Developmental toxicity of bioavailable contaminants from the Portland Harbor Superfund site: Bridging environmental mixtures and toxic effects

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BACKGROUND

The Biological Response Indicator Devices Gauging Environmental Stressors (BRIDGES) bioanalytical tool pairs passive sampling devices (PSDs) with the embryonic zebrafish developmental model to provide a quantitative measure of the toxicity of environmentally relevant contaminant mixtures.

Passive samplers sequester and concentrate freely dissolved, and therefore bioavailable, hydrophobic organic contaminants from aquatic environments[1]. They provide a time integrated measurement of chemicals in the environment[1] and samples obtained using PSDs can be applied to in-vitro and in-vivo bioassays[2].

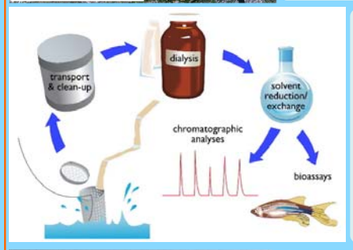
The zebrafish is a model vertebrate organism that is widely utilized for bioassays due to its small size, fecundity, rapid development and readily visible early morphology[4]. A prior study demonstrated that the BRIDGES tool can provide information about the toxicity of bioavailable contaminant mixtures[2].

The Portland Harbor Superfund site is an area impacted by numerous different chemical contaminants from historic and present day, point and non-point source inputs. It is a priority to understand the toxicity of complex environmental mixtures. The BRIDGES tool could provide valuable insight to direct research and remediation.

Objectives: 1) Utilize the BRIDGES tool to examine spatial and temporal differences in the toxicity of bioavailable chemical mixtures from sites within and outside of the Portland Harbor Superfund; 2) Associate differences in the toxic effects elicited by exposure to environmental samples with the chemicals identified in those samples.

METHODS

Study Area: PSDs for chemical analysis and bioassays were deployed at 9 sites within or outside of the Portland Harbor Superfund on the Willamette (north flow) and Columbia Rivers (west flow).



Sampling and Chemical Analysis:

Low-density polyethylene tubing PSDs, were deployed in the water column for 30 day sampling events in September and October 2009 and July, August, September and October, 2010.

PSDs were extracted and analyzed by GC-MS:

- Concentration of 33 polycyclic aromatic hydrocarbons (PAHs)

  Acute toxicity[5] of Σ_{33}PAH: Log LC_{50} (mM/L) = -1.162 log K_{ow} + 2.496

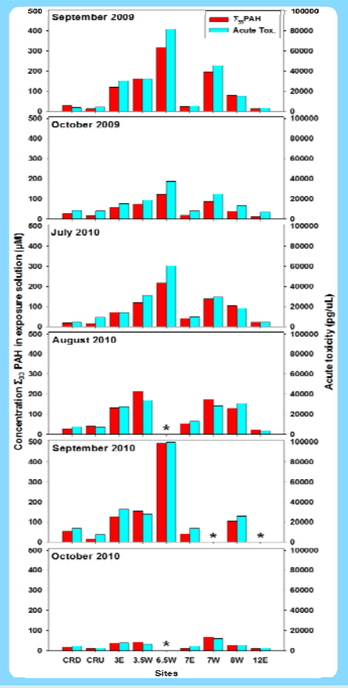
- Screening for 1201 chemicals of concern using Deconvolution Reporting Software (DRS; Agilent Technologies)

Zebrafish developmental toxicity assay: PSD extracts were prepared in DMSO at 4 concentrations: 100x (undiluted extract), 20x, 4x and 0.8x (sequential 5-fold dilutions). Dechorionated zebrafish embryos were exposed to 1% PSD extract in embryo medium, starting at 6 hours post fertilization (hpf) using a static waterborne method. Observations of mortality and 19 sub-lethal morphology endpoints were carried out at 24 hpf and 120 hpf.

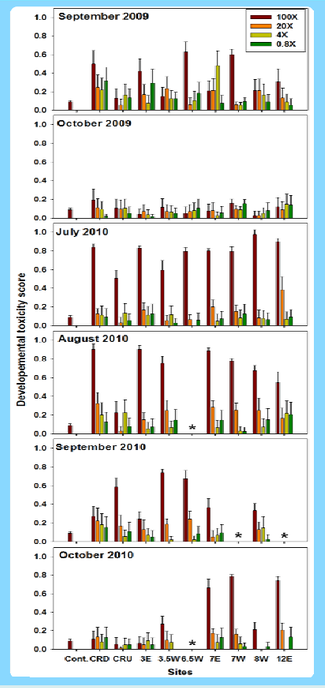
Metric for assessing overall toxicity: Embryos were scored from 0-1 based on the sum off all observed developmental endpoints:

Normal development = 0
Death at 24 hpf = 1; Death at 126 hpf = 0.95
Each sub-lethal morphological deformity = 0.045 (sum of 19 = 0.855)

RESULTS

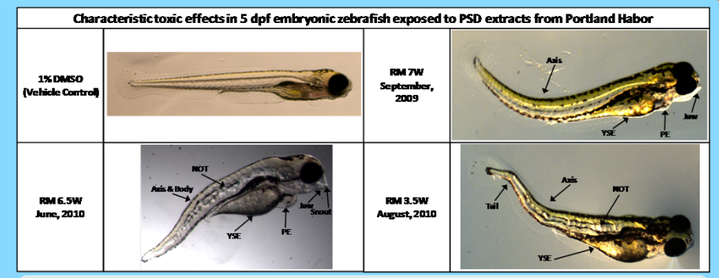


Concentration and acute toxicity of PAHs in exposure solution: Values refer to the highest exposure dose; 1% undiluted PSD extract. Acute toxicity was calculated for quantified PAH compounds only. Asterisks indicate that samples were not obtained.



Developmental toxicity of PSD extracts: Average toxicity score and SD from all exposed embryos. Scores range from 0 (normal development) to 1 (death by 24 hpf). 100X, is 1% PSD extract, other concentrations are successive 5-fold dilutions.

Characteristic toxic effects in 5 dpf embryonic zebrafish exposed to PSD extracts from Portland Habor



Characteristic toxic effects in embryonic zebrafish: 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. Other morphological deformities were observed in exposed embryos that are not pictured here.

Chemicals of concern identified in PSD extracts using DRS

		Number of samples detected at each site (out of total screened)								
Compound	Description	CRD	CRU	3E	3.5W	6.5W	7E	7W	8W	12E
Total samples screened		6	6	6	6	4	6	5	6	5
p,p'-DDE	DDT intermediate breakdown products	1	3	6	3	4	1	4	4	
o,p'-DDD		1	1	1	1	3		3	4	
p,p'-DDD			1			1		2	1	
PCB 49	Legacy organochlorine contaminants						1			
PCB 65					1		1	2	1	1
PCB 95			1				1			
PCB 110			1			1	2			1
PCB 118							1	1	1	
PCB 153							2			
9-fluorenone	Oxy-PAHs			2						
Benzofluorenone				2	2	2		2	3	
Benzathrone								1		
Benzo(cd)pyrenone						1				
Hexachlorobenzene	Fungicide – POP	2		3	3	3	3	4	4	4
Chlorfenapyr	Pro-insecticide		1							
Pendimethalin	Herbicide	1	1	2	3	1	3	1	2	2
Tonalide	Musk – PCP	3	1	5	4	2	3	2	3	2
Rabeprazole	Antiulcer pharmaceutical			1	1	2		1	1	

DISCUSSION and CONCLUSIONS

Chemical characterization of bioassay samples:

- Samples from within the Superfund had greater Σ_{33}PAH
- RM 7E (remediated in 2006) had lower Σ_{33}PAH than other sites within the Superfund area
- Σ_{33}PAH in Portland Harbor was greater during the dry season'(July-Sept.) than the wet season (October)
- 18 additional compounds were identified using DRS

Developmental toxicity of PSD extracts:

- Individual embryos are likely to express a small number (2-3) or large number (16-17) of sub-lethal deformities
- Exposure dose-response relationships were observed
- Incidences of specific biological endpoints were correlated to overall toxicity

Bridging chemistry and biological effects:

- Chemical components of mixtures were similar in all samples from Portland Harbor
- There is a significant correlation between Σ_{33}PAH and developmental toxicity scores
- Chemicals identified using DRS did not show a significant correlation with observed toxic outcomes

Conclusions

- BRIDGES is high throughput: 50 samples were analyzed for over 1200 chemical of concern and10,944 zebrafish embryos were assayed.
- BRIDGES is a sensitive bioanalytical tool capable of detecting highly resolved spatial and temporal differences in the toxicity of environmental mixtures.
- Future research should focus on determining the toxicity of samples from sites with different contaminant profiles.

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