Bridging bioavailable extracts and developing zebrafish to identify toxicants of concern

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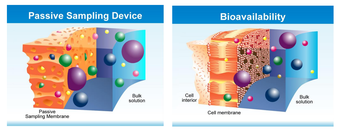
Summary
A toxicity identification evaluation benefits from methods that are neither too broad to provide meaningful results, nor too narrow to capture a potential contaminant of concern. We present an approach that integrates exposure and effects with a toolbox that evaluates potential exposure and uses non-myopic chemical and biological analyses. Passive sampling (1) narrows the search to freely dissolved hydrophobic compounds, while an embryonic zebrafish assay (2) paired with multiple GC methods allow in depth analysis (3) of such biologically relevant samples.

BRIDGES

Biological Response Indicator Device Gauging Environmental Stressors

1 Exposure

Passive Sampling Devices (PSDs)



LDPE polymer sequesters hydrophobic organic compounds much like an organism's phospholipid bilayer.

Low density polyethylene (LDPE) PSDs sample freely dissolved hydrophobic compounds, thereby collecting the **bioavailable** fraction.

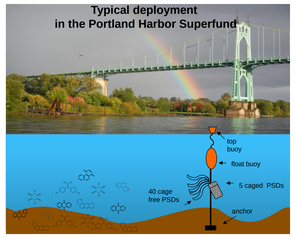
This focuses chemical analysis and bioassays on the subset of environmental chemicals that can partition to living tissues.

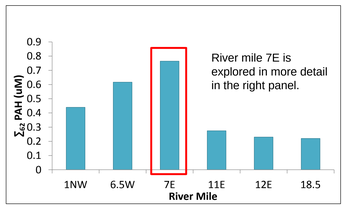
PSDs concentrate common low-level compounds and capture episodic events providing a **time-integrated** perspective at a site.

Methods

Deployment and extraction
LDPE PSDs placed in water column or sediment

- Cage-free LDPE without PRCs for bioassays; Caged LDPE with PRCs for water concentrations
- Extracted with n-hexane, samples are concentrated and ready for analysis!





Nominal PAH concentrations in bioassay exposure solutions for LDPE deployed on the Willamette River, in and around the Portland Harbor Superfund Site.

Chemical analysis
All analyses were performed with gas chromatography paired to either mass spectrometry or electron capture detection.

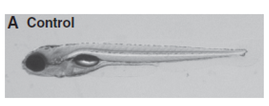
Analytes
- **62 PAHs**
- 22 OPAHs
- 60 pesticides
- 1299 other compounds
  - personal care products, pharmaceuticals, plasticizers, flame retardants

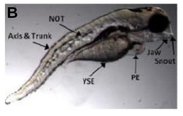
2 Effects

Zebrafish embryo assay

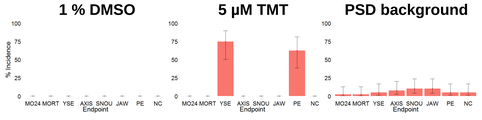
This assay is an *in vivo* assessment of teratogenicity with the technical advantages of an *in vitro* assay. Performed in 96 well plates, individual zebrafish (*Danio rerio*) embryos are exposed to test chemicals or samples from 8 hours post fertilization (hpf) to 120hpf. PSD extracts are exchanged to DMSO and diluted in aquarium water. Mortality and abnormal phenotypes are presented here but behavior and gene expression endpoints are also possible.

Key advantages of this assay include:
- Sensitive life stage
- Non-classical endpoints: *e.g.* morphological defects
- High-throughput: large *n* using small sample volume and time





Representative (A) control and (B) deformed zebrafish embryos at 120hpf. Images adapted from Goodale et al. (2013) and Allan et al. (2012).



Quality Control. A vehicle control (1 % DMSO) and a positive control (tri-methyl tin, TMT) are evaluated with every batch of exposures. Undeployed PSD extracts are non-toxic to zebrafish embryos.

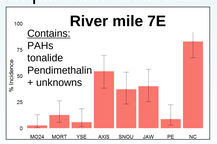
Effects-Directed Analysis (EDA)

3 Toxicant identification and mixture assessment

PSD extracts can be used directly in bioassays or to inform more targeted analyses.

Portland Harbor Superfund

What is causing the observed malformations in this sample from River Mile 7 East?

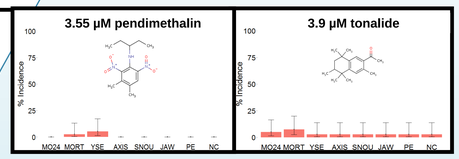


Gel permeation chromatography (GPC) separated the whole sample.

River mile 7E was teratogenic to zebrafish embryos. Separated by GPC, the fraction that contained PAHs was non-toxic.

Bioassays direct (semi-)target analysis

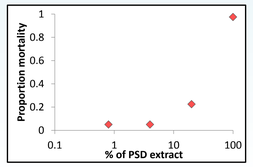
Analogous to a TIE, EDA directs chemical analysis to fractions that induce a biological effect. Here we separate PAHs from the teratogenic fraction (above) and de-confirm other target analytes (below). These results are informing further investigations into what might be causing toxicity.



Pendimethalin and tonalide were identified in the fraction of RM 7E that was toxic. Individually, these compounds were non-toxic at concentrations found in the LDPE extract.

Gulf of Mexico

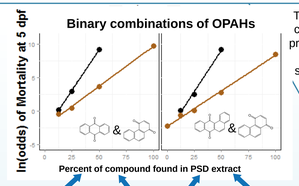
How do oxygenated-PAHs found in environmental mixtures contribute to toxicity?



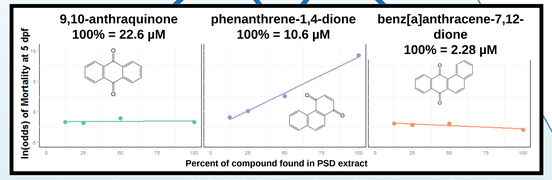
PSD extract from Louisiana is lethal to zebrafish embryos. We explored the toxicity of OPAHs in ratios derived from the PSD extract.

Target analysis informs CBA

A sample collected near-shore Louisiana after the Deepwater Horizon oil spill displayed a concentration-dependent toxicity for embryonic zebrafish. Curious about the toxicity of relevant OPAHs, we used the ratio of detected OPAHs to investigate interaction effects of this scrutinized class of compounds.



The brown lines are the concentration response predicted by the additive model. The steeper slope of the **black** lines indicate greater than additive toxicity in observed responses.



Concentration response for individual OPAHs observed in PSD extracts (bottom), and binary mixtures of the same compounds (top).

Component-Based Analysis (CBA)

Conclusions

- The BRIDGES toolbox addresses which compounds in site-specific mixtures may exert developmental toxicity.
  - We de-confirmed several compounds collected from a Superfund site and directed future analyses.
  - We identified interactive toxicity of 2 OPAH combinations with compounds that were present in the Gulf of Mexico.

References

1) Goodale BC, Tilton SC, Corvi MM, Wilson GR, Janszen DB, Anderson KA, et al. 2013. Structurally distinct polycyclic aromatic hydrocarbons induce differential transcriptional responses in developing zebrafish. Toxicol Appl Pharm. 272(3):656-70.. 2) Allan SE, Smith BW, Tanguay RL, Anderson KA. 2012. Bridging environmental mixtures and toxic effects. Environ Toxicol Chem. 31(12):2877-87. SEE ALSO: Hillwalker, W. E., S. E. Allan, et al. 2010. "Exploiting lipid-free tubing passive samplers and embryonic zebrafish to link site specific contaminant mixtures to biological responses." Chemosphere 79(1): 1-7.

Acknowledgements

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