Fractionation of passive sampling device extracts explores contribution of PAHs to zebrafish toxicity

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Background

Chemicals occur as mixtures in the environment. Evaluating whole mixture toxicity provides insight into more realistic exposure scenarios, but may not identify individual responsible toxicants. Effects directed analysis (EDA) can be applied to isolate individual or groups of chemicals to better identify responsible toxicants. Carcinogenicity is the endpoint that drives risk assessment for **PAHs,** but cancer is not the only hazard.

PAHs as re-emerging contaminants.

Our understanding of PAH mixture toxicity is moving beyond the EPA 16 priority pollutants. There are an increasing number of detectable compounds of which some have tumorigenic relative potency factors 20-60 times greater than benzo[a]pyrene. Non-cancer endpoints such as **developmental** effects are also important biological effects from PAHs (Fig. 2, Goodale et al., 2013; Incardona et al., 2006).

Passive sampling devices integrated with embryonic zebrafish

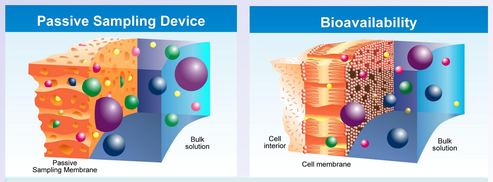


Figure 1. LDPE polymer sequesters hydrophobic organic compounds much like an organism's phospholipid bilayer.

Low density polyethylene sampling

- Samples freely dissolved fraction of hydrophobic organic compounds.
- Concentrates compounds typically found at low levels.

Zebrafish assay

- 70% genome conserved between humans and zebrafish.
- Requires small sample volumes.
- Fecundity of adult females makes large *n* easy to obtain.
- Embryos are transparent and develop outside of the mother.

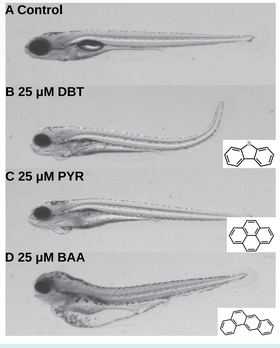


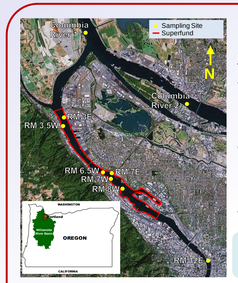
Figure 2. Zebrafish larvae after exposure to 1% DMSO control, dibenzothiophene, pyrene, and benz[a]anthracene from 6 to 48 hours post fertilization. (Goodale et al., 2013)

Hypothesis:

PAHs contribute to the developmental toxicity in freely dissolved fraction of compounds in Portland Harbor.

- **Aim 1:** Demonstrate that passive sampling and sample manipulation do not confound bioassays.
- **Aim 2:** Isolate PAHs using EDA to confirm or deny their contribution to observed toxicity.

Methods



Sampling conditions

- River mile 7E: McCormick and Baxter (**M&B**); former creosoting site; sediment cap in place
- One hundred LDPE strips were deployed at **M&B** in Winter2012-13
- Extracted with n-hexane.

Figure 3. Portland Harbor Superfund (Allan et al., 2012)

Chemical analysis- GC/MS

- 62 PAH quantitation
- 1182 analyte screen with deconvolution software

Fractionation

- Gel permeation chromatography with UV detection at 254nm
- Fractions recombined to test influence of sample manipulations.

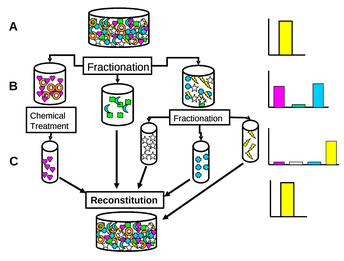


Figure 4. **Effects-directed analysis (EDA)** physically separates components of a sample to independently assess the toxicity of each. This process of elimination is an empirical complement to statistical analysis.

Toxicity Screening

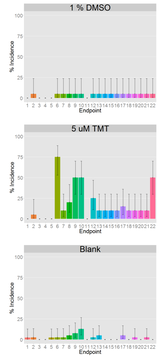
- Exposures with 1%DMSO
- 40 dechorionated zebrafish per sample, 16 for controls
- Static exposure 8-120 hours post fertilization (hpf)
- 22 developmental endpoints observed either at 24 or 120hpf

Results

Endpoint

1 Mortality 24h
2 Dev. retardation 24h
3 Spontaneous movement 24h
4 Notochord 24h
5 Mortality
6 Yolk sac edema
7 Bent Axis
8 Eye
9 Snout
10 Jaw
11 Otic nerve
12 Pericardial edema
13 Brain
14 Somites
15 Pectoral fin
16 Caudal fin
17 Pigment
18 Circulation
19 Truncation
20 Swim bladder
21 Notochord
22 Touch response

Controls



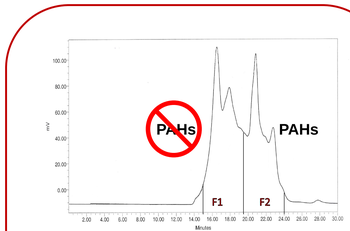


Figure 5. Typical GPC chromatogram.

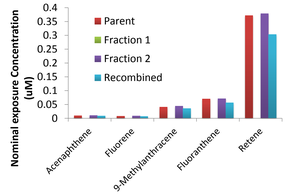
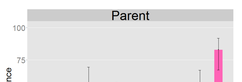
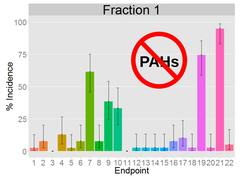
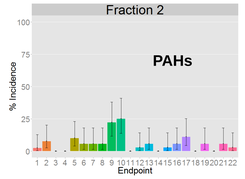


Figure 6. Concentrations of PAHs were conserved throughout fractionation.

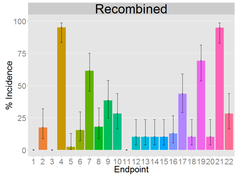


Whole sample elicits sub-lethal effects: axis (endpoints 7, 19, 21) and craniofacial malformations (endpoints 9, 10).





Fraction 1 elicits the same endpoints, to the same degree, as Parent. Fraction 2 more closely resembles the vehicle control.



The recombined sample recapitulates parent toxicity.

Conclusions

1. EDA fractionation process still conserves chemical mixture AND bioactivity.
2. LDPE artifacts nor sample manipulations impart toxicity.
3. **PAHs are not responsible** for observed effects at M&B in Winter 2012-13.

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ACKNOWLEDGEMENTS

Funding is provided by: National Institute of Environmental Health Sciences: Superfund Research Program -P42 ES016465 Training Grant- 5T32ES007060-33

Special thanks to Lane Tidwell, Glenn Wilson, and all other members of Anderson Laboratory. Thanks to Sinnhuber Aquatic Reseach Laboratory. especially Greg Gonnerman.