Project 4 – Bridging Superfund Site Based Bioavailable Extracts with Biology

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Introduction

Superfund Sites such as Portland Harbor are often contaminated with numerous chemicals, resulting in complex exposures to the inhabiting organisms. Chemicals must first be available for internalization, have a toxic mode of action, and be at sufficient concentrations to elicit a toxic effect. Discerning which chemicals in a complex mixture fit these criteria can be challenging.

Hypothesis

A minority of chemicals elicit the majority of toxicity in an environmental mixture. The responsible toxicants can be identified by pairing passive sampling with bioassays.

Background

Passive Sampling Devices (PSDs). Tool that approximates bioavailability by collecting only the freely dissolved fraction of the total mixture (Figure 1).

Figure 1. LDPE polymer sequesters hydrophobic organic compounds much like an organism's phospholipid bilayer.

Passive samplers are analyzed chemically and integrated into biological assays to **bridge** the chemistry of a complex mixture with its toxicity.

Zebrafish Assay
Static exposure in 1% DMSO of 40 fish in 96 well plates for 8-120 hours post fertilization (hpf). Mortality and other endpoints at either 24 or 120hpf are compared to 1 % DMSO control.

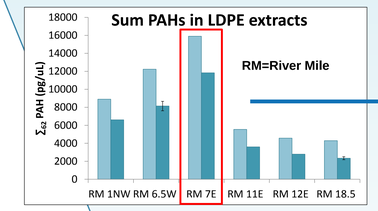
Chemical Analysis
for 62 PAHs, 22 OPAHs, 60 pesticides, and screen for 1182 miscellaneous compounds using gas chromatography.

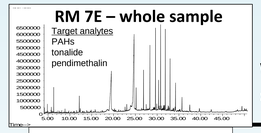
Two approaches are then used in conjunction to determine the responsible toxicants:

1. **Effects-directed Analysis (EDA)**
2. **Component-based Analysis (CBA)**

Effects-Directed Analysis

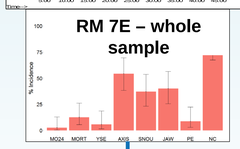
is iterative fractionation and toxicity testing to simplify complex mixtures. A technique that targets freely dissolved compounds, passive sampling is the first fractionation of an environmental mixture.

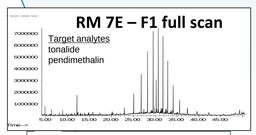


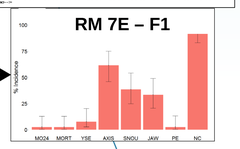


Whole Sample

Initial characterization of the whole sample determines the chemical and toxicological profiles.

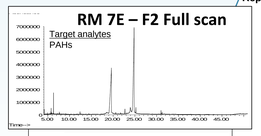


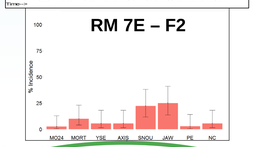




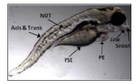
Fractionation

Gel permeation chromatography (GPC) separated the whole sample.





Representative sublethal endpoints



(De)confirm tentatively identified toxicants

Target analysis informs CBA

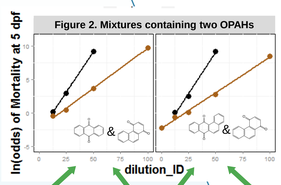
EDA fractionates the whole sample to empirically investigate the contribution of a subset of components.

EDA and CBA are complementary approaches used to identify responsible toxicants in complex environmental mixtures.

CBA can test for interaction effects between individual chemicals and confirm tentatively identified toxicants.

Binary mixtures

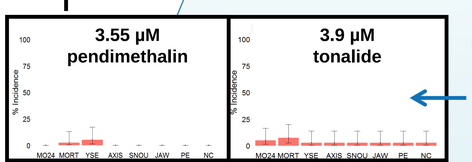
Exposure-response for binary mixtures will reveal interaction effects. The additive line shows the expected exposure-response with no interaction. The higher slope of the emperical line provides evidence of interaction effects.

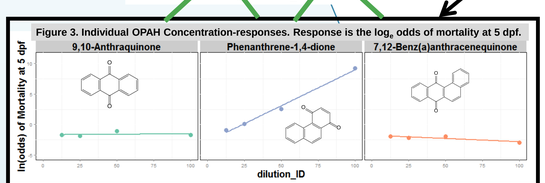


Single compounds

Exposure-response data of individual chemicals are collected to:

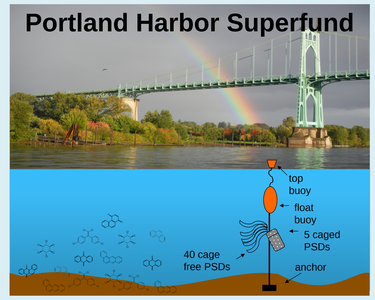
1. Confirm tentatively identified toxicants
2. Predict additive toxicity





The toxicity of individual chemicals are compared to their toxicity when in simple combinations. Passive sampling informs which chemicals are bioavailable in the environment and thus relevant to mixture toxicity studies. This approach is called:

Component-Based Analysis



Results

EDA: Fractionation of Portland Harbor samples with GPC isolated PAHs from the toxic fraction, allowing us to conclude that PAHs were not the responsible toxicants. Tonalide and pendimethalin were identified in the toxic fraction, yet did not elicit a toxic response in the zebrafish assay. The responsible toxicants have not yet been identified.

CBA: GC analysis of passive sampling reveals which contaminants are bioavailable. Selected binary combinations (Figure 2) are compared to individual exposures (Figure 3), and responses different from additivity suggest interaction effects in the tested mixtures.

Conclusions

Passive sampling in combination with the zebrafish embryo assay is a powerful bioanalytical tool that integrates exposure and effects. Bioavailable fractions are still complex, but the combination of EDA and CBA allow researchers to tease out toxicants of concern and/or mixture effects.

Acknowledgements

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