Correlating OPAH concentrations with zebrafish toxicity of Deepwater Horizon samples: a bottom-up approach

Carey E Donald, Steven G O'Connell, Sarah E Allan, Robert L Tanguay, Kim A Anderson

Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon 97331

INTRODUCTION

OPAHs in the Gulf of Mexico

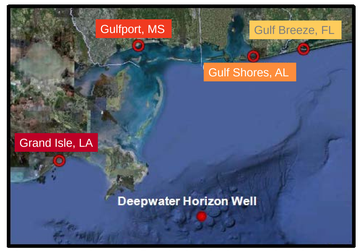


Figure 1. Passive sampling device deployment locations and site of Deepwater Horizon oil rig, Gulf of Mexico.[1]

Oxygenated polycyclic aromatic hydrocarbons (OPAHs) are found in the environment arising from both natural and anthropogenic sources (Figures 1&3).[2,3]

Passive sampling devices sample the bioavailable fraction, and extracts can be used in conjunction with bioassays to assess the toxicity of freely dissolved, environmentally-relevant contaminants (Figure 2).[5,6,7]

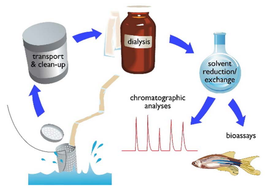


Figure 2. Passive sampling device function and attributes.

OPAHs are recognized as environmental toxicants potentially more toxic than the parent PAHs.[2,4]

Previous work shows that PAH concentrations do not correlate with toxicity.

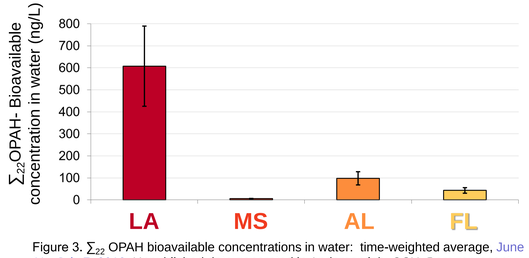


Figure 3. $\sum_{22}$ OPAH bioavailable concentrations in water: time-weighted average, June 11 - July 7, 2010. Unpublished data generated in Anderson lab, OSU. Bars represent avearge relative standard error of four replicates deployed in October 2011.

If PAHs do not correlate with developmental zebrafish toxicity, could OPAHs explain the toxicity?

Hypothesis$_A$: OPAHs are dominantly responsible for the developmental zebrafish toxicity of Gulf of Mexico passive sampler extracts.

Objectives

Further assess toxicity that can be attributed to detected OPAHs using developmental zebrafish model:

1. Create standard OPAH mixtures that mimic what we detected in the Gulf of Mexico.
2. Test bioactivity with embryonic zebrafish (*Danio rerio*) bioassay.
3. Correlate individual chemical components with zebrafish endpoints

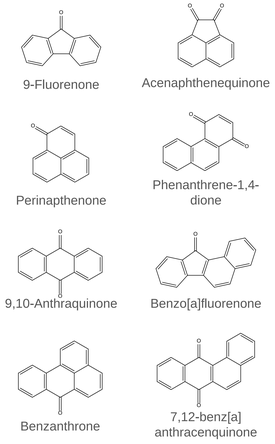
METHODS

1. Standard OPAH Mixtures

created to mimic OPAH concentrations detected in extracts of passive samplers deployed June 11 - July 7, 2010.

Table 1. Components of Standard OPAH Mixtures (ppb) used in developmental zebrafish exposures, created to mimic Gulf of Mexico passive sampler extracts .

	Standard OPAH mixture			
	LA	MS	AL	FL
9-Fluorenone	-	-	-	18
Acenaphthenequinone	-	-	44	-
Perinapthenone	-	330	140	-
Phenanthrene-1,4-dione	2200	-	-	160
9,10-Anthraquinone	4700	-	72	150
Benzo[a]fluorenone	2200	140	120	100
Benzanthrone	-	-	49	-
7,12-benz[a]anthracenquinone	590	77	59	68



2. Embryonic Zebrafish Bioassay

to assess bioactivity of standard OPAH mixtures

- Model vertebrate species (n=40)
- Embryos enzymatically dechorionated at 4 hours post-fertilization (hpf)
- Exposures from 6 to 120 hpf in final concentration of 1% DMSO
- One animal per well in 96-well plates
- 22 total endpoints at two time points (24 hpf and 120 hpf) (Figure 4).

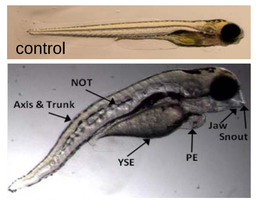
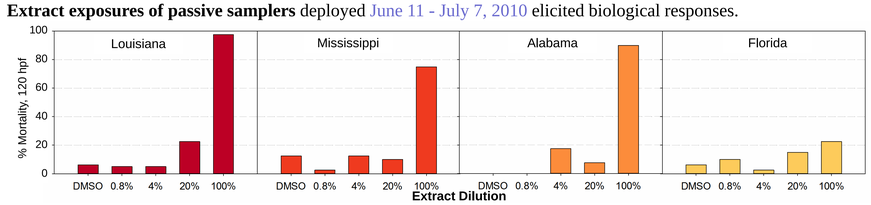


Figure 4. Examples of morphological endpoints in developmental zebrafish bioassay. YSE = yolk sac edema, PE = pericardial edema, NOT = notochord.[7]

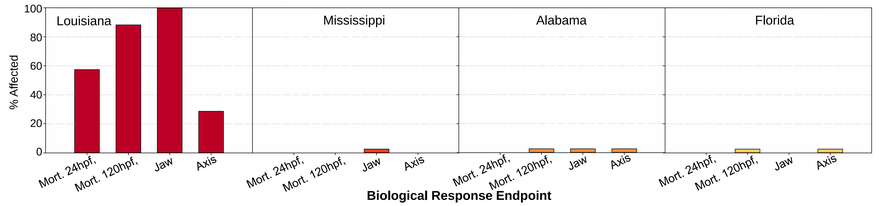
References: **1.** S.E. Allan, B.W. Smith, R.L. Tanguay, K.A. Anderson, Environmental Toxicology and Chemistry 31 (2012) 2877. **2.** B.A.M. Bandowe, W. Wilcke, Journal of Environment Quality 39 (2010) 1349. **3.** S. Lundstedt, P.A. White, C.L. Lemieux, K.D. Lynes, I.B. Lambert, L. Öberg, P. Haglund, M. Tysklind, AMBIO: A Journal of the Human Environment 36 (2007) 475. **4.** A.L. Knecht, B.C. Goodale, L. Truong, M.T. Simonich, A.J. Swanson, M.M. Matzke, K.A. Anderson, K.M. Waters, R.L. Tanguay, Toxicology and Applied Pharmacology 271 (2013) 266. **5.** K.A. Anderson, D. Sethajintanin, G.J. Sower, L. Quarles, Environ Sci Technol 42 (2008) 4486. **6.** W.E. Hillwalker, S.E. Allan, R.L. Tanguay, K.A. Anderson, Chemosphere 79 (2010) 1. **7.** S.E. Allan, B.W. Smith, K.A. Anderson, Environ Sci Technol 46 (2012) 2033. **8.** S.G. O'Connell, T. Haigh, G. Wilson, K.A. Anderson, Analytical and Bioanalytical Chemistry 405 (2013) 8885.

RESULTS

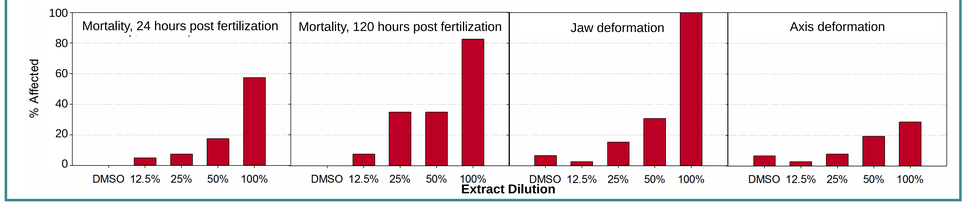
Extract exposures of passive samplers deployed June 11 - July 7, 2010 elicited biological responses.



Exposures of **Standard OPAH mixtures** based on Louisiana, Mississippi, Alabama, and Florida. Only Louisiana elicited a biological response Representative endpoints shown below of the 1x (100%) dilution.



Exposures of **Louisiana** standard OPAH mix elicit a **response over a dilution series**. Representative endpoints shown below. Vehicle control is 1% DMSO.



CONCLUSIONS

The 22 OPAHs we currently have identified and quantified appear to not be significantly causing toxicity of the Mississippi, Alabama, or Florida samples, even at concentrations 100x that of extracts. Developmental zebrafish toxicity of field-deployed passive sampling extracts must be a function of other OPAHs or compounds, or a result of the synergy of the mixture.

OPAHs appear to contribute to toxicity of Louisiana samples. These standard OPAH mixtures do elicit toxic response. One notable difference potentially contributing to the differences observed between the states is that the OPAHs in LA are different than those at other states and nominally 10-fold higher.

NEXT STEPS

Correlate individual chemical (PAH, OPAH) components with zebrafish endpoints.

Acknowledgements : C. Donald is supported by NIEHS Training Grant Fellowship T32 ES007060 from the National Institutes of Health. Support also provided by SRP. Thanks to members of the Sinnhuber Aquatic Research Laboratory, especially Greg Gonnerman. Special thanks to all members of the Food Safety and Environmental Stewardship Laboratory for data collection and laboratory and instrumental analysis.