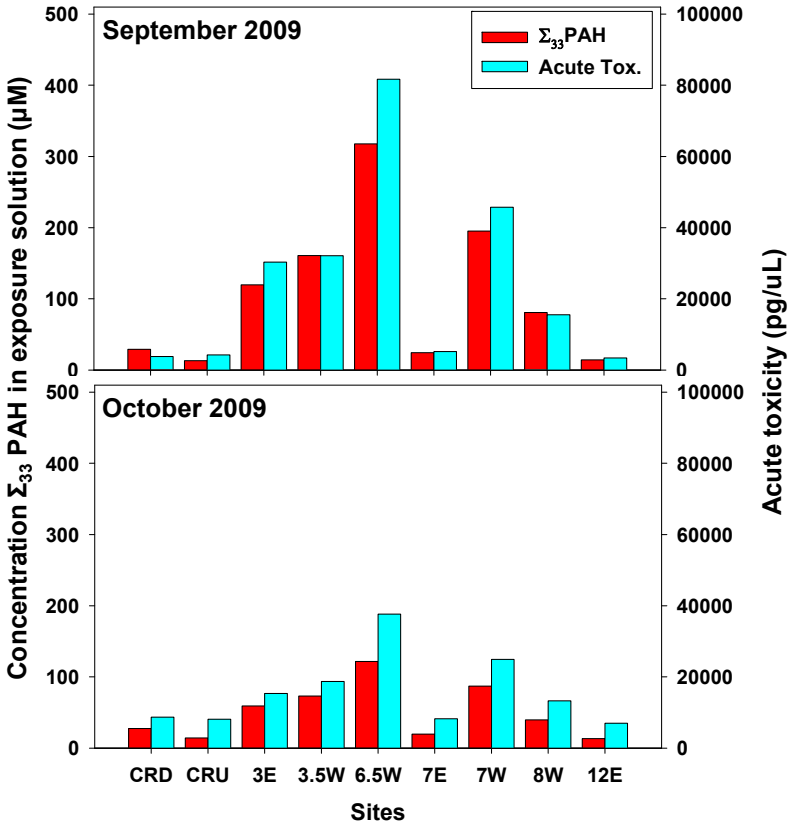


Figure 2.2 Concentration of Σ_{33}PAH in exposure solution and associated acute toxicity

The concentration of 33 PAH compounds (Σ_{33}PAH) in samples obtained from sites within and outside of the Portland Harbor Superfund site are shown in red (left Y-axis). The acute toxicity of the PAHs in the samples is shown in blue (right Y-axis). Both values refer to the highest concentration exposure solution that the zebrafish embryos were exposed to. Sampling periods in 2009 and 2010 (continued) are denoted. Asterisks indicate that samples were not obtained from the field. See Figure 2.1 for the location of the sampling sites.

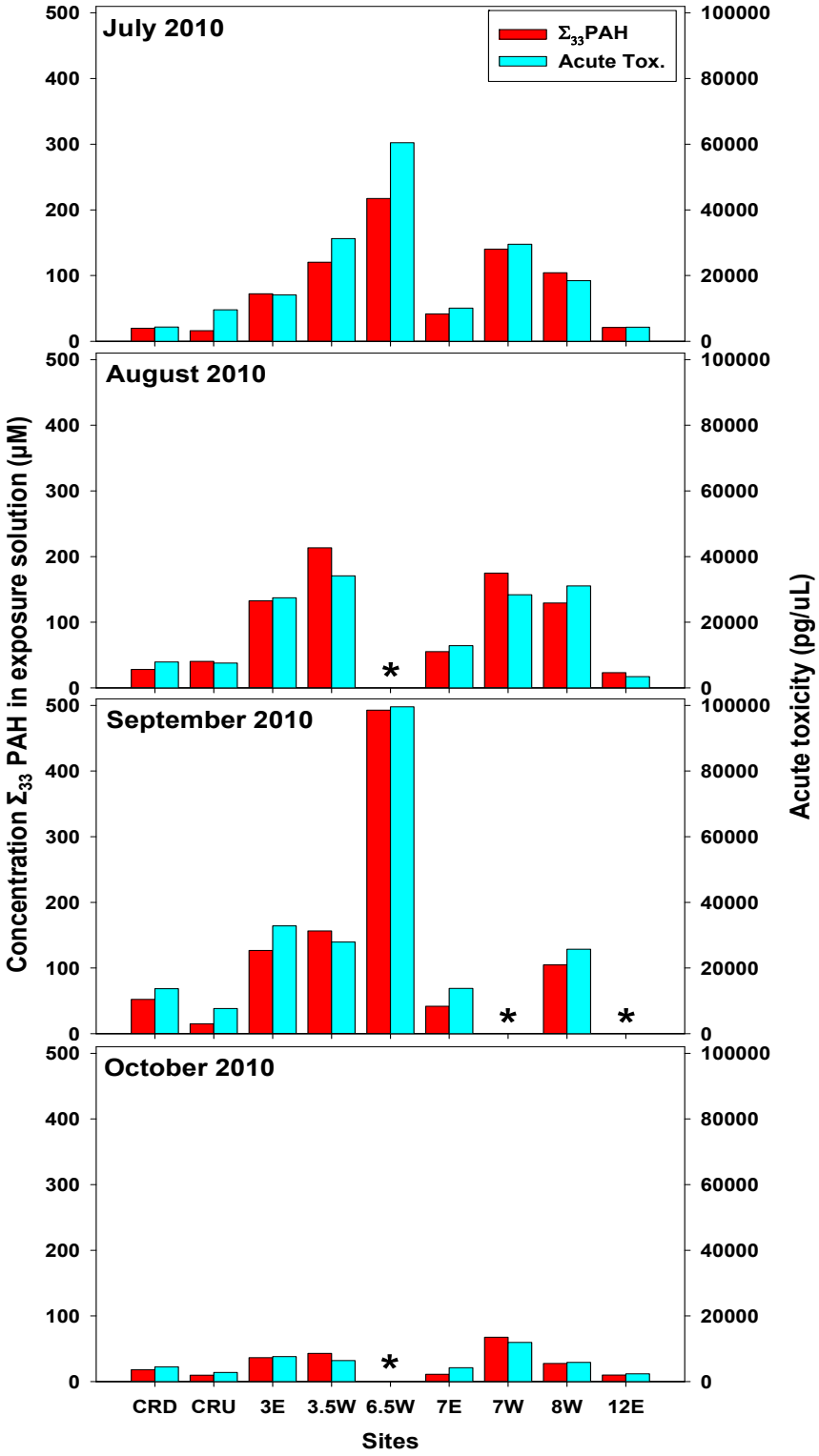


Figure 2.2 (Continued)

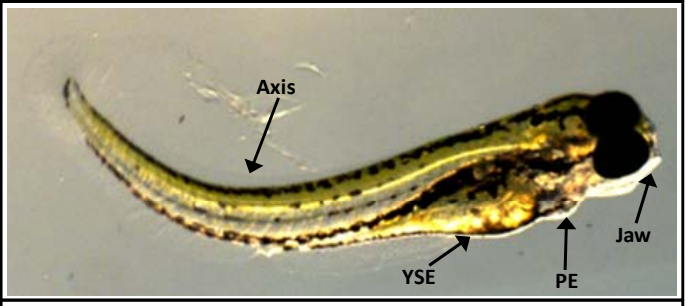
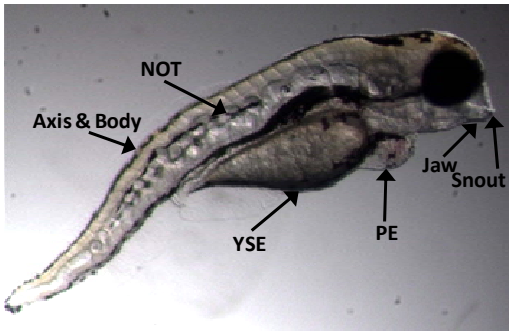
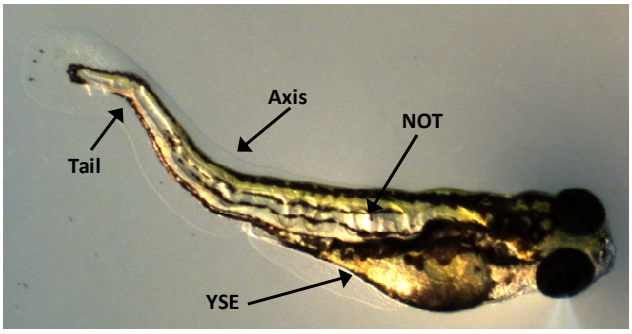
Sample	Characteristic toxic effects in embryonic zebrafish at 5 dpf observation
1% DMSO Control (Vehicle Control)	
RM 7W September, 2009	
RM 6.5W June, 2010	
RM 3.5W August, 2010	

Figure 2.3 Characteristic toxic effects in embryonic zebrafish

Some characteristic developmental effects, including yolk sac edema (YSE), pericardial edema (PE) and deformities of the body axis (Axis), body length (body), jaw, snout, notochord (NOT) and tail are pictured. Embryos were exposed to 1% PSD extract obtained from different sites and sampling events in the Portland Harbor Superfund and compared to normal development in embryos exposed to a 1% DMSO vehicle control.

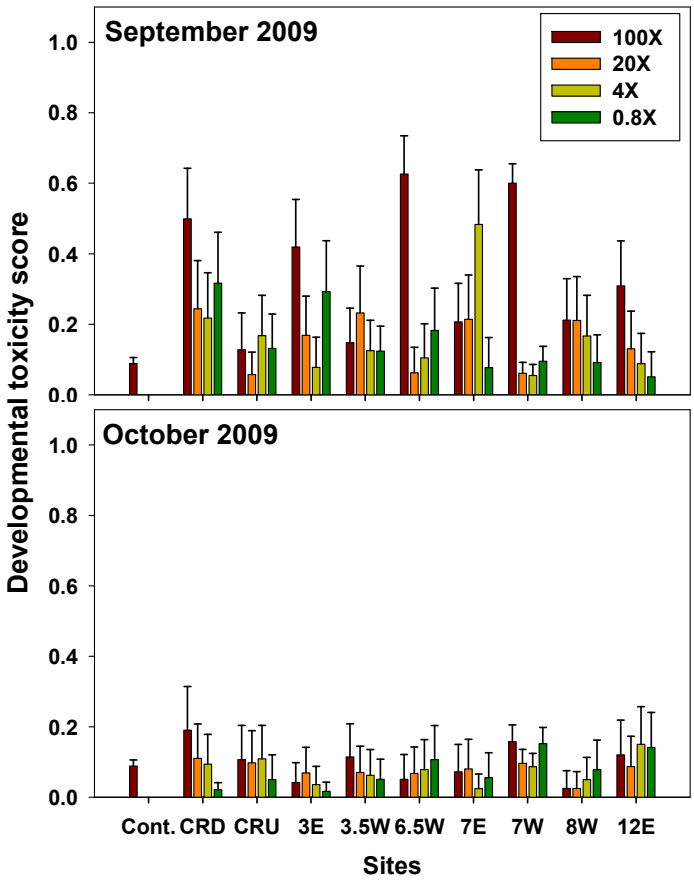


Figure 2.4 Developmental toxicity of PSD extracts

The developmental toxicity of each sample was scored based on the presence or absence of 21 toxic outcomes. Bars represent the average score of all embryos exposed to the sample (n=40 per treatment) and lines represent the standard deviation. Scores range from 0-1 where 0 is indicative of normal development and 1 represents mortality at the earliest time point. The highest concentration (100X; red bars) was 1% PSD extract and the other exposure concentrations are successive five-fold dilutions of the extract (20X, 4X and 0.8X; orange, yellow and green bars respectively). The average outcome of the 1% DMSO control (Cont.) is shown on each graph for comparison. Sampling periods in 2009 and 2010 (continued) are denoted. Asterisks indicate that samples were not obtained from the field. See Figure 2.1 for the location of the sampling sites.

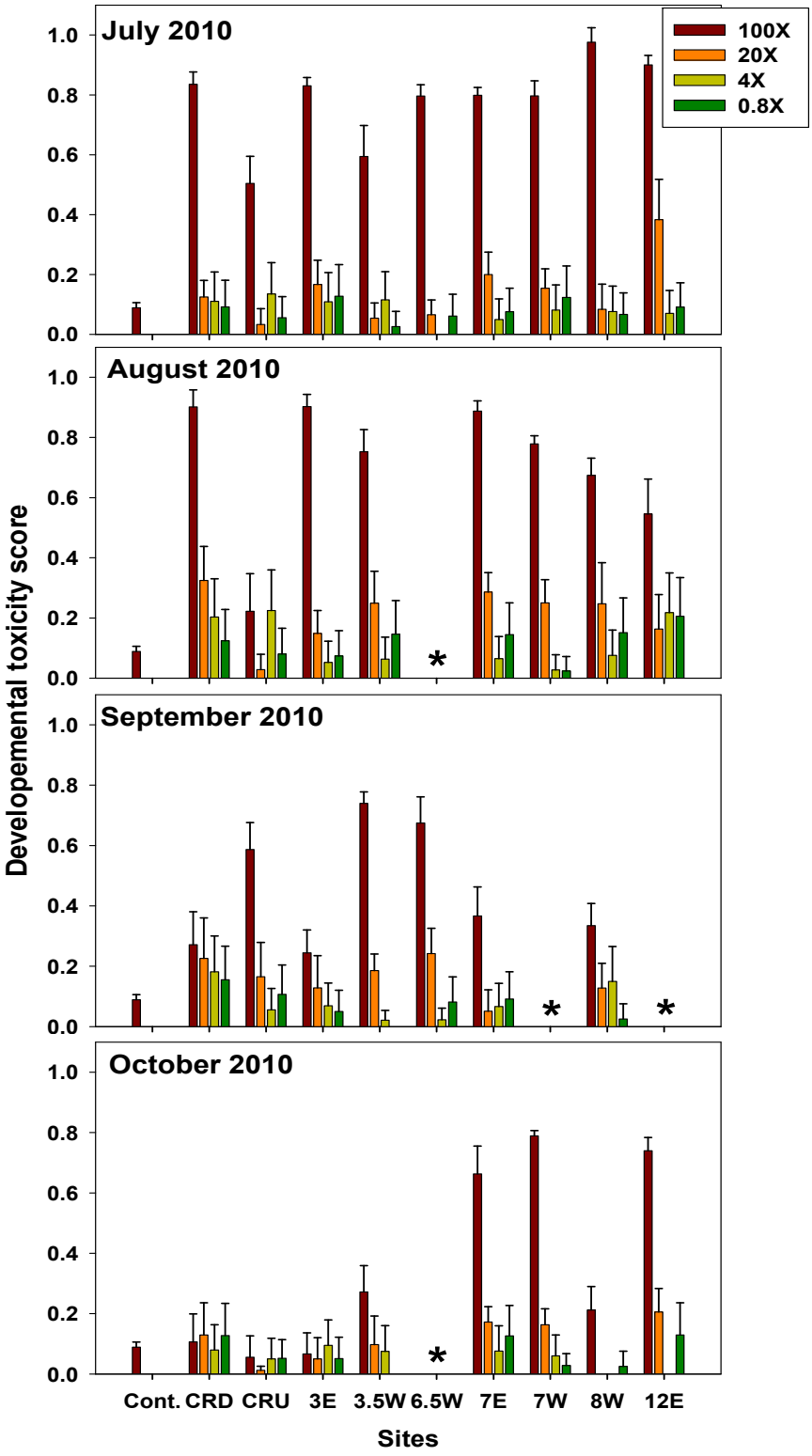


Figure 2.4 (continued)

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ESTIMATING RISK AT A SUPERFUND SITE USING PASSIVE SAMPLING DEVICES AS BIOLOGICAL SURROGATES IN HUMAN HEALTH RISK MODELS

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Chapter 3 – Estimating risk at a Superfund site using passive sampling devices as biological surrogates in human health risk models

3.1 Abstract

Passive sampling devices (PSDs) sequester the freely dissolved fraction of lipophilic contaminants, mimicking passive chemical uptake and accumulation by biomembranes and lipid tissues. Public Health Assessments that inform the public about health risks from exposure to contaminants through consumption of resident fish are generally based on tissue data, which can be difficult to obtain and requires destructive sampling. The purpose of this study is to apply PSD data in a Public Health Assessment to demonstrate that PSDs can be used as a biological surrogate to evaluate potential human health risks and elucidate spatio-temporal variations in risk. PSDs were used to measure polycyclic aromatic hydrocarbons (PAHs) in the Willamette River; upriver, downriver and within the Portland Harbor Superfund megasite for 3 years during wet and dry seasons. Based on an existing Public Health Assessment for this area, concentrations of PAHs in PSDs were substituted for fish tissue concentrations. PSD measured PAH concentrations captured the magnitude, range and variability of PAH concentrations reported for fish/shellfish from Portland Harbor. Using PSD results in place of fish data revealed an unacceptable risk level for cancer in all seasons but no unacceptable risk for non-cancer endpoints. Estimated cancer risk varied by several orders of magnitude based on season and location. Sites near coal tar contamination demonstrated the highest risk, particularly during the dry season and remediation activities. Incorporating PSD data into Public Health Assessments provides specific spatial and temporal contaminant exposure information that can assist public health professionals in evaluating human health risks.

3.2 Introduction

Urban rivers that are used by local residents for recreational purposes such as boating and sport or subsistence fishing are often heavily polluted. Public Health Assessments inform the public about the relative risks of these activities in a specific area by providing information about potential exposures and the likelihood that those exposures could lead to adverse health effects. A Public Health Assessment develops an estimated human exposure dose based on environmental and contaminant data for a specific site and existing regulatory standards *(1)* (more information about Public Health Assessments is available in Appendix 1). Currently, exposure due to consumption of resident organisms is based on tissue contaminant data from fish or shellfish harvested in the area. However, obtaining organisms for analysis can be difficult, usually requires destruction of the organism and often provides limited specific spatial or temporal information *(2)*. Studies have highlighted spatial and temporal variations in contamination and exposure *(3, 4)* and others have called for their consideration in risk assessments *(5)*. Recently, developing methodology for more accurately assessing exposure has become a priority for risk assessment *(6)*. Passive sampling devices (PSDs) can be strategically deployed to address spatial and temporal issues in bioavailable contaminant concentrations, an issue that has been shown to significantly affect risk *(2)*.

PSDs, such as semipermeable membrane devices (SPMDs), simulate biological membranes and lipid tissue and thus sequester only the freely-dissolved or bioaccessible fraction of lipophilic organic contaminants. Huckins et al. *(2)* reviewed over 30 studies with side-by-side comparisons of SPMDs with organisms and found good correlations with finfish and bivalves, though few studies have investigated PAHs specifically *(7-11)*. Correlations between PAHs in SPMDs and organisms have been found in terrestrial and aquatic systems, although investigators observed

differences in the composition of the PAHs sequestered by organisms and PSDs *(7, 8, 12)*. Baussant et al. *(7)* found that lower molecular weight PAHs predominated in caged finfish while Ke et al. *(8)* measured higher concentrations of PAHs in SPMDs compared to tissue from caged carp. While these studies demonstrate that PSD concentrations can be correlated to organism tissue concentrations, they do not link the PSD concentrations to human health risks.

Recent lab and field trials have resulted in simpler and cheaper variants of SPMDs *(13-15)*. These PSDs are constructed from low density polyethylene lay-flat tubing without triolein and designated lipid-free tubing samplers, or LFTs. PSDs, such as the LFT used in this study, offer numerous advantages over using organisms for environmental assessment including simplicity, low cost, fast and minimal extraction and clean-up procedure, no metabolic activity and no organisms are destroyed. Though numerous physical, physiological and ambient factors affect concentrations in organisms, all accumulate contaminants like PSDs: from water across biological membranes *(2)*. Also, unlike organisms, PSDs spiked with performance reference compounds provide chemical specific calibrations of time-integrated, bioavailable concentrations that can be standardized across studies *(2, 15)*. Using PSDs to determine the time integrated water concentration of contaminants is well established, however, this is the first demonstration of the direct application of PSD data for assessing potential human health risks from consumption.

PSDs are particularly useful in areas where point sources are significant contributors to contamination and where seasonal fluctuations in contaminant concentrations are suspected. To this end, the Portland Harbor Superfund megasite on the Willamette River in Portland, Oregon (river miles or RM 3.5-9.2) is an ideal model system for examining the application of PSD data to Public Health Assessments to elucidate potential exposures and risks in an urban river. Portland

Harbor is an industrialized area containing several PAH point sources including coal tar and a remediated former creosoting plant, which is its own superfund site within the larger harbor site. Additional sources of PAHs in the lower Willamette include ship, train and vehicle emissions, combined sewer overflows, urban runoff, atmospheric deposition and petroleum product leaks and spills. Additionally, significant seasonal flow and precipitation fluctuations occur on the river and seasonal variations in contamination concentrations have been observed *(13)*.

The Willamette River is used extensively for both sport and subsistence fishing. Eating contaminated fish from the harbor is considered the most significant health risk from chemical contamination at the site *(16)*. Although fish advisories have been issued for some areas, based on exposure to other industrial contaminants, the most recent Public Health Assessment did not evaluate risk from exposure to PAHs. Of 39 species of resident fish in this area, eight constitute the most likely to be caught and consumed by local sport and subsistence fishers, including walleye, black crappie, white crappie, smallmouth bass, pikeminnow, yellow bullhead, carp and largescale sucker. Clams and crayfish are also commonly harvested for consumption. Details about resident fish as well as fish consumption data for different population groups is available in the Portland Harbor Public Health Assessment *(16)*.

The purpose of this study is to apply PSD data in a Public Health Assessment to demonstrate that PSDs can be used as a biological surrogate to elucidate spatial and temporal variations in potential human health risks. To achieve this, the PSD mass concentrations of PAHs were substituted for fish tissue contaminant concentrations. The spatial and temporal distribution of PSD measured PAH concentrations were applied to cancer and non-cancer human health risk assessment models.

3.3 Materials and methods

3.3.1 Study area. The study area was the lower 18.5 miles of the Willamette River, up to its confluence with the Columbia River. Samplers were placed at 13 sites on west (W) and east (E) sides of the river channel from 2004 to 2006 (Figure 3.1). The sites were located upriver (RMs 18.5E, 17E, 15.5E, 13W, and 12E), downriver (RM 1E) and within the Portland Harbor Superfund megasite (RMs 3.5E, 3.5W, 5W, 6.5W, 7W, 7E and 8E). Residential and commercial uses dominate the upriver area whereas the Superfund megasite area is heavily industrialized and contains PAH point sources including creosote and coal tar contaminated sites at RMs 7E and 6.3W respectively. In addition, urban runoff and combined sewer overflows affect the area. Undeveloped or agricultural areas predominate downriver from the harbor.

The study period overlapped with remediation activities that were carried out at RM 6.3 from August to October, 2005. During this time submerged tar from a manufactured gas plant (MGP) site was removed by dredging and a cap was placed over the contaminated sediment. The temporal effects of this remediation activity are analyzed separately from the seasonal data and serve to highlight the importance of having specific spatial and temporal data for effective risk assessment in areas affected by sporadic peaks in contaminant inputs.

3.3.2 Chemicals and solvents. PAH standards (purities $\geq$ 99%) were obtained from ChemService, Inc. (West Chester, PA, USA) and Pesticide or Optima® grade cleanup and extraction solvents from Fisher Scientific (Fairlawn, NJ, USA) were used. The 16 target analytes, which correspond to the USEPA 16 priority PAHs, included naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene benzo(ghi)perylene, and indeno(1,2,3-c,d)pyrene.

3.3.3 Sample collection, extraction and analysis. LFT passive samplers were constructed and fortified with performance reference compounds (PRCs) using methods described in Sower and Anderson *(13)* Briefly, additive-free, 2.7 cm wide, low-density polyethylene membrane (Barefoot) from Brentwood Plastic, Inc. (St. Louis, MO, USA) was cleaned with hexanes, cut it into 100 cm strips, fortified with dibenz(ah)anthracene as a PRC and heat sealed at both ends.

From 2004 to 2006 samplers were deployed in multiple 21-d events during July or August ("dry season") and October or November ("wet season"). This period represents the transition from the lowest precipitation and river flows of the year to relatively high precipitation and flows. In 2006 two sampling events were added from May through June, the transition from high to low flow. Stainless steel cages were loaded with five LFTs and suspended 3 m above the river bottom at each site with an anchor-cage-float system described elsewhere *(17)*.

A YSI® sonde was used during sampler deployment and retrieval to collect water chemistry data including temperature, pH, specific conductivity, and oxidative-reductive potential (ORP). LFT field cleanup and laboratory extraction were carried out as described in Sower and Anderson *(18)*. Field quality control consisted of duplicate samplers at RMs 7W and 8E, field blanks, trip blanks and field cleanup blanks. Laboratory quality control included reagent blanks, high and low concentration fortifications, and unexposed fortified LFTs.

After extraction, samples were analyzed by HPLC with diode-array (DAD) and fluorescence (FLD) detectors. DAD signals were 230 and 254 nm and FLD excitation and emissions were 230 and 332, 405, 460, respectively. Flow was 2.0 mL/min beginning with 40/60% acetonitrile and water and steadily ramping to 100% acetonitrile over a 28 min run per column maker recommendations.

3.3.4 Exposure, cancer, non-cancer and ecological risk modeling. Water concentrations were calculated using equations provided in Huckins et al. *(2)*. PSD concentrations for risk models are based on the mass of contaminant collected vs. the mass of the sampler. This mass:mass concentration treats the PSD as a direct biological surrogate and represents the amount of contaminant an organism would take up through passive partitioning. PAHs do not biomagnify in fin fish and chemical uptake from water and/or pore water has been described as the most likely dominant route of uptake for fish and shellfish *(2, 19)*. LFT concentrations best reflect exposure of organisms residing in the water column; benthic fauna and infauna may be exposed to different sediment and/or pore water PAH concentrations.

Exposures and human health endpoints were calculated by substituting the PSD mass concentrations for the fish tissue contaminant concentrations in models previously used for the Portland Harbor Public Health Assessment *(16)*.

The PSD mass concentrations of PAHs that are recognized as carcinogenic by the USEPA (benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3,-c,d)pyrene, and dibenzo(a,h)anthracene) were used for cancer risk modeling. The PSD mass concentrations of PAHs that are not recognized as carcinogens were used in the non-cancer endpoint risk model. The other equation variables and default values in both the cancer and non-cancer risk models are the same as those used in the Portland Harbor Public Health Assessment *(16)* (Table A1.1).

Exposure (ug $kg^{-1}day^{-1}$) was calculated using Equation 1 where C is the mass concentration (PSD substituted for fish), CF is a conversion factor, EF and ED are exposure frequency and duration, respectively, BW is body weight and AT is the averaging time (Table A1.1).

$$Exposure = \frac{C \times CF \times IR \times EF \times ED}{BW \times AT} \qquad \text{Eq. 1}$$

The ingestion rates (IR) are the 90th (17.5 g day^{-1}) and 99th percentiles (142.4 g d^{-1}) for fish consumption that were used in the Portland Harbor Public Health Assessment *(16)* that evaluated local sport and subsistence angling populations. These rates may not apply to other situations. In order to assess potential public health implications of exposure, estimates of exposure can be compared to estimates of a dose that is likely to be without appreciable risk of deleterious effects, such as minimal risk level (MRLs) or reference doses (RfD) *(16)*.

Excess cancer risk was determined by normalizing the slope factors for carcinogenic PAHs to benzo(a)pyrene and then multiplying by the sum contaminant exposure (Equation 2). Unacceptable cancer risk, as a matter of policy, was set at an excess of one in one million (1 x 10^{-6}).

$$Excess \quad Lifetime \quad Cancer \quad Risk = Exposure \times Slope \quad Factor \qquad \text{Eq. 2}$$

For the non-cancer endpoint each contaminant's exposure was divided by a chronic RfD or MRL to determine a hazard quotient (HQ) for the chemical (Equation 3). The sum of the HQs for the individual chemicals yields the hazard index (HI) and, as a matter of policy, a HI exceeding one represents an unacceptable risk.

$$Hazard \ Quotient = \frac{Exposure}{Rfd \ or \ MRL} \qquad \text{Eq. 3}$$

Analysis of exposure data was carried out using S-plus® (8.0, Insightful Corp.); Wilcoxon rank sum tests were used for seasonal comparisons and Kruskal-Wallis was used for analysis of spatial differences in exposure, followed by multiple comparisons using the Tukey 95% simultaneous confidence intervals method. Spatial and temporal

differences in cancer risk were analyzed using Mann-Whitney rank sum tests in SigmaStat®. SigmaPlot® was used for graphing.

3.4 Results

A total of 110 samples, from 3 years and 10 different sampling events are included in this study: six dry (summer) and four wet season (fall and spring) events, defined by river flow. The wet season is defined as flow greater than 300 m^3/s; median flows were higher during the wet season (494 $m^3\ s^{-1}$) than during the dry season (246 $m^3\ s^{-1}$, $p<0.001$). Results for water chemistry parameters support the seasonal delineation; the dry season had higher temperature (22 vs. 16° C), higher specific conductivity (0.1 vs. 0.08 mS cm^{-1}), lower ORP (139 vs. 196 mV; all n = 17, $p<0.05$), but no difference in pH (7.4, $p = 0.9$).

Relative standard deviation (RSD) for the PAH concentrations at duplicate sites averaged 19%. Target compounds in blanks were either non-detect or below levels of quantitation. Results are recovery corrected. Recoveries from method spikes range from an average 43% for NAP, the lowest molecular weight and most volatile PAH, to 108% for IPY, with an overall average of 77%.

A detailed analysis of spatial and temporal variations of water concentrations of PAHs in the lower Willamette River can be found in Sower and Anderson *(13)*. Briefly, the sum concentration of 16 PAH analytes (Σ_{16}PAH) in the Superfund area (11.4 ng L^{-1}) is significantly higher than upriver sites (3.1 ng L^{-1}, $p<0.001$), but not downriver sites. The upriver area does not exhibit significant variation among sites, but Superfund sites do. RMs 7W, 6.5W and 5W are consistently the most contaminated sites. None of the average concentrations for any site exceed the EPA human health Water Quality Criteria for consumption of water (3.8 ng L^{-1}) or 'water +

organism' (18 ng L^{-1}) *(20)* for the total carcinogenic PAHs, though some sites exceeded the threshold seasonally or during specific sampling events.

3.4.1 Comparison of PSD and fish tissue concentration data. While it is widely understood that humans do not consume passive samplers, comparisons of PAH concentrations in PSDs and fish tissue from the Portland Harbor Superfund site demonstrate that using PSD concentrations in a public health assessment would provide a reasonable and conservative estimate of exposure that would be protective of human health without significantly overestimating risk. Table 3.1 presents fish tissue data from the Lower Willamette Group *(21)*, some of which was used in the Portland Harbor Public Health Assessment *(16)* as well as PSD data from this study. The fish and shellfish were collected from Portland Harbor during a period that overlapped with the PSD study; however these two studies are unrelated to one another. Furthermore, it is important to highlight that PAHs were not included in the Portland Harbor Public Health assessment because of insufficient data *(16)*; therefore, the data presented in Table 3.1 is based on a limited sample set. The side-by-side comparison demonstrates that PSDs from this study captured the magnitude, range and variability of PAH concentrations that have been reported in a variety of fish and shellfish tissues from the harbor and provide an estimate of exposure that is realistic and protective.

3.4.2 Spatial and temporal variations in PAH exposure. As detailed in the methods section, exposure to PAHs from consumption of fish is dependent on a number of factors; some of which have standard values in risk assessment models, and others that are determined for specific human populations, such as consumption rates of organisms. In this study, the mass:mass concentrations of PAHs in LFT passive samplers are substituted for fish tissue concentrations in the exposure formula.

Exposure is therefore a factor of consumption rate on the measured PSD concentrations.

To avoid confounding the interpretation of spatial differences in exposure to PAHs, data that were acquired during the tar removal dredging in the superfund have been removed from these analyses. The effects of remediation activities on exposure and risk are discussed later in the results.

Significant differences in PSD concentrations of the Σ_{16}PAH were observed within and outside of the Superfund megasite ($p<0.001$). A median Σ_{16}PAH concentration of 603 ug kg^{-1} in the Superfund was significantly greater than 431 ug kg^{-1} at upriver sites ($p<0.001$) but not greater than the downriver area. Similarly, significant differences in carcinogenic PAHs were observed ($p<0.001$), where median exposure was greater within the Superfund (12.1 ug kg^{-1}) than upriver (5.7 ug kg^{-1}) but not downriver.

A more detailed analysis of PSD concentrations shows significant differences between sites, both within and outside of the Superfund megasite for Σ_{16}PAH and carcinogenic PAHs ($p=0.002$ and $p<0.001$ respectively). Exposure to Σ_{16}PAH is greater at RM 7W and 3.5W (1110 and 1150 ug/kg medians respectively) than three upriver sites, which had median concentrations between 353 and 466 ug kg^{-1}. A similarly high median concentration of Σ_{16}PAH was observed in PSD extracts from RM 6.5W (1270 ug kg^{-1}); however this site was not differentiated from other sites in the analysis, likely due to a smaller sample size. Furthermore, the PSD concentration at RM 7W was significantly greater than at RM 8E (448 ug kg^{-1} median), though both sites are located within a mile of each other in the Superfund megasite (Figure 3.2).

Median PSD concentrations of carcinogenic PAHs were greater at RM 3.5W (22.3 ug kg^{-1}) than RMs 18.5E, 17E and 12E (4.2, 5.5 and 6.4 ug kg^{-1} respectively).

Additionally, it was greater than at RM 8E (10.1 ug kg^{-1}), which is also located in the Superfund megasite. Interestingly, RM 7W did not differentiate itself from other sites with regards to carcinogenic PAHs, although it had significantly higher levels of total PAHs than sites located both within and outside of the Superfund area (Figure 3.2).

No differences in exposure to total or carcinogenic PAHs were observed between the wet and dry seasons at the upriver or downriver sites. In contrast, significant differences were observed between the wet and dry season for both carcinogenic and total PAHs within the Superfund megasite ($p<0.001$). PSD concentrations of PAHs in the Superfund megasite were greater during the dry than the wet season (1470 and 442 ug kg^{-1} respectively). Similarly, median concentrations of carcinogenic PAHs were 33.5 ug kg^{-1} in the dry season compared to 8.5 ug kg^{-1} in the wet season.

3.4.3 Spatial and temporal variations in cancer and non-cancer risk. All areas exceed the established threshold of one excess cancer risk in 1,000,000 (1×10^{-6}). Estimated risk of cancer in excess of the background rate for the Superfund megasite and downriver (1.3×10^{-5} and 1.7×10^{-5}, respectively, for average consumption) were significantly higher than upriver sites (4.5×10^{-6}, $p<0.001$) (Figure 3.3). Within the Superfund megasite, estimated excess risk was up to five times greater at RM 7E than 7W; sites located on opposite banks and separated by only a few hundred meters (Table 3.2). These estimated numbers of cancer cases in excess of the background are based on the assumption, from the risk assessment model applied here, that all individuals are equally exposed.

Non-cancer risk from PAHs was also higher at Superfund and downriver sites than urban areas ($p<0.001$) with RMs 7W, 6.5W, and 3.5W exhibiting the highest hazard quotients, though all were below one by more than two orders of magnitude (Table 3.2).

The increased PAH concentrations in the Superfund area during the dry season result in significantly elevated risk (Figure 3.2). Both non-cancer HQs and excess cancers increased during the dry period ($p=0.004$ and $p<0.001$ respectively), however the non-cancer HQs remained below unacceptable risk levels (Table 3.2). The cancer model predicts four times greater cancer risk from fish consumption in the Superfund area during the dry season compared to wet season. Notably, the excess cancer risk at RM 7W from average consumption of PSD measured mass concentrations increases by seven-fold during the dry season from 7.8×10^{-6} to 6.0×10^{-5} ($n = 18$, $p = 0.005$).

3.4.4 Effects of remediation activities on risk. Dredging of a coal tar contaminated site at RM 6.3W removed more than 11,500 m^3 of submerged tar contamination from August to October 2005. LFT samplers downriver at RM 3.5W and upriver at RM 7W during this period accumulated significantly elevated Σ_{16}PAH and carcinogenic PAH concentrations (Figure 3.2). September 2005 samples, taken during the middle of the dredging activity, from RM 7W down to RM 1 are the highest concentrations of Σ_{16}PAH and carcinogenic PAHs recorded during this study (Figure 3.2). The median Σ_{16}PAH and carcinogenic PAH pre- and post-tar removal are significantly lower than the tar removal median. The highest observed carcinogenic PAH concentrations in water measured during this study occurred at RMs 7W and 5W in September 2005 (71 ng L^{-1} and 20ng L^{-1}, respectively). At RM 3.5W, chrysene and benz(a)anthracene exceed the US EPA Water Quality Criteria limit of 3.8 ng L^{-1} for the consumption of water and organism, while at RM 7W benzo(b)fluoranthene and benzo(a)pyrene exceeded this limit and chrysene and benz(a)anthracene exceeded the 18 ng L^{-1} limit for the consumption of organisms.

3.5 Discussion

PSDs are well established for determining the water concentrations of freely dissolved and thus bioavailable, organic contaminants *(2, 15, 22)*. Their use for risk assessment is less well established, however, they respond to the need for biologically relevant exposure data *(6)* and they can be standardized across studies. Furthermore, initial comparisons of PAH concentrations in PSDs and fish tissue demonstrate that PSDs capture the magnitude and variability of PAH exposure, and thus are an adequate surrogate for this parameter in some risk models. Obtaining PSD data from sites within and outside of the Superfund area provided a more representative range of concentrations for highly mobile fish species that are likely to move through large areas of the river and might avoid contaminated areas. Conversely, less motile or sessile organisms, such as crayfish and clams from the Superfund area had concentrations of PAHs in their tissues more closely aligned with PSD data from the Superfund area. Fin fish, unlike PSDs, ingest and metabolize PAHs, however passive partitioning has been shown to be the principal route of uptake *(19)* and the results of this study concord with other publications that demonstrate the comparability of PSDs (SPMDs) with finfish and bivalves *(2)*. As mentioned by other researchers *(7, 8)* a comparison of this study to fish tissue data from the area (Table 3.1) demonstrated higher concentrations of low molecular weight PAHs in fin fish than PSDs. This observation merits further study; however, due to these compounds classification as non-carcinogenic and their relatively high MRLs, the lower concentrations observed in PSDs do not have a significant effect on the outcomes of a public health assessment based on the PSD data.

Using PSDs as direct biological surrogates by measuring unmetabolized parent compounds through mass:mass concentrations reveals a more complete exposure potential. In Portland Harbor, the large number of PSD samples over several seasons

and years, provided a much more complete understanding of risk for the area, with specific spatial and temporal resolution that proved to be significant. Notably, risk from exposure to PAHs from consumption of fish had not been evaluated in the Public Health Assessment for Portland Harbor due to insufficient fish data. Using PSDs in place of organisms eliminates problems associated with capturing samples, destructive sampling and analyzing compounds in an analytically complex biological matrix.

Temporal disparities in exposure and estimated risk were observed in the Superfund area. Several studies have observed higher PAH concentrations with increasing precipitation, flows, and urban runoff *(3, 4, 23)* and Stout et al. *(24)* note that storm water is the greatest contributor to sediment PAHs over time. However, our data demonstrate an opposite tendency, where the dry season is associated with higher water concentrations, higher exposure, and consequently higher risk, in the Superfund area. Dilution does not explain the concentration and risk disparities between wet and dry seasons in the Superfund area either. Unlike the Superfund sites, upriver and downriver areas do not demonstrate seasonal variations. If the observed differences in the Superfund were due to dilution, this should be a uniform effect in the river. One potential explanation for the seasonal differences observed only within the Superfund site, and especially at 7W, 6.5W and 3.5W, is that contaminant diffusion from sediments into overlying water is responsible for high concentrations. The contamination may be from riverbank sediments and higher wet season flows could inhibit groundwater movement into the river due to hydraulic pressure and bank storage *(13, 25)*. Another possible explanation is that higher summer temperatures cause greater contaminant diffusion from the sediment to the water column. Further investigation is required to elucidate sources of seasonal disparities in PAH contamination in the Superfund area.

A sediment cap over creosote contaminated sediments at RM 7E, installed prior to this study, was found to be effective in preventing PAH contamination into the overlying water column *(13)* but did not diminish RM 7W high concentrations. The cause of the significant difference observed between sites located in close proximity to one another, such as RMs 7E, 7W and 8E, merits further study. It also highlights the importance of considering spatial differences in risk on a small scale, which can be achieved by taking PSD data into account in risk assessments.

While remediation of contaminated sites is desirable, few studies have assessed the potential impacts of dredging on exposure and risk during and after remediation *(26)*. This study provided an opportunity to evaluate the effects of dredging on PAH bioavailability and potential human health risks from exposure. Prior to capping, dredging at RM 6.3W removed significant quantities (> 11,500 m^3) of coal tar; however the area remains a higher risk with higher freely-dissolved PAH concentrations than surrounding areas, particularly in the dry season.

This study demonstrates an association between variable flows, sediment disturbance and freely-dissolved and, thus, bioavailable contamination in the water column. Although the dredging produced a spike in exposure to PAHs, and a corresponding increase in risk values, the duration of the effect was limited to the time that it took to complete the operation. The short duration of the disturbance would only be expected to have an immediate and more substantial effect on aquatic organisms. Though fish kills were observed within the containment area, none were observed outside the barriers *(27)*.

The site downriver from the Superfund megasite, RM 1E, is not significantly different in concentration from the Superfund sites. While the Portland Harbor Public Health Assessment only sampled within the Portland Harbor Superfund sites, our data demonstrate that the downriver site has similar concentrations and could pose

similar health risks. Seasonal and spatial information like this could be useful to public health officials when constructing a health assessment or determining where to post warning signs.

3.6 Uncertainty evaluation

Some degree of uncertainty is inherent in the risk assessment process. This uncertainty can be introduced at various stages and can lead to over- or underestimation of risk. Some of these uncertainties are a result of the assumptions used in the model, whereas others are the results of the characteristics of the data. A risk assessment performed using PSDs has some of the same uncertainties as one conducted using fish tissue data; however there are other sources of uncertainty that are unique to this approach.

Uncertainty in the exposure assessment in this study is partially due to substituting PSD concentrations for fish tissue data. Although this study demonstrates that PSDs could be an adequate surrogate for fish tissue data in some risk assessment models, there is uncertainty about the exact contaminant concentrations that would be present in fish tissue that is consumed. Unlike fish, PSDs do not move in the environment nor do they metabolize or excrete PAH compounds. PSDs represent passive uptake by organisms, however in cases where dietary intake, metabolism and/or excretion are predominantly determinant in body burden further research could help elucidate the ability of PSDs to serve as surrogates for fish tissue. Initial comparisons of PSDs and resident organism tissue data suggest the PSDs may overestimate exposure to PAHs in the case of finfish and underestimate it in the case of shellfish. Additionally, PSDs do not provide species-specific information, which can significantly affect exposure.

Concentrations of contaminants in PSDs, as well as fish and shellfish, present uncertainty in risk assessments because they represent a site- and time-specific value that may not be representative of lifetime exposure. For example, inputs to the Portland Harbor area can change and remediation may significantly affect short- and long-term contaminant levels in biota. Concentrations of contaminants that are below the detection limit (DL) of the analytic method are another source of uncertainty. Results below the DL do not necessarily indicate the complete absence of a compound. The EPA recommends using one-half DL in cases where a contaminant is below DL because other substitutions have a greater potential to lead to a significant over- or underestimation of exposure.

Another area of significant uncertainty is the consumption rates of fish and shellfish that were applied to the assessment. The rates applied in this study were those used in the Public Health Assessment for the Portland Harbor Superfund, which were the 90^{th} and 99^{th} percentile per capita ingestion rates for people age 18 or older in the United States. These nation-wide values may not be representative of the local population. For example, higher fish consumption rates have been documented for Native American communities in the area (28). Furthermore, most people that eat fish consume a variety of different species, collected from different areas, and interspecies and spatial differences could significantly affect exposure. Exposure duration and body weight were standard values that may not be representative of all individuals in the population being considered.

Consumption patterns and preparation methods are another area of uncertainty in the exposure calculation. For example, consuming a small number of large portions of fish in a short time span may present a different risk than consuming the same quantity of fish in smaller portions spread over a longer period of time. Preparation and cooking processes can significantly affect the amount of

chemical intake by consumers. Removing internal organs and high-fat tissues could significantly decrease the concentrations of lipophilic contaminants such as PAHs compared to the whole-body concentration. Certain cooking methods may decrease exposure to PAHs, whereas others, such as smoking fish, may introduce additional PAHs.

3.7 Conclusions

PSDs provide spatially and temporally resolved contaminant exposure information that, as demonstrated here, can be incorporated into risk assessment models. This study revealed significant spatial and temporal differences in risk that would not have been elucidated in a traditional risk assessment, such as the Portland Harbor Public Health Assessment. Although it is clear that humans do not consume PSDs, their application as a biological surrogate in risk assessment models has the potential to provide specific spatial and temporal contaminant exposure information that can assist public health professionals in accurately evaluating human health risks. Furthermore, using PSDs for risk assessment has the advantages of larger sample size, non-destructive sampling and comparability across studies. PSDs provide biologically relevant exposure data for risk assessment that could be used when organism data is not available or to complement, and further refine, other measures of exposure.

3.8 Acknowledgements

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3.9 Supporting information

Supporting information and tables about public health assessments and the values used in the exposure calculation are available in Appendix 2.

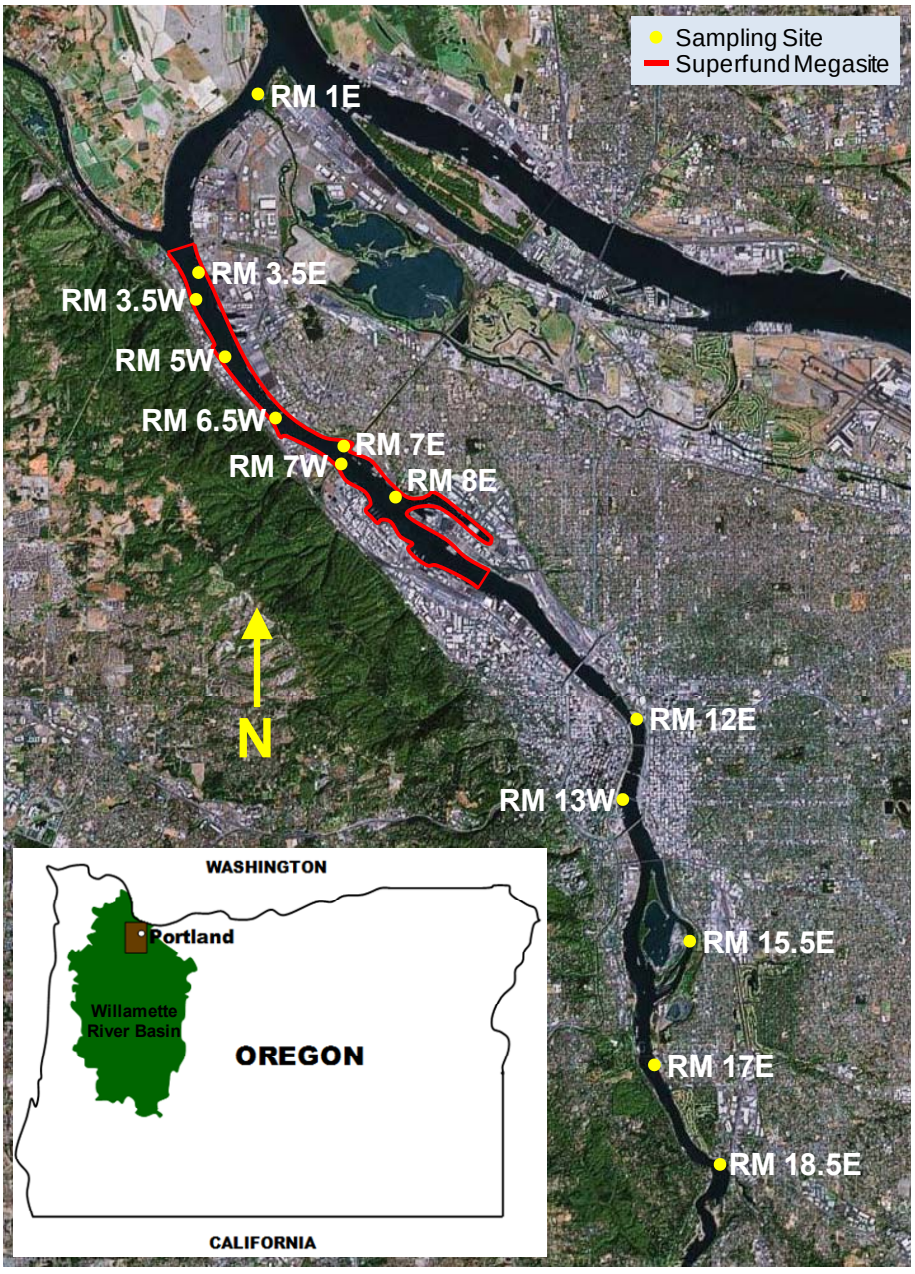

Figure 3.1 Sampling sites on the lower Willamette River 2004-2006.

Each site is designated by a yellow circle. Not all sites were used every deployment. The red line indicates the approximate boundaries of the Portland Harbor Superfund megasite.

Table 3.1 Concentrations of PAHs in PSDs and fish and shellfish tissue from the Portland Harbor Superfund site

	Concentration (ug/kg) - Average (Maximum)						
	PSD[1]		Fish and Shellfish from Superfund[2]				
Chemical	Superfund	Upstream	Smallmouth Bass	Carp	Sculpin	Crayfish	Clam
Naphthalene	1.0 (6.5)	0.7 (3.8)	10 (86)	20 (56)	19 (250)	0.82 (2.9)	25 (78)
Acenaphthene	5.5 (54)	0.02 (1.1)	13.7 (95)*	34.1 (75)*	NA	NA	NA
Fluorene	13 (84)	5.4 (70)	9.31 (69)*	22.3 (53)*	NA	NA	NA
Phenanthrene	44 (219)	4.9 (24)	20 (85)	10 (16)	6.8 (33)	52 (97)	35 (300)
Fluoranthene	170 (850)	24 (57)	2.77 (36)*	NA	NA	10.2 (130)*	NA
Benz(a)anthracene	51 (504)	10 (44.6)	NA	NA	NA	2.01 (80)*	NA
Chrysene	36 (172)	10 (28)	20 (85)	NA	NA	2.16 (87)*	NA
Pyrene	170 (733)	35 (92)	2.9 (39)*	NA	NA	4.02 (83)	NA
Benzo(a)pyrene	14 (70)	4.1 (21)	0.64 (1.3)	NA	NA	1.1 (7.5)	34 (490)
Σ_{16}PAH	819 (3094)	397 (1147)	71.5 (308)	85.5 (222)	52.3 (550)	71.2 (477)	478 (4980)
Σcarcinogenic PAH	23 (123)	7.6 (25.2)	2.5 (6.8)	2.1 (2.8)	3.18 (9.8)	22 (170)	220 (2700)

[1] PSD measured concentrations of PAH analytes (this study)

[2] Data from the Lower Willamette Group Portland Harbor RI/FS *(21)* except where noted (*)

*Data from Portland Harbor Public Health Assessment *(16)*

NA indicates that data was not available for this publication

Notes: PSD average and maximum concentrations are based on measurements made during the study period; data obtained in the superfund during tar removal remediation were not included. Concentrations in organisms correspond to reported whole body measurements in fish and shellfish obtained from the Portland Harbor Superfund site in an unrelated study. Not all analytes used to compute reported totals were available to be shown in this table.

Table 3.2 Cancer and non-cancer risk associated with consumption of fish estimated by PSD[a,b]

River location and Season		N	Non-cancer hazard quotient Consumption Average	Non-cancer hazard quotient Consumption High	p-value	Excess cancer risk Consumption Average	Excess cancer risk Consumption High	p-value
Upriver		37						
	Wet	13	$2.0x10^{-4}$	$1.3x10^{-3}$		$3.8x10^{-6}$	$3.1x10^{-5}$	
	Dry	24	$4.0x10^{-4}$	$3.3x10^{-3}$	0.07	$5.6x10^{-6}$	$4.6x10^{-5}$	0.31
Superfund Megasite		64						
	Wet	28	$9.3x10^{-4}$	$7.6x10^{-3}$		$6.7x10^{-6}$	$5.4x10^{-5}$	
	Dry	36	$3.3x10^{-3}$	$2.7x10^{-2}$	0.004	$2.6x10^{-5}$	$2.1x10^{-4}$	<0.001
Superfund No tar events		45						
	Wet	22	$3.6x10^{-4}$	$3.0x10^{-3}$		$6.5x10^{-6}$	$5.3x10^{-5}$	
	Dry	23	$2.9x10^{-3}$	$2.4x10^{-2}$	<0.001	$2.2x10^{-5}$	$1.8x10^{-4}$	<0.001
RMs 7W & 5W		28						
	no tar	19	$1.1x10^{-3}$	$8.5x10^{-3}$		$1.5x10^{-5}$	$1.2x10^{-4}$	
	tar	9	$4.5x10^{-3}$	$3.7x10^{-2}$	0.02	$9.1x10^{-5}$	$7.4x10^{-4}$	<0.001
Downriver		10						
	Wet	4	$3.4x10^{-4}$	$1.5x10^{-2}$		$9.1x10^{-6}$	$7.4x10^{-5}$	
	Dry	6	$1.9x10^{-3}$	$2.8x10^{-3}$	0.11	$1.8x10^{-5}$	$1.5x10^{-4}$	0.18
			Threshold = 1			Threshold = $1.0 x10^{-6}$		

[a] Mann-Whitney rank sum tests within location between seasons.
[b] P-values are for comparisons between seasons
All test are at $\alpha = 0.05$.

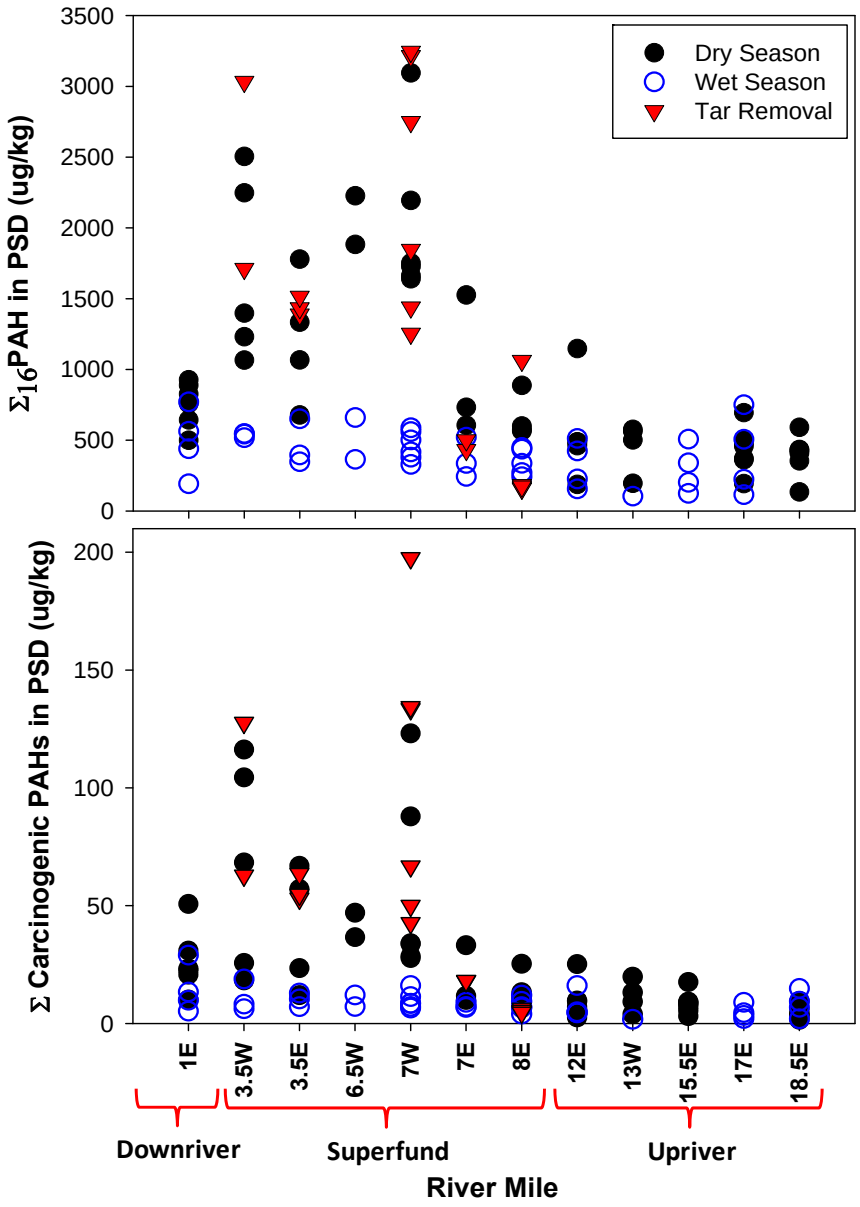

Figure 3.2 Σ_{16}PAH and carcinogenic PAHs in PSDs

Mass-to-mass concentration of sum PAHs and sum carcinogenic PAHs in passive sampling devices (PSDs) at sites downriver, upriver and within the Portland Harbor Superfund megasite. Each point represents one observation during the dry season (closed circles), wet season (open circles) or tar removal remediation (triangles). These values were used in place of fish tissue concentrations to calculate exposure for risk assessment models.

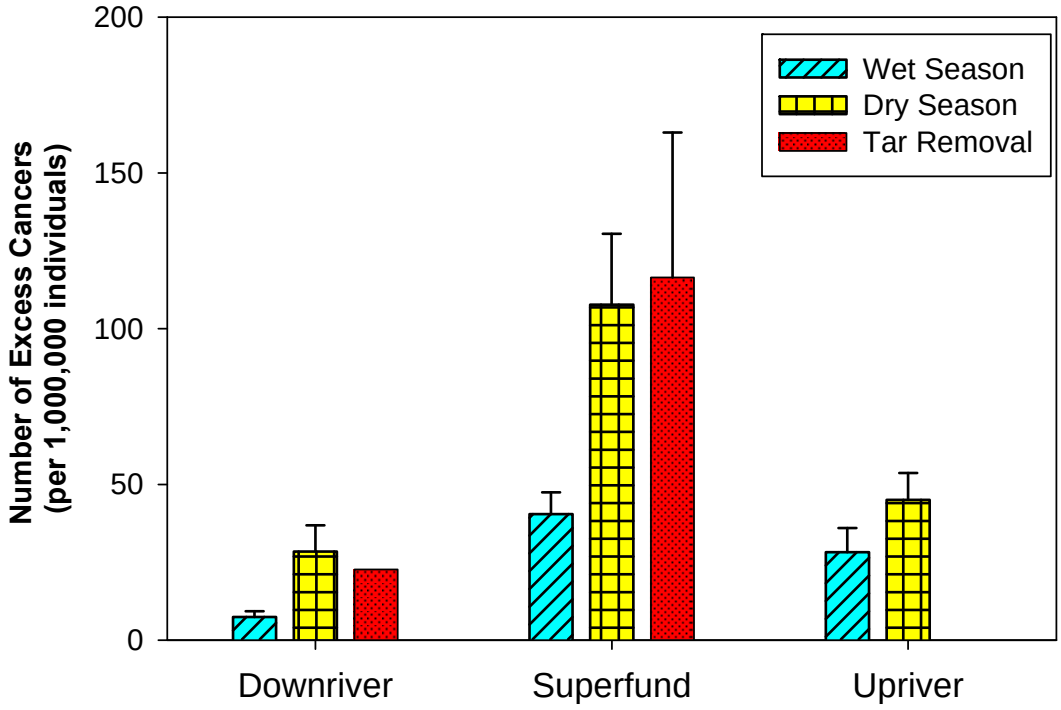

Figure 3.3 Estimated number of cancers, in excess of the background rate, per 1,000,000 individuals exposed to carcinogenic PAHs

Calculations are based on average fish consumption rates; where LFT concentrations have been substituted for fish tissue concentrations. Data from all sites located in each area of the river (upriver, downriver and within the Superfund) were averaged for the wet and dry seasons and observations associated with tar removal remediation activities are presented separately. Error bars represent 95% confidence intervals, based on variability in the PSD measured concentrations for each site-season, and only one observation was made at the downriver site during tar removal. See Table 3.2 for statistical analyses of these data.

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Chapter 4 - Bioavailable polycyclic aromatic hydrocarbons in coastal waters of the Gulf of Mexico

4.1 Abstract

An estimated 4.4 million barrels of oil and 2.1 million gallons of dispersants were released into the Gulf of Mexico during the Deepwater Horizon oil spill and there is a continued need for information about the impacts and long-term effects of the disaster on the Gulf of Mexico. The objectives of this study were to assess bioavailable polycyclic aromatic hydrocarbons (PAHs) in the coastal waters of four Gulf Coast states that were impacted by the spill and apply source-modeling techniques to elucidate potential sources of chemicals of concern. For over a year, beginning in May 2010, passive sampling devices were used to monitor the bioavailable concentration of PAHs. Prior to shoreline oiling, baseline data was obtained at all the study sites. Significant increases in bioavailable PAHs were observed following the oil spill, however, pre-oiling levels were observed at all sites by March, 2011. A return to elevated PAH concentrations, accompanied by a chemical fingerprint similar to that observed while the site was being impacted by the spill, was observed in Alabama in summer, 2011. Chemical forensic modeling demonstrated that elevated PAH concentrations are associated with a petrogenic signature and distinctive chemical profiles.

4.2 Introduction

On April 20th, 2010 a lethal explosion at the Deepwater Horizon oil drilling rig, managed by BP and located 66 km southeast of the Louisiana coast in Mississippi Canyon Block 252, led to the largest marine oil spill in United States history. Estimates of the amount of oil spilled into the ocean vary, however calculations from independent researchers indicate that approximately 4.4 million barrels of oil (7.0 x

10^5 m^3) were released *(1)*. Furthermore, an estimated 2.1 million gallons of dispersants were applied at the ocean surface and wellhead *(2)*.

Crude oil may contain up to 7% PAHs by weight *(3)* and many PAH compounds are toxic and/or carcinogenic to humans and wildlife. The water solubility and volatility of PAHs decreases as their molecular weight increases; however, low water concentrations of PAHs can be environmentally relevant due to their potential to bioaccumulate in organisms *(4, 5)*. In the case of marine oil spills, such as the Deepwater Horizon spill, there is an initial, acute risk to organisms that can become covered in viscous crude as well as acute and chronic risks from exposure to toxic chemicals through air, water and food. Even after the oil is no longer visible, chemicals of concern can persist in the environment *(3)* and affect exposed organisms *(5, 6)*. It is the freely dissolved fraction of chemicals that is bioavailable to enter organisms and the food web *(7)*. The application of chemical dispersants during the Deepwater Horizon oil spill was a source of contention, in part because it has been demonstrated that their application to crude oil makes PAHs and other hydrophobic compounds more soluble in water, increasing their bioavailability *(8-11)*.

Passive sampling devices (PSDs) were developed to address the issue of quantifying the bioavailable fraction of hydrophobic compounds in environmental media. They sequester and accumulate the freely dissolved, and therefore bioavailable fraction of hydrophobic organic contaminants, such as PAHs; mimicking passive uptake and accumulation of these compounds by biomembranes and lipid tissues. PSDs provide a time integrated measure of the concentration of chemicals in the environment and, by effectively sampling a large volume of water, allow for the detection of chemicals that are present at low concentrations *(12)*. Fortifying PSDs with performance reference compounds (PRCs) prior to field deployment allows for an accurate determination of sampling rates, which can be used to calculate the bioavailable concentrations of chemicals in the water *(12, 13)*. A variety of different

polyethylene membrane PSDs have been applied a range of environmental media *(12, 14-18)* including petroleum-contaminated water *(19-22)*. More recently, variants of the semi-permeable membrane device (SPMD) sampler that do not contain triolene have been developed and validated *(14, 23-25)*. These lipid-free tubing (LFT) PSDs are cheaper and require less clean-up prior to analysis than SPMDs.

The objective of this study was to assess the impact of the Deepwater Horizon oil spill on bioavailable PAHs at coastal sites in Gulf of Mexico. A rapid response to news of the explosion on the drilling rig allowed for baseline data from coastal waters to be collected prior to the oil reaching any of the study sites. Understanding spatial and temporal changes in bioavailable PAHs provides information about potential exposures to contamination that can be broadly applied to many areas including biology, ecology, public health and seafood safety in the Gulf of Mexico. A second objective was to apply forensic chemistry source modeling techniques to elucidate sources of the bioavailable chemicals of concern that were observed before, during and after the oil spill.

4.3 Materials and Methods

4.3.1 Sample collection. Lipid-free tubing PSDs were constructed from low-density polyethylene tubing and fortified with deuterated performance reference compounds (PRCs) using methods detailed elsewhere *(26)*. The PRCs allow for the determination of *in situ* uptake rates *(12)*. Stainless steel cages containing five, 1 m PSDs were deployed from piers in coastal marine waters. The samplers were suspended in the water column at least 1 m above the bottom. Water depths varied by site and tide cycle between 2-8 m. When necessary, precautionary measures were taken to prevent direct contact of the sampling material with crude oil floating on the surface of the water.

Samplers were deployed at four sites: Grand Isle, Louisiana, Gulfport, Mississippi, Gulf Shores, Alabama and Gulf Breeze, Florida (Figure 4.1). The sampling sites in Alabama and Florida were at the mouth of Mobile Bay and Pensacola Bay, respectively. The first sampling event began on May 10th, 2010 and was carried out after the explosion of the Deepwater Horizon drilling rig but prior to shoreline oiling at any of the study sites. A total of nine sampling events were conducted over the course of more than a year (names in quotes refer to the shorthand used in text and figures): 'May 2010' (May 10-13, 2010), 'June (1)' (June 8-11, 2010), 'June(2)' (June 11-July 7, 2010), 'July' (July 7-August 5, 2010), 'August' (August 5-September 8, 2010), 'September' (September 8-October 13, 2010), 'March 2011' (February 9-March 15, 2011), 'April' (March 15-April 29, 2011), 'May' (April 29-June 8, 2011).

4.3.2 Sample preparation. PSDs were retrieved from the field, transported to laboratory and cleaned with hydrochloric acid and isopropanol to remove superficial fouling and water. The 5 PSDs from each cage were extracted together as one sample to increase detection capabilities. Samplers were extracted by dialysis in n-hexane; 40 mL per PSD for 4 hours, the dialysate was decanted then dialysis was repeated for 2 hours and the dialysates were combined. Samples were quantitatively concentrated to a final volume of 1 mL.

4.3.3 Chemicals. Solvents used for pre-cleaning, clean-up and extraction were Optima® grade or better (Fisher Scientific, Pittsburgh, PA). The following 33 PAH analytes were included in analyses: naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 1,2-dimethylnaphthalene, 1,6-dimethylnaphthalene, acenaphthylene, acenaphthene, fluorene, dibenzothiophene, phenanthrene, 1-methylphenanthrene, 2-methylphenanthrene, 3,6-dimethylphenanthrene, anthracene, 2-methylanthracene, 9-methylanthracene, 2,3-dimethylanthracene, 9,10-dimethylanthracene, fluoranthene, pyrene, 1-methylpyrene, retene, benz(a)anthracene, chrysene, 6-methylchrysene, benzo(b)fluoranthene,

benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and dibenzo(a,l)pyrene. The deuterated PAH compounds used as PRCs were fluorene-D10 p,p'-DDE-D8 and benzo(b)fluoranthene-D10. The following deuterated PAHs were used as surrogate recovery standards: naphthalene-D8, acenaphthylene-D8, phenanthrene-D10, fluoranthene-D10, pyrene-D10, benzo(a)pyrene-D12 and benzo(g,h,i)perylene-D12; and perylene-D12 was the internal standard.

4.3.4 Sample analysis. PSD extracts were analyzed using Agilent 5975B Gas Chromatograph-Mass Spectrometer (GC-MS); with a DB-5MS column (30 m x 0.25 mm x 0.25 μm) in electron impact mode (70 eV) using selective ion monitoring (SIM). The GC parameters were as follows: injection port maintained at 300 °C, 1.0 mL min^{-1} helium flow, 70 °C initial temperature, 1 min hold, 10 °C min^{-1} ramp to 300 °C, 4 min hold, 10 °C min^{-1} ramp to 310 °C, 4 min hold. The MS temperatures were operated at 150, 230 and 280 °C for the quadrupole, source and transfer line respectively. Sample concentrations were determined by the relative response of the deuterated surrogate to the target analyte in a nine point calibration curve with a correlation coefficient greater than 0.98.

4.3.5 Quality assurance/control. Quality control accounted for over 30% of the samples analyzed and included laboratory preparation blanks, field and trip blanks for each deployment/retrieval, laboratory clean-up blanks and reagent blanks. All target compounds were below the detection limit in all blank quality control samples.

Mean surrogate standard recoveries varied between 48-102% for naphthalene-D8 and benzo(g,h,i)perylene-D12 respectively. Lower recoveries were observed for 2-3 ring PAHs, which are relatively volatile, due to losses during sample preparation. Target analytes were recovery corrected during concentration determination based on the measured recovery of the surrogate with the most

similar structure. The average relative standard deviation (RSD) for all analytes from replicate samples was 7.5%. Napthalene and its methylated derivatives had the highest RSDs; averaging 21%

4.3.6. Water concentration calculation. Water concentrations were calculated using the empirical uptake model with PRC-derived sampling rates detailed elsewhere *(12)*. Fluorene-D10 and benzo(b)fluoranthene-D10 PRCs were used in the calculations. During longer deployments, 2 and 3 ring PAHs were at or near equilibrium, based on the complete dissipation of fluorene-D10, and their concentration was calculated based on an improved model for calculating *in situ* sampling rates when recoveries approach 0 or 100% that is detailed by Booij and Smedes *(13)*.

4.3.7 Data modeling. For comparisons of total PAHs, all 33 PAH analytes were summed. Two-way comparisons between different sampling events at the same site were carried out using the Wilcoxon signed-rank test. For sums and two-way comparisons, analyte concentration values below the detection limit were equal to zero. Probabilities less than p=0.005 were considered significant.

For other analyses, data were standardized to avoid a magnitude bias when analyzing chemical profiles. Sample measurements were scaled to reflect relative abundances by representing individual analyte concentrations as percentages of the total PAHs measured in a given sample.

Principal component analysis (PCA) is a multivariate variable reduction technique in which principal components (PCs) are calculated as combinations of the original variables. The goal of PCA is to express as much of the total variation as possible with a few uncorrelated PCs. Use of PCA can reveal important features obscured within the original data and has been applied to PAH fingerprinting and allocation studies *(22, 27)*. In this case PCA was used to explore similarities, differences and changes in chemical profiles of samples obtained from the study sites

over the course of more than a year. PCA was performed using all of the analytes from each sample. The resulting PCs were analyzed graphically for apparent similarities and differences between samples including spatial and temporal tendencies.

Statistical analyses and modeling were carried out using SAS 9.2 (SAS Institute Inc.) and graphics were created using Sigma Plot 11.0 (Systat Software Inc.).

4.4 Results and discussion

4.4.1 Bioavailable PAHs in coastal waters of four Gulf Coast states. The sum of the measured bioavailable water concentrations of all 33 PAH analytes considered in this study is denoted as Σ_{33}PAH. Prior to shoreline oiling at Grand Isle, LA, the measured Σ_{33}PAH in May, 2010, was 3.76 ng/L. Samplers were in the water column during heavy shoreline oiling in the month of June (samples June-1 and June-2), during which time the highest concentrations of bioavailable Σ_{33}PAH measured in this study were recorded. The maximum concentration of 174 ng/L, which was significantly greater than the initial baseline measurement ($p<0.001$), was observed in the June-1 sample. The concentration decreased from the observed maximum in June-2 ($p=0.049$) to 141 ng/L, which was still significantly greater than the pre-oiling observation ($p=0.004$). The samplers at Grand Isle were lost in the month of July. The Σ_{33}PAH concentration at the site decreased in August and September but remained significantly elevated. By March, 2011, Σ_{33}PAH was not significantly different from pre-oiling levels ($p=0.098$) and this trend was maintained through the conclusion of the study (Figure 4.2).

During the first sampling event in Gulfport, MS the Σ_{33}PAH was 7.33 ng/L. This increased to 20.5 ng/L in June-1, at which time the site was being visibly impacted by oil, and remained significantly elevated above the initial observation during June-2 and July ($p<0.05$). From August 2010 through May 2011, none of the samples taken

at this site demonstrated Σ_{33}PAH concentrations greater than pre-oiling measurements ($p>0.05$) (Figure 4.2).

The temporal progression of bioavailable PAHs at the site in Gulf Shores, AL demonstrated a different trend than the other sites. The Σ_{33}PAH concentration at the site was 9.14 ng/L in May 2010 and did not change significantly until July ($p=0.0005$) when it reached 19.8 ng/L. The concentration remained significantly elevated above pre-impact levels ($p<0.002$) through September, when a maximum concentration for the site, 25.7 ng/L, was observed. The Σ_{33}PAH concentration in March, 2011 was not different from the initial observation at the site ($p=0.112$); however, samples from April and May showed significantly elevated Σ_{33}PAH concentrations ($p<0.008$) that were comparable ($p>0.100$) to samples taken from the site during the oil spill (Figure 4.2). This observed increase in bioavailable PAHs may be due to re-suspension of contaminated sediments due to recorded high wind events and/or continued near-shore restoration activities, both of which were observed during those sampling periods. It could also be explained by increased inputs from other sources and other climactic factors.

The coastal water at Gulf Breeze, FL had an initial Σ_{33}PAH concentration of 3.85 ng/L. No significant change in this concentration was observed until August, 2010, when it reached 15.6 ng/L and remained significantly higher through September ($p<0.001$). A significant decrease from the maximum observed concentration was recorded in March, 2011 ($p<0.001$) and bioavailable PAHs were not different from pre-spill levels at this site in April or May of 2011 ($p>0.30$) (Figure 4.2).

It is important to note that while ng/L, or parts per trillion, concentration levels may seem low, the methodology used in this study only measures freely dissolved chemicals in the water column, which does not include oil slicks, tar balls,

suspended droplets or any other undissolved fraction. The reported concentrations are representative of what an organism in the water column would be exposed to through passive partitioning, which is the dominant route of uptake for fish and shellfish *(6, 7)*. Furthermore, because PAHs bioaccumulate in organisms, the concentration of these compounds in biological tissues will be much greater than the surrounding water concentration; likely more similar to mass:mass concentrations in the PSD itself, which are in the ug/kg to mg/kg range, for organisms whose predominant route of accumulation is passive uptake *(28)*.

4.4.2. Source modeling – discerning PAH assemblage types. One of the most difficult issues to address with oil and other chemical spills is demonstrating the source of contamination *(27)*. This is especially difficult in a system like the Gulf of Mexico, which has a multitude of natural and anthropogenic sources of PAHs and other chemicals associated with oil *(29)*. A variety of modeling and chemical forensic techniques were applied to this data set to help elucidate sources of PAHs at the study sites.

The first step in this forensic PAH allocation was to differentiate between source types; petrogenic or pyrogenic PAH assemblages. This differentiation is based on the distribution of alkylated PAH compounds compared to unsubstituted PAHs. Pyrogenic PAHs, such as those formed through wood and fuel combustion, are characterized by assemblages where the unsusbstituted PAHs are found at higher concentrations than the alkylated compounds. Petrogenic, or petroleum and coal PAHs, are formed at lower temperatures which leads to the relative abundance of alkylated PAHs exceeding that of the unsubstituted compound *(27)*.

Over the course of this study, the predominant source type changed at the study sites. In May, 2010, the relative abundances of parent-to-alkylated PAHs indicated pyrogenic or mixed pyro-petrogenic sources at all four sites. The sites in

Louisiana and Mississippi both had chemical profiles indicating mixed pyro-petrogenic sources prior to being impacted by the spill then strong petrogenic profiles during June-1 when both sites were being impacted by the spill and high concentrations of bioavailable PAHs were recorded. A year after the spill, in May, 2011, the chemical profile suggested a predominately pyrogenic PAH assemblage. The site in Alabama demonstrated a distinctive petrogenic signal in May, 2010, a mixed source profile in September, during the highest recorded PAH concentrations and a mixed pyro-petrogenic signal in May, 2011, when a return to elevated PAH levels was observed. Chemical profiles in Gulf Breezes, FL changed from pyrogenic during the first sampling event to predominately petrogenic in September and demonstrated a mixed pyro-petrogenic profile in May, 2011(Figure 4.3).

Based on the analytes that were quantified in this study, it was possible to assess three groups of unsubstituted-alkylated PAHs. Only C1- and C2- (one and two alkyl groups respectively) PAHs were analyzed, which is sufficient for demonstrating the profile trend. An increase in the petrogenic chemical signature during the sampling events that recorded maximum PAH concentrations was consistently observed; Figure 4.3 illustrates this change for the naphthalene compounds. The trend towards petrogenic source profiles in samples from months with higher PAH concentrations suggests that the additional inputs are the result of an influx of oil. This conclusion supported visual observations in Louisiana and Mississippi in June, but was less visible in Alabama and Florida in September.

4.4.3 Source modeling – chemical profile PCA. To further refine PAH sourcing, principal component analysis (PCA) was used to explore similarities, differences and changes in the chemical profiles of samples obtained from the study sites. PCA is a multivariate variable reduction technique in which principal components (PCs) are calculated as combinations of the original variables in order to express the maximum

total variation with a few uncorrelated PCs. This modeling approach has been applied to PAH fingerprinting and allocation studies *(22, 27, 30)*.

There are a number of trends that were revealed during visual analysis of the PCA output from this data (Figure 4.4). Prior to shoreline oiling at any of the sites (May 2010; month 1), the chemical profiles at all four sites were similar and group closely on the PCA figure. For the three sites that were not impacted in June-1; AL and FL, the chemicals profiles for month 2 also group closely with month 1. During shoreline oiling in Louisiana, the chemical profile of the samples from that site changed (months 2 and 3), as shown by the distancing of these points from the baseline observation on the PCA plot. There is no data for month 4 at the Louisiana site. Interestingly, in months 5 and 6, the concentration of PAHs at LA had decreased significantly from the maximums observed in June and July but the chemical profile remains similar, as seen by the proximity of these four observations. This suggests an attenuation of the input but a similar source. The other three sites were impacted by oiling throughout the year and showed a tendency for PAH chemical profiles from the sampling events with the highest recorded concentrations to be most distant from the pre-oiling observations. However, the PAH assemblage that impacted the more easterly shorelines had a different chemical profile than what was seen in Louisiana, likely due to aging of the oil and relatively more significant inputs from other sources. The tendency for the chemical profiles at these three sites to change in the same way, as demonstrated by their closely grouped temporal displacement in the same direction on the PCA plot, suggests at least one similar, significant source. Samples from Alabama, taken in April and May, 2011, when a renewed increase in PAH concentrations was observed, group closely with samples taken when the site was being visibly impacted by oil. This, along with the mixed-petrogenic signal (Fig. 3), supports the hypothesis that oil from the Deepwater Horizon spill may still be

affecting this site. Analysis of other PCs, especially PC 3 (not shown), suggests that inter-state differences are a secondary contributor to variability in the data set.

Taken together, it is clear that the Deepwater Horizon oil spill impacted coastal waters of the Gulf of Mexico and contributed to temporary increases in the bioavailable concentration of PAHs. The persistently elevated levels of contamination at Gulf Shores, AL, observed after a decrease to pre-oiling levels, merit further study. Though this study demonstrates a nearly complete attenuation of Deepwater Horizon oil inputs by the one year anniversary of the spill at three of the four coastal sites, it does not preclude contamination of sediments or other media not contemplated here nor the possibility that residual oil could become re-suspended and dissolved in the water column.

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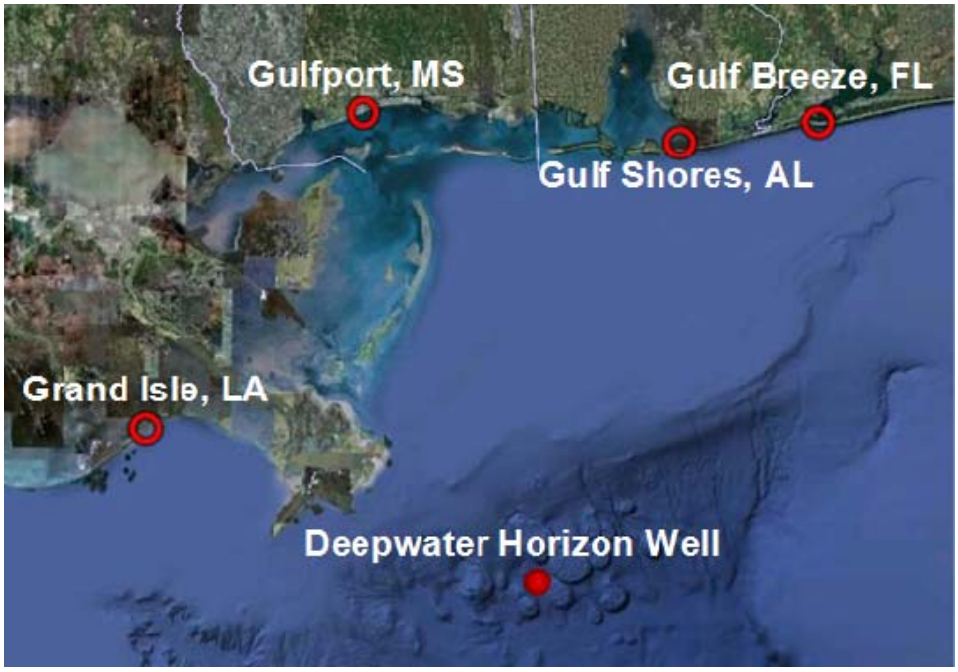

Figure 4.1. Sampling locations at four states in the Gulf of Mexico

Sampling sites are indicated by open circles and the filled circle corresponds to the location of the Deepwater Horizon well.

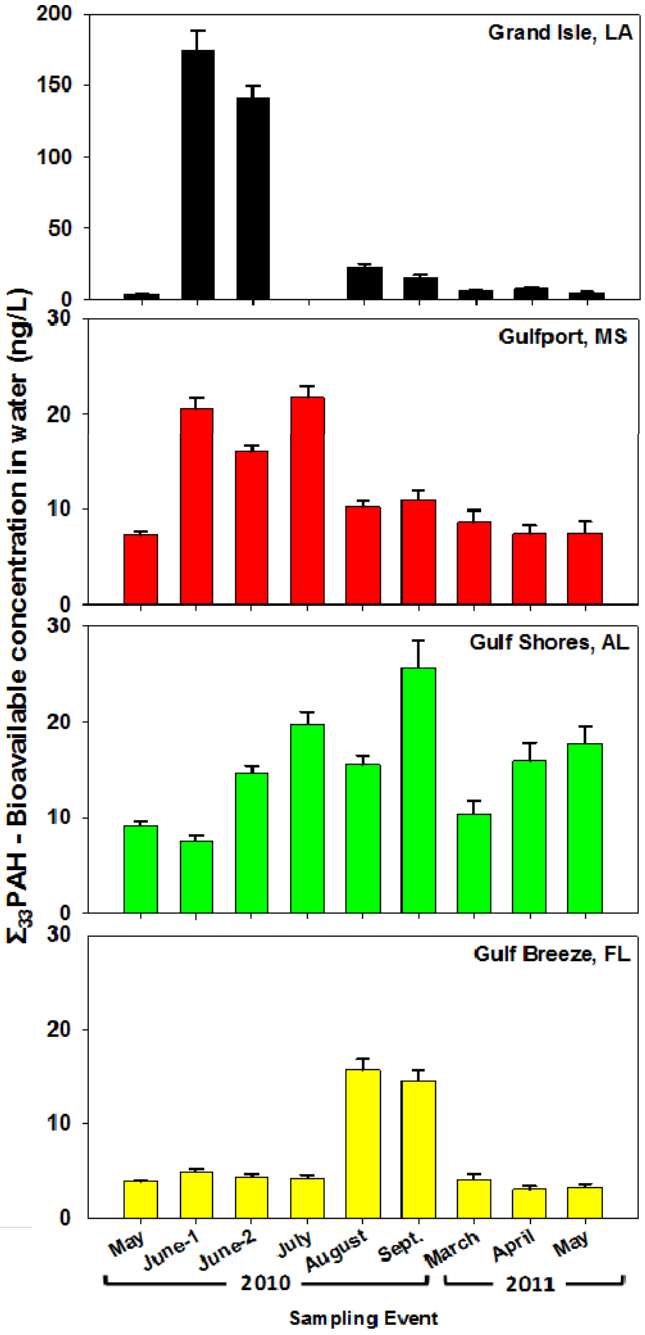

Figure 4.2. Bioavailable PAHs in coastal waters of the Gulf of Mexico

Bars represent the dissolved concentration of the sum of 33 PAH compounds and lines represent the 95% confidence interval. Note that the scale is different for Grand Isle, LA. Exact sampling dates can be found in the methods.

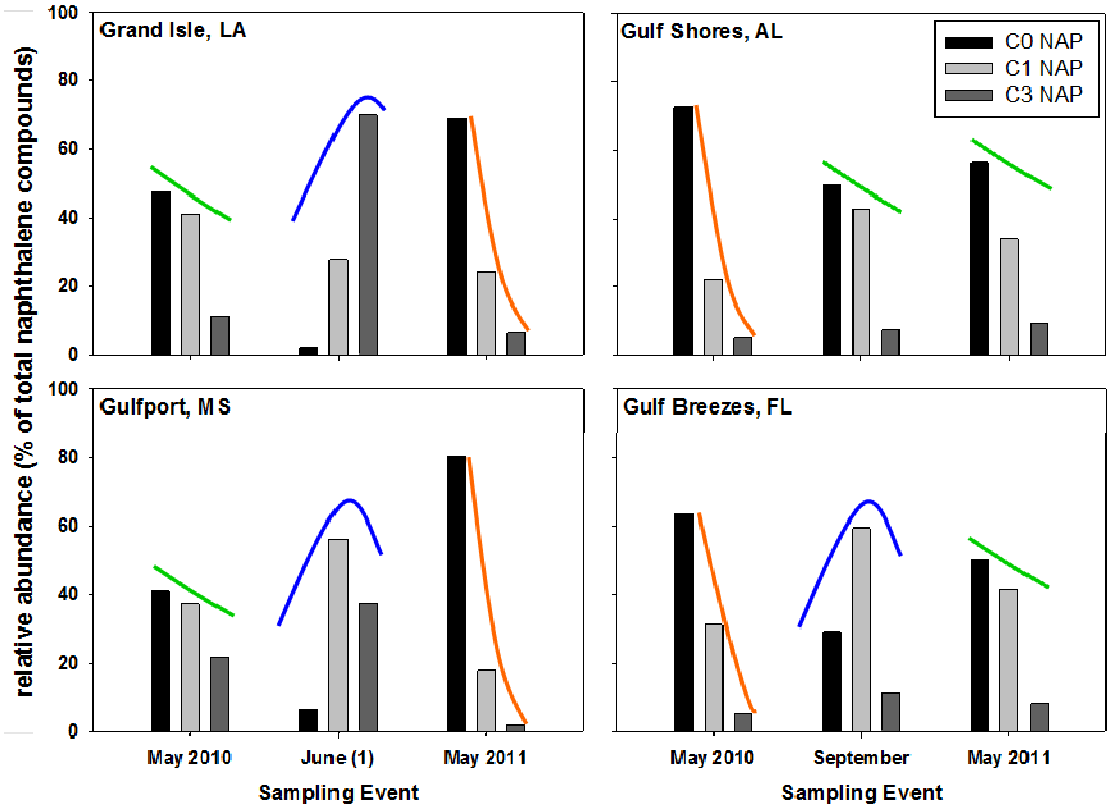

Figure 4.3. Relative abundance of unsubstituted and alkyl- PAHs

Profiles from samples obtained pre-oiling (May, 2010), during maximum observed Σ_{33}PAH and a year after the oil spill (May, 2011). A high relative abundance of the unsubstituted naphthalene, compared with the alkyl-napthalenes (C1 and C2) indicates a primarily pyrogenic source (orange line). A high relative abundance of the alkylated compounds compared to the unsubstituted PAH indicates a primarily petrogenic source (blue line). Samples with approximately equal relative abundances are a result of a mix of petrogrenic and pyrogenic sources (green line).

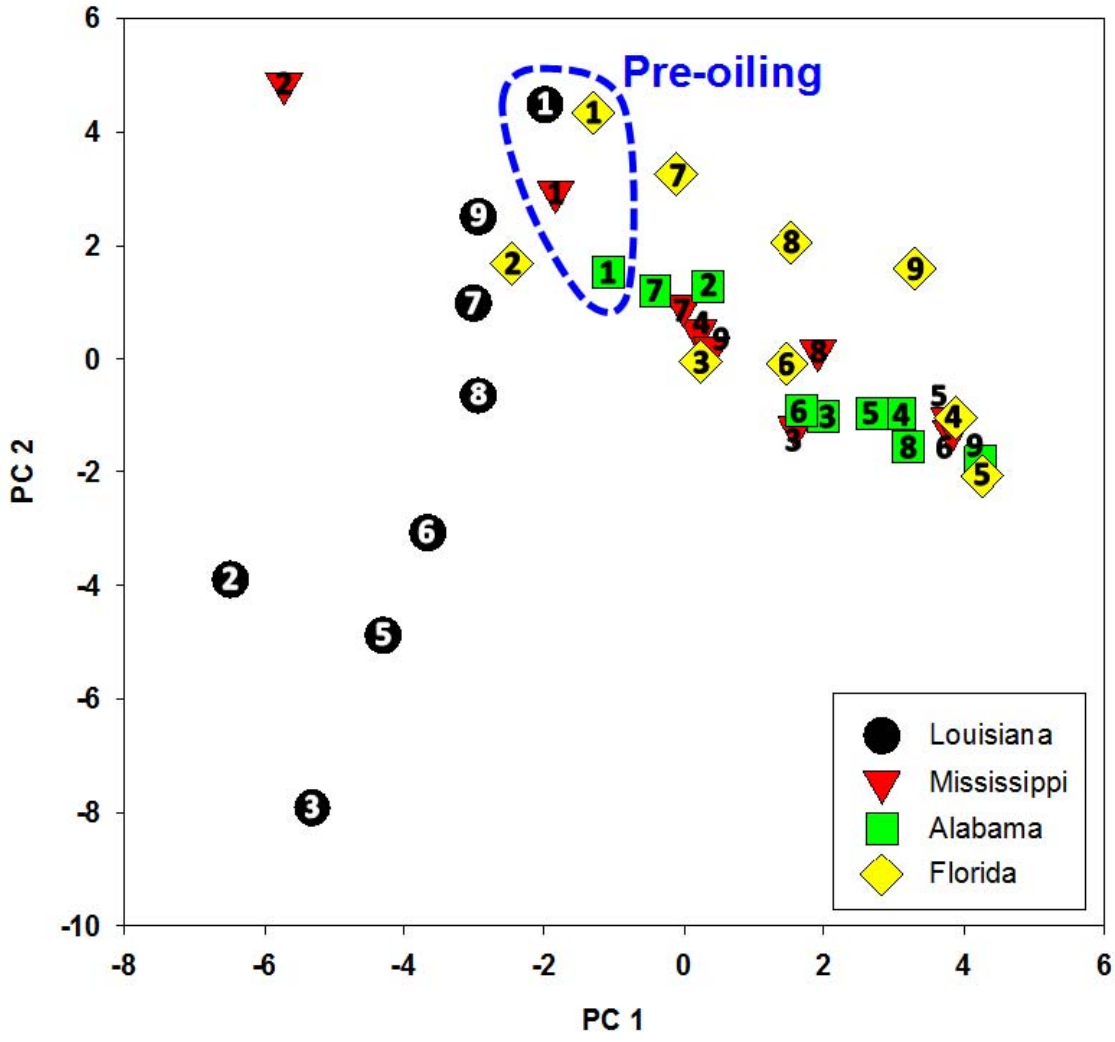

Figure 4.4. Principal components analysis of PAH chemical profiles

Principle component 1 and 2, together representing 49% of the variability in the data set, are plotted. States are differentiated by symbols and the numbers indicate the sampling events in chronological order. There is no month 4 sample for Louisiana. Data from samples taken during month 1, prior to shoreline oiling, are enclosed by a dotted line labeled 'Pre-oiling'.

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Chapter 5 – Conclusion

Passive sampling devices (PSDs) are widely recognized as a valuable tool for quantifying freely dissolved contaminants in environmental media. The work presented in this dissertation demonstrates novel applications for PSDs that advance research in the areas of environmental monitoring, mixture toxicity and risk assessment.

The BRIDGES tool directly integrates passive sampling with a vertebrate organism bioassay. It provides site-specific, temporally resolved information about the toxicity of complex mixtures of environmental contaminants. When the BRIDGES model was applied in the Portland Harbor Superfund and adjacent areas, it proved to be a sensitive, high throughput method, capable of detecting differences in the toxicity of environmentally relevant mixtures on a reduced spatial and temporal scale. In addition to observed differences between sites located within and outside of the Superfund area, significant differences were detected in the toxicity of PSD extracts from sites located on opposite banks of the Superfund area, separated by a few hundred meters. Furthermore seasonal changes in the concentration of contaminants in the harbor were paralleled by differences in the toxicity of the samples. Higher concentrations of polycyclic aromatic hydrocarbons (PAHs) were observed during the dry season, when river flows were lower, than in the wet season. Similarly, the toxicity of samples obtained during the dry season was greater than the wet season. Associations between chemical characteristics of environmental mixtures and observed toxic outcomes were identified; however these associations could be further refined in future studies by performing chemical fractionation of PSD extracts or deconvolution and reconstruction of mixtures.

Another innovative application for PSDs that was developed and tested was using them as biological surrogates in risk assessment models. Though humans do not consume passive samplers, comparisons of PAH concentrations in PSDs and fish tissue from the Portland Harbor Superfund site demonstrate that using PSD

concentrations in a public health assessment would provide a reasonable and conservative estimate of exposure that would be protective of human health without significantly overestimating risk. Significant spatial and temporal differences in risk from consuming PAHs in fish and shellfish from Portland Harbor were determined in this study. This information complemented the Public Health Assessment for the area, which could not evaluate risk from exposure to PAHs due to insufficient fish data. Using PSDs as direct biological surrogates has advantages over sampling organisms including: larger sample size, non-destructive sampling and comparability across studies. PSDs provide biologically relevant exposure data for risk assessment that could be used when organism data is not available or to complement, and further refine, other measures of exposure.

In the tragic aftermath of the explosion of the Deepwater Horizon oil well there was an opportunity to take advantage of past experience and the relative simplicity of deploying PSDs in the environment to obtain much needed data about the impacts of the oil spill. Samplers deployed at four Gulf Coast states prior to shoreline oiling provided a measure of baseline contamination that proved unique and invaluable for assessing the impacts of the spill. Bioavailable PAHs were monitored at sites located along more than 500 miles of the Gulf coast for over a year. Significant increases in bioavailable PAHs were observed, following the oil spill, in June and July, 2010; however, pre-oiling levels were observed at all sites by March, 2011. A return to elevated PAH concentrations, accompanied by a chemical fingerprint similar to that observed while the site was being impacted by the spill, was observed in Alabama in summer, 2011. Elevated PAH concentrations were associated with a petrogenic signature and distinctive chemical profiles. The ability to respond rapidly to an environmental disaster as well as the spatial and temporal scope of this project and the forensic chemistry techniques applied to the data

demonstrate multiple fit-for-purpose advantages of utilizing passive sampling techniques for environmental monitoring.

Innovative applications for PSDs, a widely utilized environmental monitoring tool, were developed and tested in the Portland Harbor Superfund site and during the Deepwater Horizon oil spill in the Gulf of Mexico. The research presented in this dissertation advances the science of bioavailability assessment, demonstrates new methods for determining the toxicity of complex environmental mixtures and provides techniques for modeling associations between mixtures and toxic effects. It fulfills a stated objective of Oregon State University's department of Environmental and Molecular Toxicology; to create, disseminate and apply new knowledge to ensure protection of environmental and public health.

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APPENDIX

***Appendix 1 – Supporting information: Bioavailable contaminant* mixtures and toxic effects: using passive sampling and the embryonic zebrafish model to bridge exposure and effects**

Table A1.1 Chemicals in AMDIS library used in DRS screening

2-Chlorobiphenyl	3,3',4-Trichlorobiphenyl	2,3',4,6-Tetrachlorobiphenyl
3-Chlorobiphenyl	3,3',5-Trichlorobiphenyl	2,3',4',5-Tetrachlorobiphenyl
4-Chlorobiphenyl	3,4,4'-Trichlorobiphenyl	2,3',4',6-Tetrachlorobiphenyl
2,2'-Dichlorobiphenyl	3,4,5-Trichlorobiphenyl	2,3',5,5'-Tetrachlorobiphenyl
2,3-Dichlorobiphenyl	3,4',5-Trichlorobiphenyl	2,3',5',6-Tetrachlorobiphenyl
2,3'-Dichlorobiphenyl	2,2',3,3'-Tetrachlorobiphenyl	2,4,4',5-Tetrachlorobiphenyl
2,4-Dichlorobiphenyl	2,2',3,4-Tetrachlorobiphenyl	2,4,4',6-Tetrachlorobiphenyl
2,4'-Dichlorobiphenyl	2,2',3,4'-Tetrachlorobiphenyl	2',3,4,5-Tetrachlorobiphenyl
2,5-Dichlorobiphenyl	2,2',3,5-Tetrachlorobiphenyl	3,3',4,4'-Tetrachlorobiphenyl
2,6-Dichlorobiphenyl	2,2',3,5'-Tetrachlorobiphenyl	3,3',4,5-Tetrachlorobiphenyl
3,3'-Dichlorobiphenyl	2,2',3,6-Tetrachlorobiphenyl	3,3',4,5-Tetrachlorobiphenyl
3,4 Dichlorobiphenyl	2,2',3,6'-Tetrachlorobiphenyl	3,3',5,5'-Tetrachlorobiphenyl
3,4'-Dichlorobiphenyl	2,2',4,4'-Tetrachlorobiphenyl	3,4,4',5-Tetrachlorobiphenyl
3,5-Dichlorobiphenyl	2,2',4,5-Tetrachlorobiphenyl	2,2',3,3',4-Pentachlorobiph
4,4'-Dichlorobiphenyl	2,2',4,5-Tetrachlorobiphenyl	2,2',3,3',5-Pentachlorobiph
2,2',3-Trichlorobiphenyl	2,2',4,6-Tetrachlorobiphenyl	2,2',3,3',6-Pentachlorobiph
2,2',4-Trichlorobiphenyl	2,2',4,6'-Tetrachlorobiphenyl	2,2',3,4,4'-Pentachlorobiph
2,2',5-Trichlorobiphenyl	2,2',5,5'-Tetrachlorobiphenyl	2,2',3,4,5-Pentachlorobiphenyl
2,2',6-Trichlorobiphenyl	2,2',5,6'-Tetrachlorobiphenyl	2,2',3,4,5'-Pentachlorobiph
2,3,3'-Trichlorobiphenyl	2,2',6,6'-Tetrachlorobiphenyl	2,2',3,4,6-Pentachlorobiphenyl
2,3,4-Trichlorobiphenyl	2,3,3',4-Tetrachlorobiphenyl	2,2',3,4,6'-Pentachlorobiph
2,3,4'-Trichlorobiphenyl	2,3,3',4'-Tetrachlorobiphenyl	2,2',3,4',5-Pentachlorobiph
2,3,5-Trichlorobiphenyl	2,3,3',5-Tetrachlorobiphenyl	2,2',3,4',6-Pentachlorobiph
2,3,6-Trichlorobiphenyl	2,3,3',5'-Tetrachlorobiphenyl	2,2',3,5,5'-Pentachlorobiph
2,3',4-Trichlorobiphenyl	2,3,3',6-Tetrachlorobiphenyl	2,2',3,5,6-Pentachlorobiphenyl
2,3',5-Trichlorobiphenyl	2,3,4,4'-Tetrachlorobiphenyl	2,2',3,5,6'-Pentachlorobiph
2,3',6-Trichlorobiphenyl	2,3,4,5-Tetrachlorobiphenyl	2,2',3,5',6-Pentachlorobiph
2,4,4'-Trichlorobiphenyl	2,3,4,6-Tetrachlorobiphenyl	2,2',3,6,6'-Pentachlorobiph
2,4,5-Trichlorobiphenyl	2,3,4',5-Tetrachlorobiphenyl	2,2',3',4,5-Pentachlorobiph
2,4,6-Trichlorobiphenyl	2,3,4',6-Tetrachlorobiphenyl	2,2',3',4,6-Pentachlorobiph
2,4',5-Trichlorobiphenyl	2,3,5,6-Tetrachlorobiphenyl	2,2',4,4',5-Pentachlorobiph
2,4',6-Trichlorobiphenyl	2,3',4,4'-Tetrachlorobiphenyl	2,2',4,4',6-Pentachlorobiph
2',3,4-Trichlorobiphenyl	2,3',4,5-Tetrachlorobiphenyl	
2',3,5-Trichlorobiphenyl	2,3',4,5'-Tetrachlorobiphenyl	

2,2',4,5,5'-Pentachlorobiph
2,2',4,5,6'-Pentachlorobiph
2,2',4,5',6-Pentachlorobiph
2,2',4,6,6'-Pentachlorobiph
2,3,3',4,4'-Pentachlorobiph
2,3,3',4,5-Pentachlorobiphenyl
2,3,3',4',5-Pentachlorobiph
2,3,3',4,5'-Pentachlorobiph
2,3,3',4,6-Pentachlorobiphenyl
2,3,3',4',6-Pentachlorobiph
2,3,3',5,5'-Pentachlorobiph
2,3,3',5,6-Pentachlorobiphenyl
2,3,3',5',6-Pentachlorobiph
2,3,4,4',5-Pentachlorobiphenyl
2,3,4,4',6-Pentachlorobiphenyl
2,3,4,5,6-Pentachlorobiphenyl
2,3,4',5,6-Pentachlorobiphenyl
2,3',4,4',5-Pentachlorobiph
2,3',4,4',6-Pentachlorobiph
2,3',4,5,5'-Pentachlorobiph
2,3',4,5',6-Pentachlorobiph
2',3,3',4,5-Pentachlorobiph
2',3,4,4',5-Pentachlorobiph
2',3,4,5,5'-Pentachlorobiph
2',3,4,5,6'-Pentachlorobiph
3,3',4,4',5-Pentachlorobiph
3,3',4,5,5'-Pentachlorobiph
2,2',3,3',4,4'-Hexachlorobi
2,2',3,3',4,5-Hexachlorobip
2,2',3,3',4,5-Hexachlorobip
2,2',3,3',4,6-Hexachlorobip
2,2',3,3',4,6'-Hexachlorobi
2,2',3,3',5,5'-Hexachlorobi
2,2',3,3',5,6-Hexachlorobip
2,2',3,3',5,6'-Hexachlorobi
2,2',3,3',6,6'-Hexachlorobi
2,2',3,4,4',5-Hexachlorobip
2,2',3,4,4',5'-Hexachlorobi
2,2',3,4,4',6-Hexachlorobip
2,2',3,4,4',6'-Hexachlorobi
2,2',3,4,5,5'-Hexachlorobip
2,2',3,4,5,6-Hexachlorobiph
2,2',3,4,5,6'-Hexachlorobip
2,2',3,4,5',6-Hexachlorobip
2,2',3,4,6,6'-Hexachlorobip
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2,2',3,4',5,6'-Hexachlorobi
2,2',3,4',5',6-Hexachlorobi
2,2',3,4',6,6'-Hexachlorobi
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2,2',3,5,6,6'-Hexachlorobip
2,2',4,4',5,5'-Hexachlorobi
2,2',4,4',5,6-Hexachlorobip
2,2',4,4',6,6'-Hexachlorobi
2,3,3',4,4',5-Hexachlorobip
2,3,3',4,4',5'-Hexachlorobi
2,3,3',4,4',6-Hexachlorobip
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2,3,3',4,5',6-Hexachlorobip
2,3,3',4',5,5'-Hexachlorobi
2,3,3',4',5,6-Hexachlorobip
2,3,3',4',5',6-Hexachlorobi
2,3,3',5,5',6-Hexachlorobip
2,3,4,4',5,6-Hexachlorobiph
2,3',4,4',5,5'-Hexachlorobi
2,3',4,4',5',6-Hexachlorobi
3,3',4,4',5,5'-Hexachlorobi
2,2',3,3',4,4',5-Heptachlor
2,2',3,3',4,4',6-Heptachlor
2,2',3,3',4,5,5'-Heptachlor
2,2',3,3',4,5,6-Heptachloro
2,2',3,3',4,5,6'-Heptachlor
2,2',3,3',4,5',6-Heptachlor
2,2',3,3',4,6,6'-Heptachlor
2,2',3,3',4',5,6-Heptachlor
2,2,3,3',5,5',6-Heptachloro
2,2',3,3',5,6,6'-Heptachlor
2,2',3,4,4',5,5'-Heptachlor
2,2',3,4,4',5,6-Heptachloro
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2,2',3,4,4',5',6-Heptachlor
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2,2',3,3',4,4',5,5'-Octachl
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2,2',3,3',4,4',5,6'-Octachl
2,2',3,3',4,4',6,6'-Octachl
2,2',3,3',4,5,5',6-Octachlo
2,2',3,3',4,5,5',6'-Octachl
2,2',3,3',4,5,6,6'-Octachlo
2,2',3,3',4,5',6,6'-Octachl
2,2',3,3',5,5',6,6'-Octachl
2,2',3,4,4',5,5',6-Octachlo
2,2',3,4,4',5,6,6'-Octachlo
2,3,3',4,4',5,5',6-Octachlo
2,2',3,3',4,4',5,5',6-Nonac
2,2',3,3',4,4',5,6,6'-Nonac
2,2',3,3',4,5,5',6,6'-Nonac
2,2',3,3',4,4',5,5',6,6'-De
Diethylene glycol
Aniline
p-Dichlorobenzene
Dicyclopentadiene
Dimefox
o-Dichlorobenzene
2-Methylphenol

4-Methylphenol
m-Cresol
1,2-Dibromo-3-chloropropane
2,4-Dimethylaniline
2,6-Dimethylaniline
2,4-Dichlorophenol
1,2,4-Trichlorobenzene
Ethiolate
3-Chloroaniline
4-Chloroaniline
2-Ethyl-1,3-hexanediol
p-Nitrotoluene
Methamidophos
Dichlorvos
Allidochlor
2,3,5-Trichlorophenol
2,6-Dichlorobenzonitrile
Nicotine
2,4,6-Trichlorophenol
EPTC
Dichlormid
2,4,5-Trichlorophenol
Phenoxyacetic acid
Biphenyl
Propamocarb
2-Phenoxypropionic acid
3,5-Dichloroaniline
Mevinphos
Butylate
3,4-Dichloroaniline
Acephate
Chlormefos
Vernolate
Dimethylphthalate
Propham
Nitrapyrin
Etridiazole
Pebulate
Metolcarb
Trichlorfon
Butylated hydroxyanisole
Methacrifos
Chloroneb
o-Phenylphenol
Crimidine
Dicamba methyl ester
2-(2-Butoxyethoxy)ethyl
Pentachlorobenzene
2-(Octylthio)ethanol
Molinate
Isoprocarb
Demephion
2,3,5,6-Tetrachlorophenol
Mecoprop methyl ester
Ethylenethiourea
2,3,4,5-Tetrachlorophenol
2,3,4,6-Tetrachlorophenol
Methomyl
MCPA methyl ester
Tetraethylpyrophosphate (TEPP)
Heptenophos
N,N-Diethyl-m-toluamide
Chlorfenprop-methyl
Omethoate
Diethyl phthalate
Tecnazene
Thionazin
Propachlor
4,6-Dinitro-o-cresol (DNOC)
Dichlorprop methyl ester
Demeton-S-methyl
Diphenylamine
Azobenzene
Benzophenone
Cycloate
Ethoprophos
2,4-D methyl ester
Fenuron
Tributyl phosphate
Dicamba
Chlorpropham
2-[3-Chlorophenoxy] propiona
Chlordimeform
2,3,5-Trimethacarb
Ethalfluralin
Dicrotophos
Thiofanox
Bromoxynil
Bendiocarb
Trifluralin
Benfluralin
Monocrotophos
Sulfotep
Tebutam
Desbromo-bromobutide
Promecarb
Di-allate I
Phorate
Triclopyr methyl ester
BHC alpha isomer
Methyl-1-naphthalene acetate
Di-allate II
Thiometon
Hexachlorobenzene
Desmedipham
Dazomet
Dicloran
Pentachloroanisole
Demeton-S
Diethyl dithiobis(thionofor
Dimethoate
Atraton
Dichlorprop
3,4,5-Trimethacarb
Ethoxyquin
Simazine
Prometon
Carbofuran
Swep

Chlorbufam
Dimethipin
Atrazine
BHC beta isomer
Clomazone
Pentachlorophenol
Fenoprop methyl ester
Propazine
Cyromazine
Lindane
Terbumeton
Chloramben methyl ester
N-Methyl-N-1-naphthyl acetate
Isocarbamide
Aminocarb
Cycluron
Di-n-propyl phthalate
Pentachloronitrobenzene
Cyanophos
Terbufos
Pyroquilon
Terbuthylazine
Trietazine
Fonofos
Propetamphos
Propyzamide
2,4,5-T methyl ester
MCPB methyl ester
Profluralin
Dinoterb
Pyrimethanil
Pindone
Dichlone
Phosphamidon I
Diazinon
BHC delta isomer
Disulfoton
Dinoseb
Methyl paraoxon
Fenfuram
Fluchloralin
2,4-D sec-butyl ester
Secbumeton
Terbacil
Chlorothalonil
Dinitramine
Tri-allate
Isazophos
Carbofuran-3-keto
Etrimfos
Bromocyclen
Isobornyl thiocyanoacetate
Sebuthylazine
Oxabetrinil
Endosulfan ether
Iprobenfos
Monalide
Metobromuron
Pentachloroaniline
Formothion
2,4-DB methyl ester
Ethiofencarb
Furmecyclox
Pirimicarb
Dinoseb methyl ether
Butoxycarboxim
N-1-Naphthylacetamide
Dioxacarb
Benfuresate
Desmetryn
Chlorthiamid
Propanil
Dichlofenthion
Dimethachlor
Cyprazine
Phosphamidon II
Bromobutide
Metribuzin
Prothoate
Bentazone methyl derivative
Acetochlor
Fuberidazole
Methyl parathion
Chlorpyrifos Methyl
Vinclozolin
Plifenat
Terbucarb
Chloranocryl
3-Hydroxycarbofuran
Heptachlor
Carbaryl
Tolclofos-methyl
Simetryn
Fenoprop
Malathion-o-analog
Alachlor
Isoproturon
Ametryn
Flurenol-methylester
Dimetilan
Tridiphane
Fenchlorphos
Oxydemeton-methyl
Metalaxyl
Paraoxon
Prometryn
Dinoseb acetate
2-(1-naphthyl)acetamide
Demeton-S-methylsulfon
Tycor (SMY 1500)
Picloram methyl ester
Ioxynil
Dinoterb acetate
Terbutryn
Methiocarb
Fenitrothion
Dithiopyr
Quinoclamine
Linuron
Pentanochlor

Esprocarb
Pirimiphos-methyl
Ethofumesate
Probenazole
Bromacil
Chlorotoluron
Dichlofluanid
Di-n-butylphthalate
9,10-Anthraquinone
Aldrin
Amidithion
Benthiocarb
Dipropetryn
Oxamyl
Malathion
Metolachlor
Kinoprene
Fenthion
Diethofencarb
Dimethylvinphos(Z)
4,4'-Dichlorobenzophenone
Tetrapropyl thiodiphosphate
Chlorpyrifos
Fepropimorph
Parathion
Isomethiozin
Cyanazine
Triadimefon
Dicapthon
Isobenzan
Chlorthal-dimethyl
Methfuroxam
Carbetamide
Tiocarbazil I
Fenson
Tiocarbazil II
Bentazone
Chlorthion
Phthalide
Trichloronat

Nitrothal-isopropyl
Drazoxolon
Crufomate
Dodemorph I
Flurochloridone I
Sulfur (S8)
Pyracarbolid
Isodrin
Bromophos
Flurochloridone II
Naphthalic anhydride
Diphenamid
Butralin
Endosulfan lactone
Octachlorostyrene
Pirimiphos-ethyl
Heptachlor exo-epoxide isom
Isopropalin
Oxychlordane
Dodemorph II
Metazachlor
Methoprene I
Mefluidide
Thiabendazole
Benazolin-ethyl
Anilazine
Chlorbromuron
Pendimethalin
Penconazole
Dimethametryn
Tributyl phosphorotrithioite
Phosfolan
Captan
Tolylfluanid
Pyrifenox I
Methyldymron
Diuron
Mephosfolan
Chlozolinate
Chlorfenvinphos

Folpet
Isofenphos
Pyridinitril
Quinalphos
Triadimenol
Phenthoate
Mecarbam
Bioallethrin S-cyclopenteny
Chlorbenside
Bioallethrin
Chinomethionat
Furalaxyl
Procymidone
Dinobuton
trans-Chlordane
Chlorflurecol-methyl ester
Flurenol-butyl ester
Crotoxyphos
Methidathion
Methoprene II
Triflumizole
o,p'-DDE
Bromophos-ethyl
Paclobutrazol
Endosulfan (alpha isomer)
Pyrifenox II
Vamidothion
cis-Chlordane
Tetrachlorvinphos
2,4-Dichlorophenyl benzenes
TCMTB
Flutriafol
Ditalimfos
Butachlor
Chlorfenson
Flumetralin
Napropamide
Diamyl phthalate
Hexaconazole
Jodfenphos

Butamifos
Tricyclazole
Fenamiphos
Diethatyl ethyl
Fluorodifen
Prothiofos
Imazalil
Flutolanil
Bisphenol A
Dieldrin
Isoprothiolane
Profenofos
Uniconizole-P
p,p'-DDE
Barban
S,S,S-Tributylphosphorotrit
Pretilachlor
Carboxin
o,p'-DDD
Flubenzimine
Diclobutrazol
Myclobutanil
Oxadiazon
Metamitron
Azaconazole
Flamprop-methyl
Buprofezin
Flusilazole
Methoprotryne
Tryclopyrbutoxyethyl
Nitrofen
Erbon
Isoxathion
Ancymidol
Endosulfan (beta isomer)
Perthane
Binapacryl
Fluazifop-p-butyl
Acifluorfen methyl ester
Chlorobenzilate
Chloropropylate
Fensulfothion
Fenthion sulfoxide
Diniconazole
Cyprofuram
p,p'-DDD
Methiocarb sulfoxide
Etaconazole
o,p'-DDT
Flamprop-isopropyl
Oxadixyl
Endrin aldehyde
Methiocarb sulfone
Triamiphos
Benodanil
Ethion
Chlordecone
Tetrasul
Chlorthiophos
Fenazaflor
Mepronil
Sulprofos
Triazophos
Chlornitrofen
2,3,7,8-Tetrachlorodibenzo
Carbophenothion
Famphur
Benalaxyl
Edifenphos
Endosulfan sulfate
Cyanofenphos
Bromoxynil octanoic acid ester
Lenacil
Propiconazole-I
Diethylstilbestrol
Norflurazon
Hexestrol
p,p'-DDT
Butyl benzyl phthalate
Pyrazon
2,3,7,8-Tetrachlorodibenzo-
Propiconazole-II
Piperalin
Hexazinone
Tebuconazole
Nuarimol
Thenylchlor
Captafol
Diclofop methyl
Fluroxypyr-1-methylheptyl
Propargite
Diflufenican
Oxycarboxin
Dinocap I
Piperonyl butoxide
Resmethrin
Bioresmethrin
Epoxiconazole
Fluotrimazole
Nitralin
Endrin ketone
Dinocap II
Pyributicarb
Benzoylprop ethyl
Iprodione
Dichlorophen
Hexabromobenzene
Phosmet
Pyridaphenthion
Leptophos oxon
Chlorthiophos sulfoxide
Menazon
Tetramethrin I
Bromopropylate
Dinocap III
EPN
Carbosulfan
Fenoxycarb
Tetramethrin II
Bifenthrin

Piperophos
Methoxychlor
Phenkapton
Dinocap IV
Fenpropathrin
Bifenox
Dicyclohexyl phthalate
Chlorthiophos sulfone
Tetradifon
d-(cis-trans)-Phenothrin-I
d-(cis-trans)-Phenothrin-II
Furathiocarb
Bis(2-ethylhexyl)phthalate
Azinphos-methyl
Phosalone
Leptophos
Mirex
Mefenacet
Amitraz
Cyhalothrin I (lambda)
Fenarimol
Azinphos-ethyl
Pyrazophos
b-Estradiol
Isoxaben
Dialifos
Bitertanol I
Oryzalin
Bitertanol II
Permethrin I
Pyridaben
Permethrin II
17a-Ethynylestradiol
Coumaphos
Prochloraz
Tamoxifen
Dioxathion
Fenbuconazole
Cyfluthrin I
Cyfluthrin II
Cyfluthrin III
Cyfluthrin IV
Cypermethrin I
Cypermethrin II
Quizalofop-ethyl
Cypermethrin III
Cypermethrin IV
Flucythrinate I
Benzo(a)pyrene
Hexachlorophene
5,7-Dihydroxy-4'-methoxyiso
Flucythrinate II
Decachlorobiphenyl
Fluridone
2-Hydroxyestradiol
Pyridate
Fenvalerate I
Fenvalerate II
Esfenvalerate
Fluvalinate-tau-I
Fluvalinate-tau-II
Difenoconazol I
Difenoconazol II
Deltamethrin
Temephos
Phenol
2-Chlorophenol
1,3-Dichlorbenzene
3-Trifluormethylaniline
Triethylphosphate
2-Nitrophenol
4-Chlorophenyl isocyanate
2,4-Dimethylphenol
Naphthalene
3-Chloro-4-fluoroaniline
4-Isopropylaniline
Carvone
2-ethyl-6-methylaniline
Profenofos metabolite
3-Aminophenol
4-Chloro-3-methylphenol
Thymol
4-Chloro-2-methylaniline
2,4,5-Trimethylaniline
4-Bromoaniline
Carbofuran-7-phenol
Diuron Metabolite
Promecarb artifact
2,4,6-Trichloroanisole
Eugenol
2,3,6-Trichloroanisole
Indoxacarb and Dioxacarb
1,3,5-Tribromobenzene
3-Chloro-4-methoxyaniline
Acenaphthylene
Phthalimide
Tetrahydrophthalimide, cis-
Acenaphthene
Cashmeran
1-naphthalenol
Tebuthiuron
4-Nitrophenol
XMC (3,4-Dimethylphenyl)
Amitraz metabolite
2,4,5-Trichloroaniline
Benzenesulfonamide
Benzene, 1,3-bis(bromomethyl)
XMC
Tolyltriazole
Benzoximate metabolite
Fluorene
2-Methyl-4,6-dinitrophenol
Phorate-oxon
2,4,6-Tribromoanisole
2,4,5,6-Tetrachloro-m-xylene
Chlorethoxyfos
2,4,6-Tribromophenol
Dichlofluanid metabolite
Naled
Methabenzthiazuron

Atrazine-desethyl
2,3,4,5-Tertrachloronitrobe
Dioxabenzofos
2,6-Dichlorobenzamide
Terbuthylazine-desethyl
Cadusafos
Sulfallate
Fenclorim
Celestolide
Fluoroimide
Empenthrin IV
Empenthrin V
Schradan
Fenazaflor metabolite
4-Aminodiphenyl
Sebuthylazine-desethyl
Tolylfluanid metabolite (DMST)
Tris(2-chloroethyl) phosphate
Phantolide
Benzyl benzoate
Bufencarb
Phenanthrene-d10
Phenanthrene
Diazinon-oxon
Anthracene
Chlordene, trans-
3-Indolylacetonitrile
Cyclopentadecanone
Aziprotryn metabolite
BHC epsilon isomer
Tefluthrin, cis-
Exaltolide
Azoxybenzene
Musk amberette
Methyl (2-naphthoxy)acetate
Cyclafuramid
Caffeine
Tebupirimifos
Benoxacor
Traseolide
Tridemorph , 4-tridecyl-
p,p'-DDM
Fenchlorphos-oxon
Silthiopham
Theobromine
Diisobutyl phthalate
4-Nonylphenol
Musk xylene
Tonalide
PCB 30
PCB 31
Hydroprene
Fenitrothion-oxon
Ethofumesate
Dimethenamid
Flurprimidol
Spiroxamine I
Musk Moskene
Dipropyl isocinchomeronate
Azibenzolar-S-methyl
Simeconazole
Cymiazole
Flurochloridone, deschloro-
Transfluthrin
Propisochlor
Propargite metabolite
Bis(2,3,3,3-tetrachloroprop
Fipronil, Desulfinyl-
Prosulfocarb
2,4'-Dichlorobenzophenone
Fenpropidin
Orbencarb
Musk Tibetene (Moschustibeten)
Spiroxamine II
Quintozene metabolite
Bifenazate metabolite
PCB 49
Triapenthenol
Prodiamine
Phorate sulfoxide
Terbufos-oxon-sulfone
Difenoxuron
Phorate sulfone
Sulfanilamide
Musk Ketone
Thiazopyr
Rabenzazole
PBB 15
Isocarbophos
Flufenacet
Isofenphos-oxon
Isoxaflutole
Tetraconazole
Fosthiazate I
Ethidimuron
Fosthiazate II
Cyprodinil
Phenothiazine
Chlorbicyclen
Fluoranthene
Terbufos-sulfone
Chlorfenvinphos, cis-
Fipronil-sulfide
Dimepiperate
Irgarol
Chlorfenvinphos, trans-
Fluazinam
MCPA-butoxyethyl ester
Fipronil
Zoxamide decomposition product
Beflubutamid
Triclosan
Pyrene
DDMU
4,4'-Oxydianiline
Prallethrin, cis-
Benzidine
Propaphos
PCB 101
Prallethrin

Fenothiocarb
Trichlamide
Haloxyfop-methyl
Triclosan-methyl
Disulfoton sulfone
Nonachlor, trans-
Mepanipyrim
Bromfenvinphos-(E)
Triazamate
Bromfenvinphos-(Z)
Picoxystrobin
PCB 81
Fluazolate
Metominostrobin (E)
PCB 136
Fludioxonil
PCB 110
PCB 77
Fensulfothion-oxon
Prothioconazole-desthio
Iprovalicarb I
Aramite I
p,p'-Dibromobenzophenone
Toxaphene Parlar 26
Imazamethabenz-methyl I
Endrin
Fipronil-sulfone
Bupirimate
Thifluzamide
Metominostrobin (Z)
Cyproconazole
Kresoxim-methyl
Iprovalicarb II
Fensulfothion-oxon -sulfone
Aramite II
Imazamethabenz-methyl II
Carpropamid
Chlorfenapyr
Cyflufenamid
Fenoxanil

PCB 118
ortho-Aminoazotoluene
Nonachlor, cis-
Fenthion-sulfone
Aclonifen
Pyriminobac-methyl (Z)
PCB 153
PCB 131
fensulfothion-sulfone
PCB 127
Chlorbenside sulfone
PBB 52 Tetrabrombiphenyl
Isoxadifen-ethyl
Ofurace
Pyrethrin I
Quinoxyfen
Methoxychlor olefin
Diofenolan I
Fenhexamid
Di-n-hexyl phthalate
Carfentrazone-ethyl
Diofenolan II
PCB 138
Clodinafop-propargyl
Trifloxystrobin
PCB 126
Pyraflufen-ethyl
Pyriminobac-methyl (E)
Triphenyl phosphate
Resmethrine I
Toxaphene Parlar 50
Tris(2-butoxyethyl) phosphate
Resmethrine II
Zoxamide
Mefenpyr-diethyl
Fenpiclonil
Spiromesifen
Benzo(a)anthracene
Fenamiphos sulfoxide
Bromuconazole I

Sudan I
Chrysene
Fenamiphos-sulfone
Ethoxyfen-ethyl
Dimoxystrobin
Tris(2-ethylhexyl) posphate
Bis(2-butoxyethyl) phthalate
Picolinafen
Cloquintocet-mexyl
o-Dianisidine
Etoxazole
Sulfentrazone
Metconazole I
Fenamidone
Fenazaquin
Tebufenpyrad
PCB 180
Bromuconazole II
Anilofos
Fenchlorazole-ethyl
Phenothrin I
Toxaphene Parlar 62
Phenothrin II
Diphenyl phthalate
Potasan
Flurtamone
PCB 169
Pyriproxyfen
PCB 170
Cyhalofop-butyl
Naproanilide
Ioxynil octanoate
Trifenmorph
Lactofen
PBB 101
Pyriftalid
Acrinathrin
Fluoroglycofen-ethyl
Benfuracarb
Tricresylphosphate, ortho-

Pyraclofos
Metrafenone
Fenoxaprop-ethyl
Tricresylphosphate, meta-
Spirodiclofen
Sudan II
Tricresylphosphate
Fluquinconazole
Di-n-octyl phthalate
2,4,5-Trichloro-p-terphenyl
Butafenacil
Benzo[b]fluoranthene
Cafenstrole
2,3,5,6-Tetrachloro-p-terph
Fluoxastrobin cis-
Boscalid (Nicobifen)
Halfenprox
Cekafix
Ethofenprox
Acequinocyl
Silafluofen
Pyrimidifen
Di-n-nonyl phthalate
Flumiclorac-pentyl
Azoxystrobin
Famoxadon
Dimethomorph-(Z)
Tolfenpyrad
Dimethomorph-(E)
Indeno[1,2,3-cd]pyrene
Dibenz[a,h]anthracene
Cinidon-ethyl
Benzo[g,h,i]perylene
PBB 169 Hexabrombiphenyl
Rotenone
Spiroxamine metabolite (4-t)
Etridiazole, deschloro- (5-)
Tolyltriazole
PCB 105
Benzo[k]fluoranthene

Ipconazole
Triticonazole
Metconazole II
Norflurazon, Desmethyl-
Empenthrin I
Empenthrin II
Empenthrin III
Diphacinone
Pyrazoxyfen
Fenobucarb
Propoxur
Cymoxanil
Fluometuron
Monolinuron
Aziprotryne
Chlorfenethol
Oxyfluorfen
Azamethiphos
acetamiprid
Cyphenothrin cis-
Cyphenothrin trans-
Sudan Red
Fluthiacet-methyl
Diclocymet I
Diclocymet II
p,p'-Dicofol
Fluacrypyrim
Flumioxazin
Furilazole
Cyhalothrin (Gamma)
Imibenconazole
Imibenconazole-desbenzyl
Prohydrojasmon I
Prohydrojasmon II
Jasmolin I
Cinerin I
Cinerin II
Jasmolin II
Pyrethrin II
Heptachlor epoxide isomer A

Dimethylvinphos(E)
5,12-Napthacenequinone
9-Fluorenone-D8
1,4-Anthraquinone
Benzanthrone
9-Fluorenone
1,4-Anthraquinone
Acenapthenequinone
4H-
cyclopenta[def]phenanthrene
9,10-phenthrenequinone
9,10-Anthraquinone
Phenanthrene-1,4-dione
9,10-Anthraquinone-d8
Benzofluorenone
7,12-
benz[a]anthracenquinone
Chrysene-d12
Benzo[c]phenanthrene-
[1,4]quinone
1,6-Benzo(a)pyrene-
quionone
Benzo(cd)pyrenone
Aceanthracenequinone
Naphthalene-D8
2-Methylnaphthalene
1-Methylnaphthalene
1,6-Dimethylnaphthalene
Acenaphthylene-D8
1,2-Dimethylnaphthalene
Dibenzothiophene
Retene
1-Methylpyrene
6-Methylchrysene
Dibenzo(a,l)pyrene
Fluoranthene-D10
Pyrene-D10
Benzo(a)pyrene-D12
Benzo[ghi]perylene-D12
Perylene-D12
1-Nitronaphthalene
2-Nitronaphthalene

3-Nitrobiphenyl
4-Nitrobiphenyl
5-Nitroacenaphthene
2-Nitrofluorene
9-Nitroanthracene
9-Nitrophenanthrene
3-Nitrophenanthrene
2-Nitroanthracene
3-Nitrofluoranthene
1-Nitropyrene
2-Nitropyrene
7-Nitrobenz[a]anthracene
6-Nitrochrysene
3-Nitrobenzanthrone
1,3-Dinitropyrene
1,6-Dinitropyrene
1,8-Dinitropyrene
6-Nitrobenzo[a]pyrene
5-Nitroacenaphthene-D9
2-Nitrofluorene-D9
1-Nitropyrene-D9
6-Nitrochrysene-D11
2-Nitrobiphenyl
2-Methylanthracene
1-Methylphenanthrene
p,p' DDE-D8
PCB 77-D6
Acenaphthene-D10

Appendix 2 – Supporting information: Estimating risk at a Superfund site using passive sampling devices as biological surrogates in human health risk models

A2.1 Public health assessments The initial Portland Harbor Public Health Assessment was conducted by the Agency for Toxic Substances and Disease Registry (ATSDR) and then further developed by the Superfund Health Investigation and Education program of the Oregon Department of Human Services. A public health assessment is formally defined as "The evaluation of data and information on the release of hazardous substances into the environment in order to assess any [past], current, or future impact on public health, develop health advisories or other recommendations, and identify studies or actions needed to evaluate and mitigate or prevent human health effects" (42 Code of Federal Regulations, Part 90, published in 55 Federal Register 5136, February 13, 1990). According to the ATSDR "A public health assessment is conducted to determine whether and to what extent people have been, are being, or may be exposed to hazardous substances associated with a hazardous waste site and, if so, whether that exposure is harmful and should be stopped or reduced." *(1)*.

Public health assessments are more exposure driven than quantitative risk assessments; they aim to explain whether exposures are likely to be harmful under site-specific conditions. According to the ATSDR, public health assessments consider the same environmental data as EPA risk assessments, but focus on site-specific exposure conditions, community health concerns and health outcome data to provide an evaluation of possible public health hazards considering past, current and future exposures *(1)*.

Table A2.1 Established values for calculating estimated exposure doses[1]

Parameter	Abbreviation	Adult	Units
Concentration	C		μg/g
Conversion factor	CF	0.001	
Ingestion Rate Avg	IR-AVG	17.5	g/day
Ingestion Rate High	IR-high	142.4	g/day
Body Weight	BW	70	kg
Exposure Frequency	EF	365	days/year
Exposure Duration	ED	30	years
Averaging Time	AT-non-cancer	10950	days (30 yrs)
Averaging Time	AT-cancer	25550	days (70 yrs)

[1]From Portland Harbor Public Health Assessment *(2)*

A2.3 Literature cited

(1) ATSDR, *Public Health Assessment Guidance Manual (2005 Update)*. 2005, ATSDR: Atlanta, GA. Available on line at http://www.atsdr.cdc.gov/hac/PHAManual.

(2) ATSDR, *Public Health Assessment: Portland Harbor, Multnomah County, Oregon, EPA Facility ID: OR0001297969*. 2006, Oregon Department of Human Services Superfund Health Investigation and Education Program, U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry: Atlanta, GA. Available on line at http://www.atsdr.cdc.gov/HAC/pha/PortlandHarbor/PortlandHarborPHA032206.pdf.