

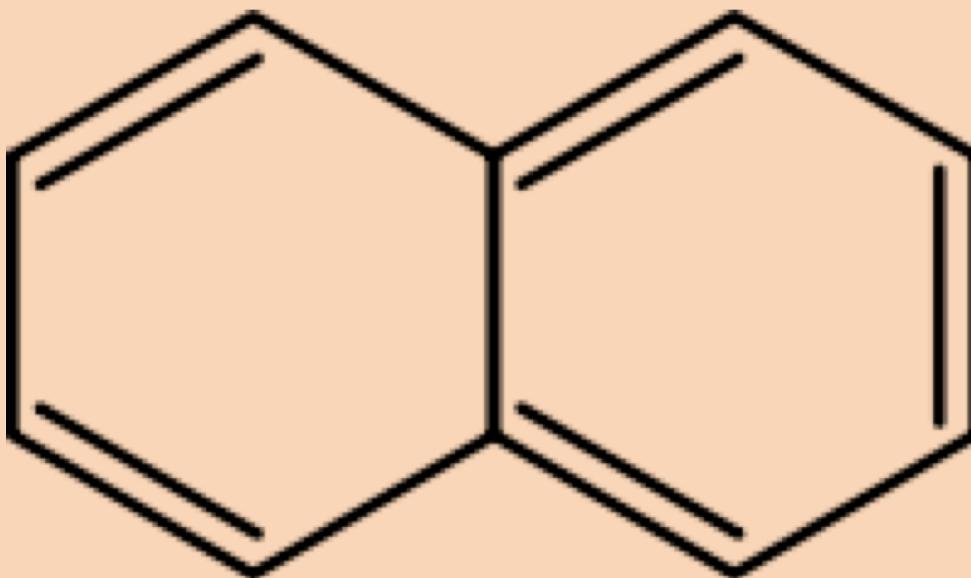
Investigating the Toxicity of Oxygenated Polycyclic Aromatic Hydrocarbons (OPAHs) in Developing Zebrafish

Researched by
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Mentor
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PAHs

- Polycyclic aromatic hydrocarbons
 - 2 or more rings
 - Named by functional groups



Naphthalene

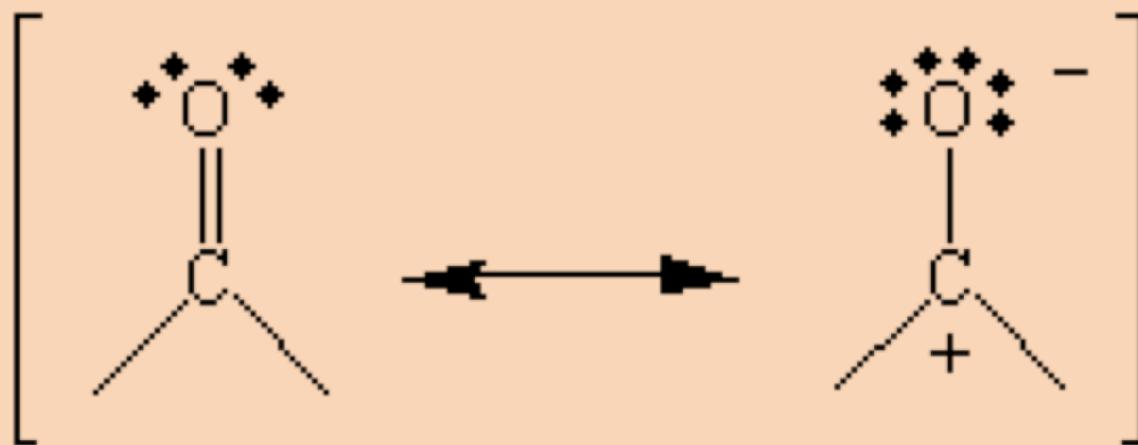
PAHs

- Environmental contaminants
 - Carcinogenic
 - Mutagenic
 - Teratogenic
- PAH production
 - Automobile exhaust
 - Industry emissions
 - Wood burning
 - Tobacco smoke



OPAHs

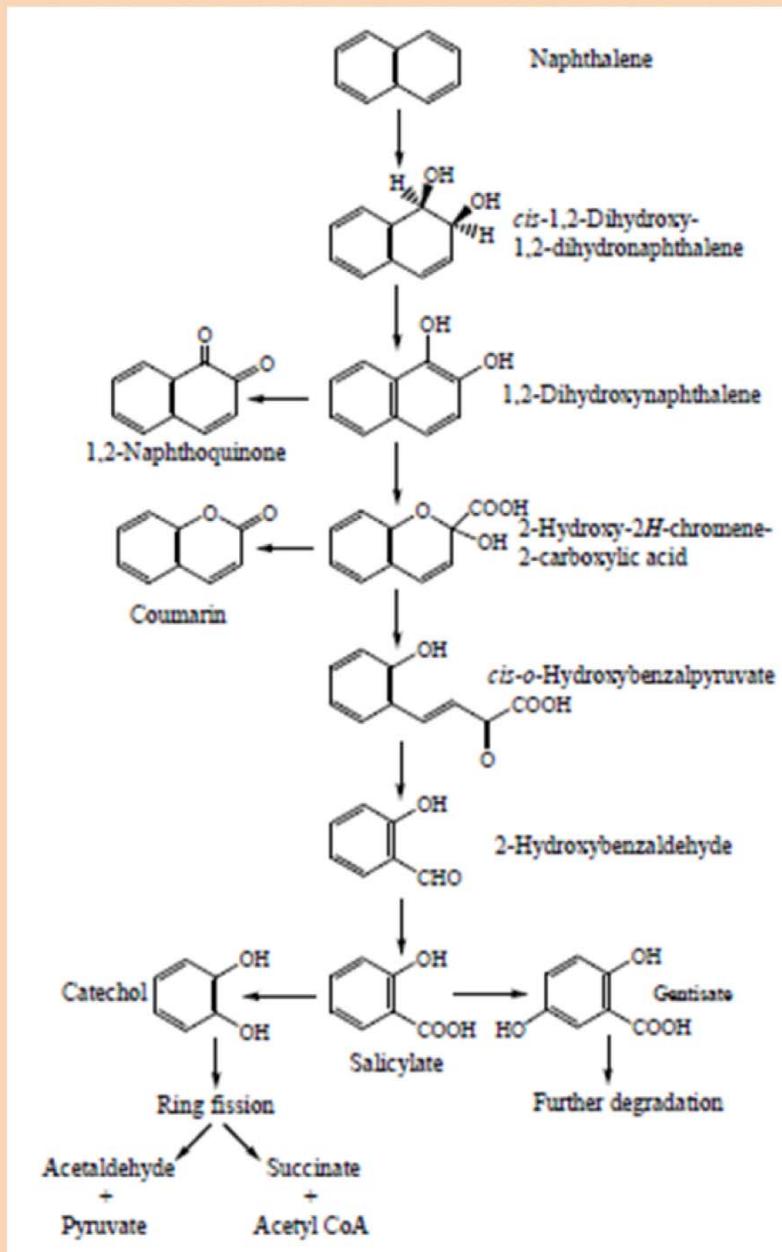
- Oxygenated polycyclic aromatic hydrocarbons
- PAHs with oxygen additions
 - Functional groups
 - Carbonyl, hydroxyl, carboxyl



OPAH Formation

- Incomplete combustion processes
- Transformation of PAHs
 - Chemical oxidation
 - Photo-oxidation
 - Biological/microbial transformation

Bacteria Degradation of Naphthalene



Research Objectives

- Analyze the toxicity of OPAHs in developing zebrafish
 - AHR activation
 - CYP1A induction
 - Molecular structures
- Expand the limited scientific knowledge about OPAH toxicity



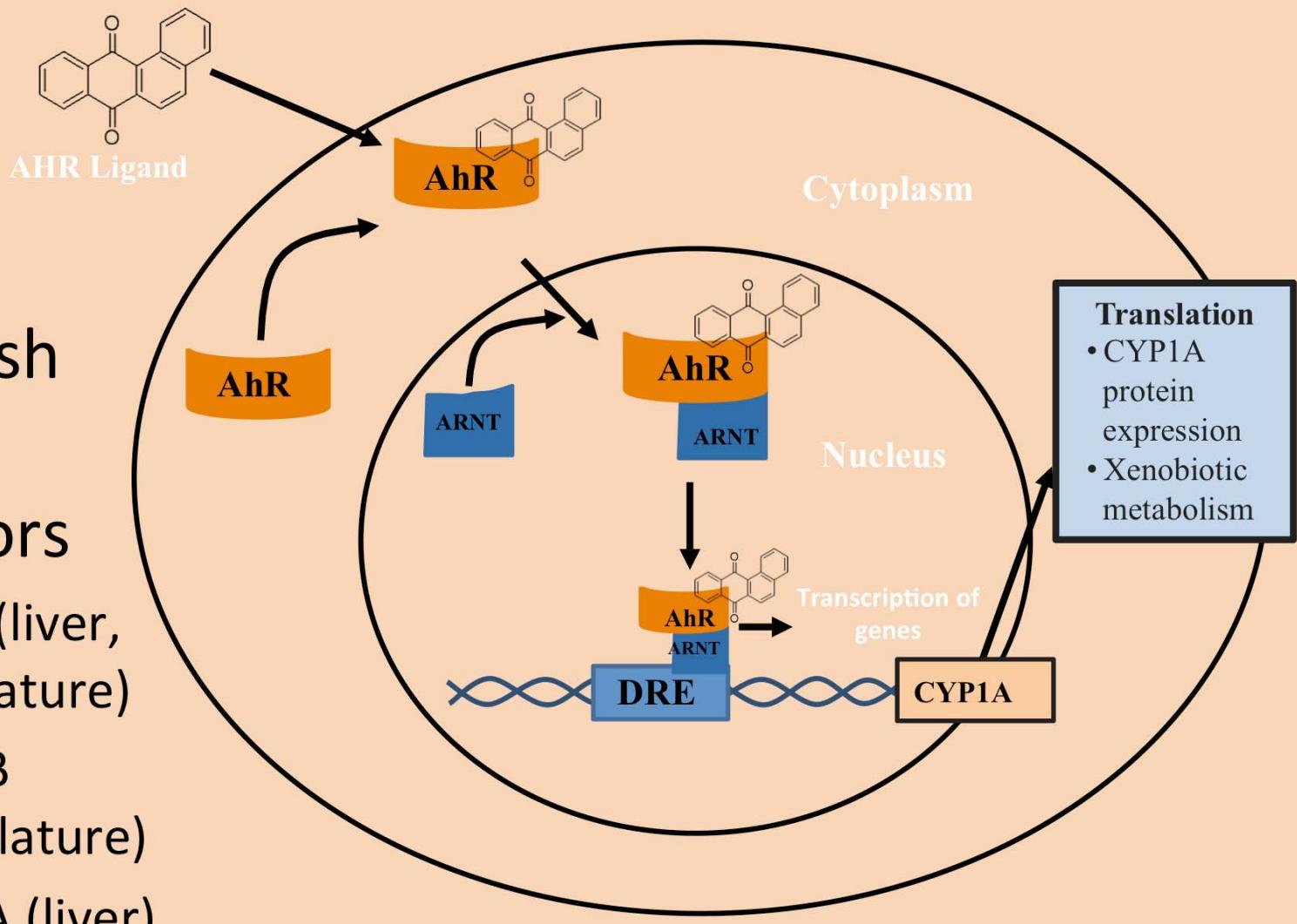
TANGUAY LABORATORY MOLECULAR TOXICOLOGY

- Zebrafish
- Development and toxicity



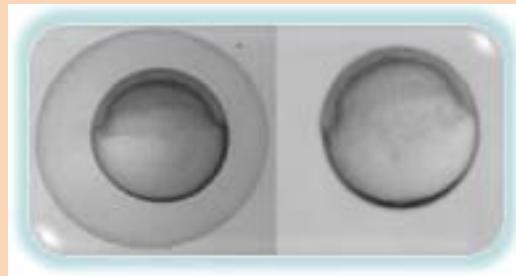
The AHR Pathway

- Three Zebrafish AHR receptors
 - AHR2 (liver, vasculature)
 - AHR1B (vasculature)
 - AHR1A (liver)

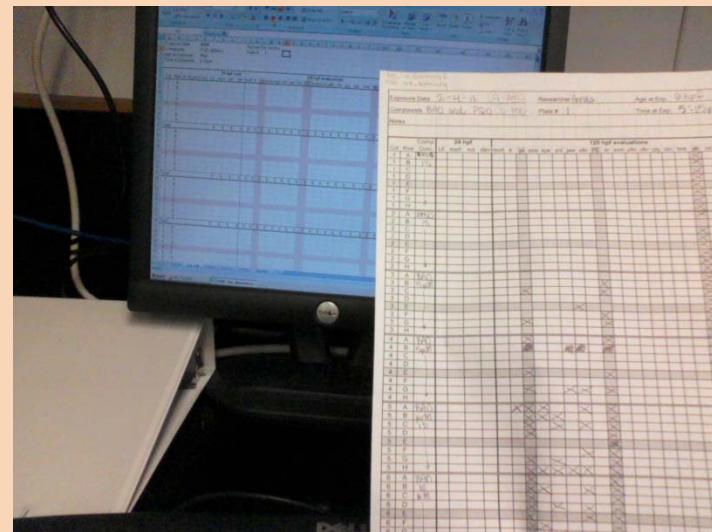


Experiment Setup

- Dechorionate embryos



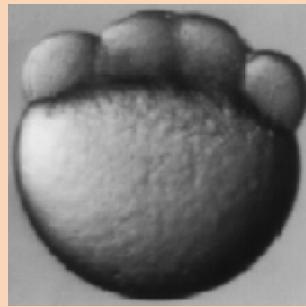
- Expose to the chemical in solution
- Make 24 hour and 5 day evaluations
- Fix for IHC analysis



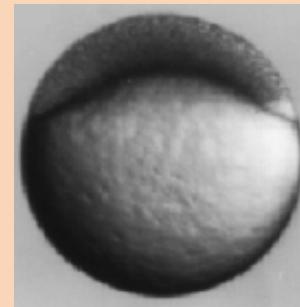
Stages of Development



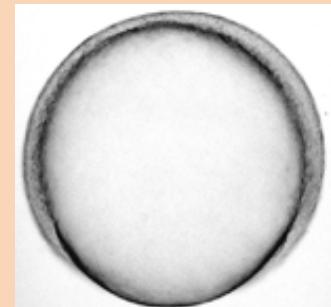
3 min



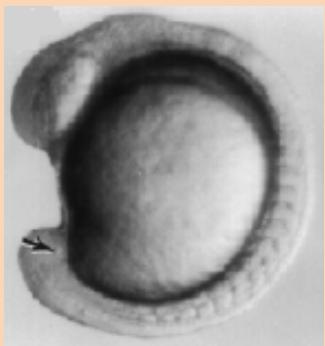
1.25 hr



4 hr



6 hr



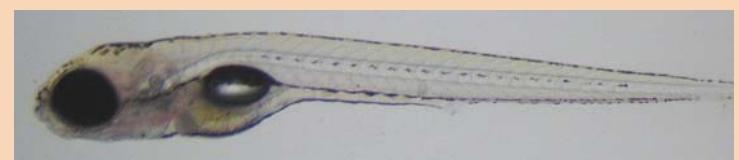
19 hr



48 hr



24 hr



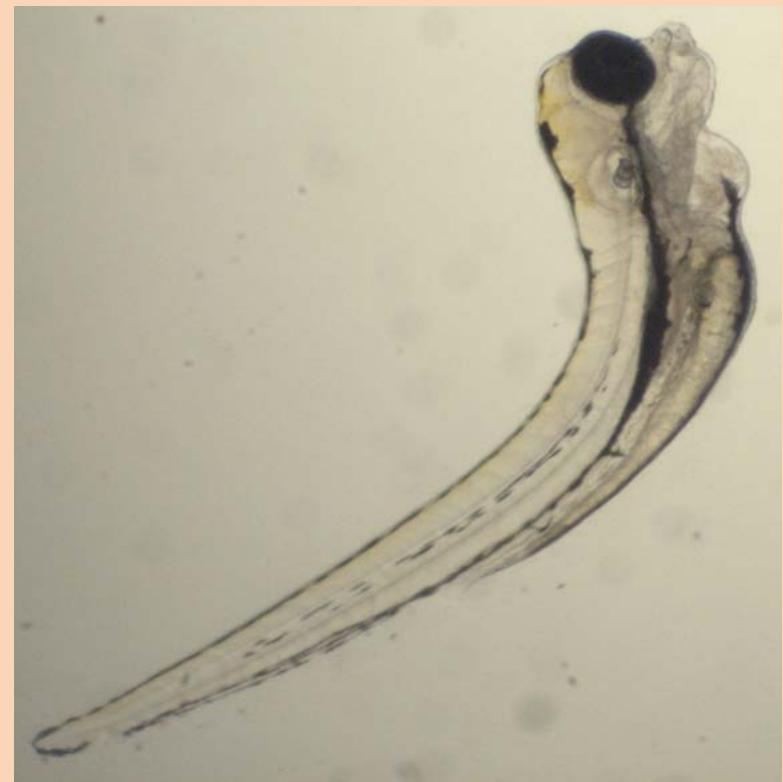
5 day

9,10-Phenanthrenequinone

Five Day Observations



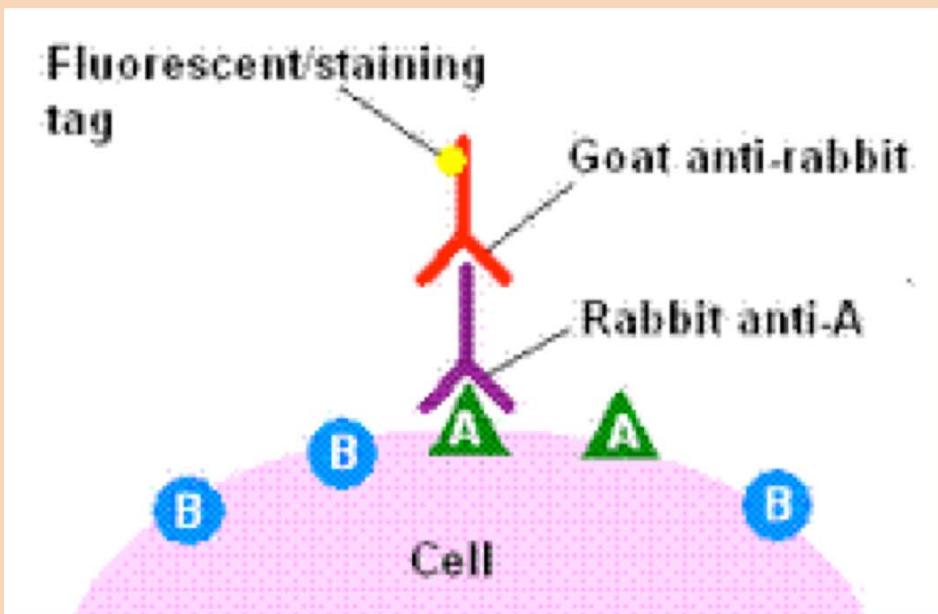
1% DMSO (Control)



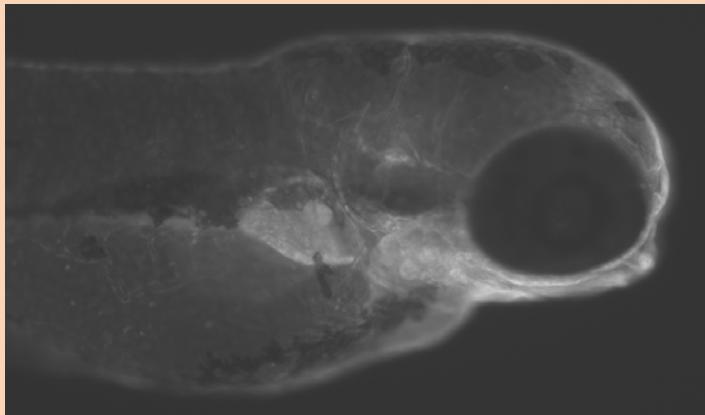
2.0 μ M PQO in 1% DMSO

Immunohistochemistry

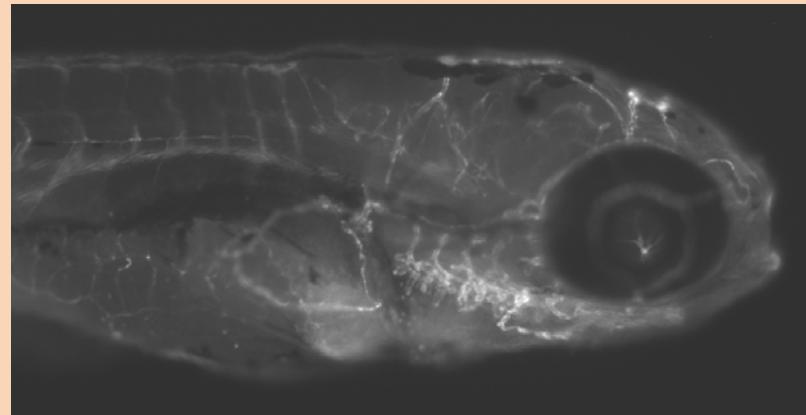
- IHC
 - Tag CYP1A protein



IHC

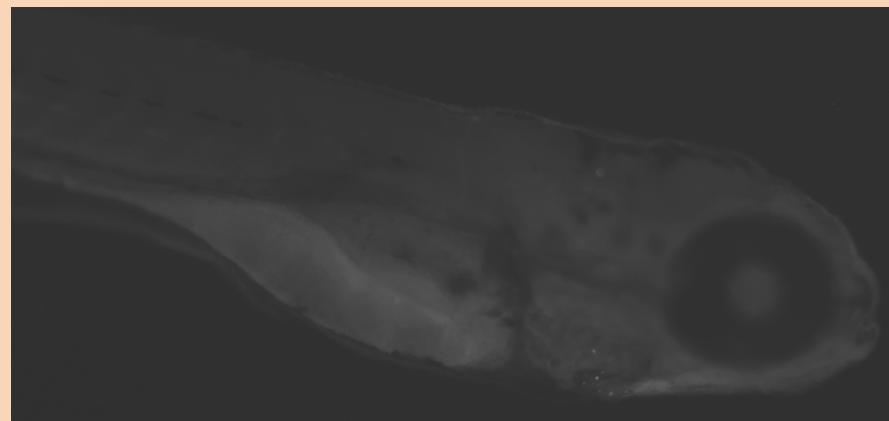


10 μM Leflunomide



25 μM Benz(a)anthracene

Positive Controls (Vehicle: 1% DMSO)

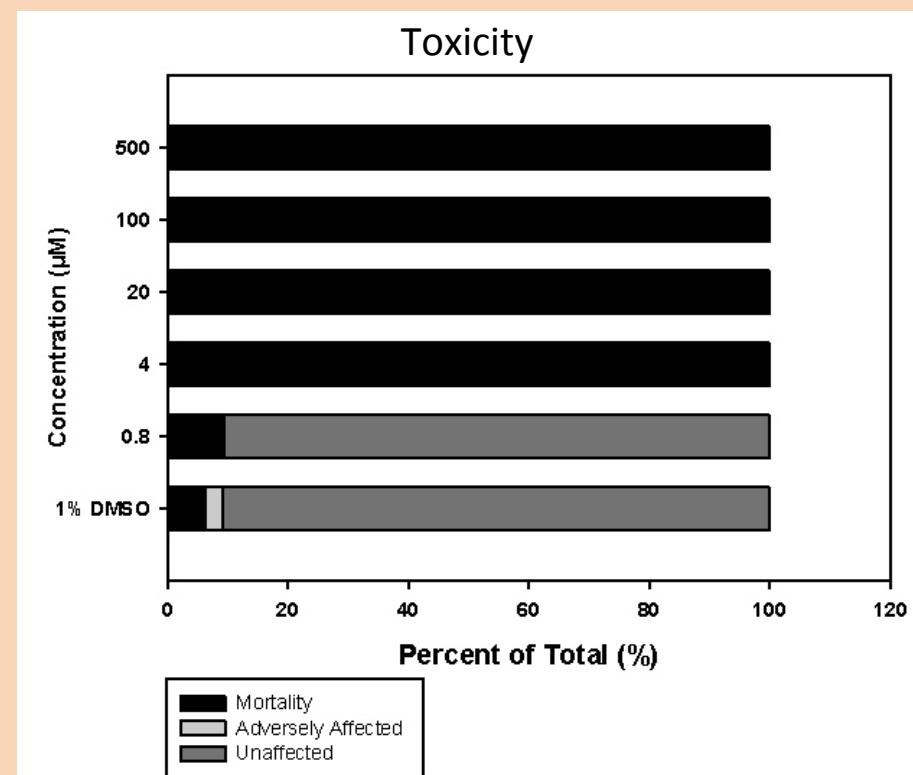
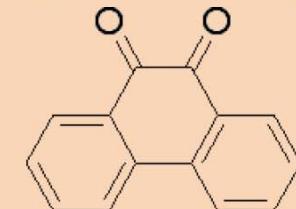
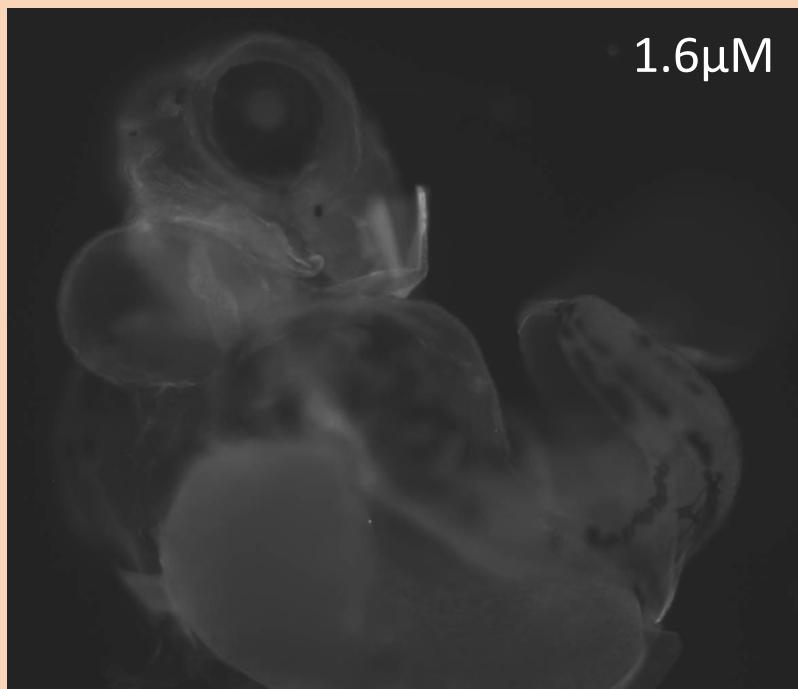


Negative Control (1% DMSO)

Results

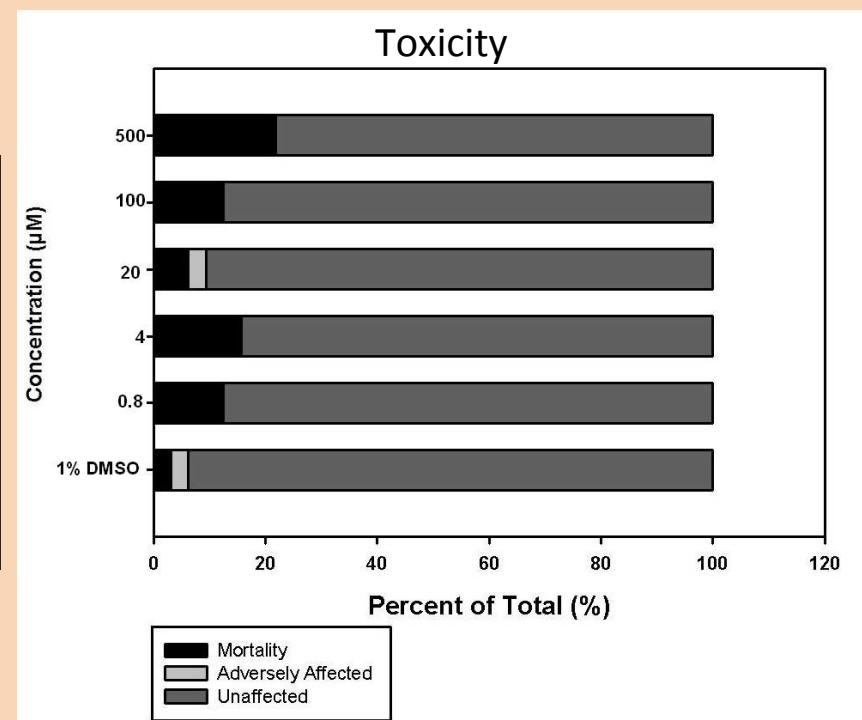
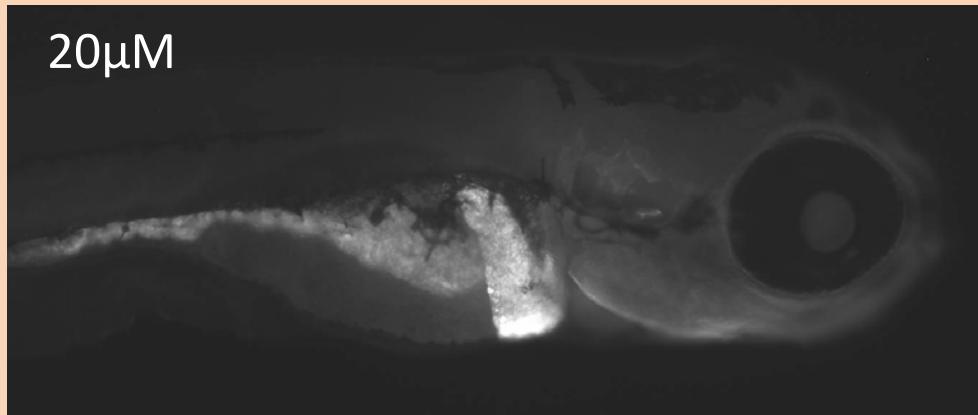
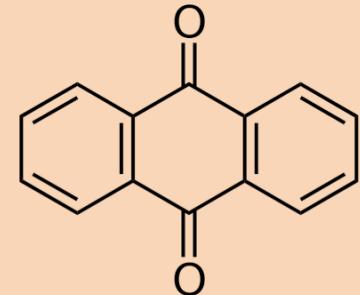
9,10-Phenanthrenequinone (PQO)

- $C_{14}H_{18}O_2$
- Toxic chemical in diesel exhaust emissions (DEP)
- Quinone



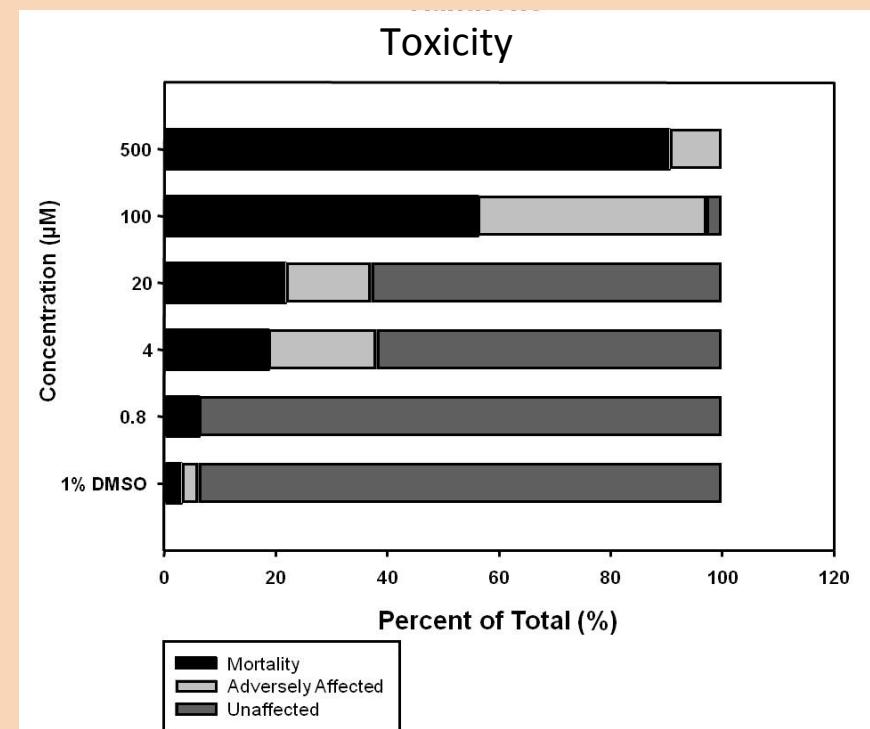
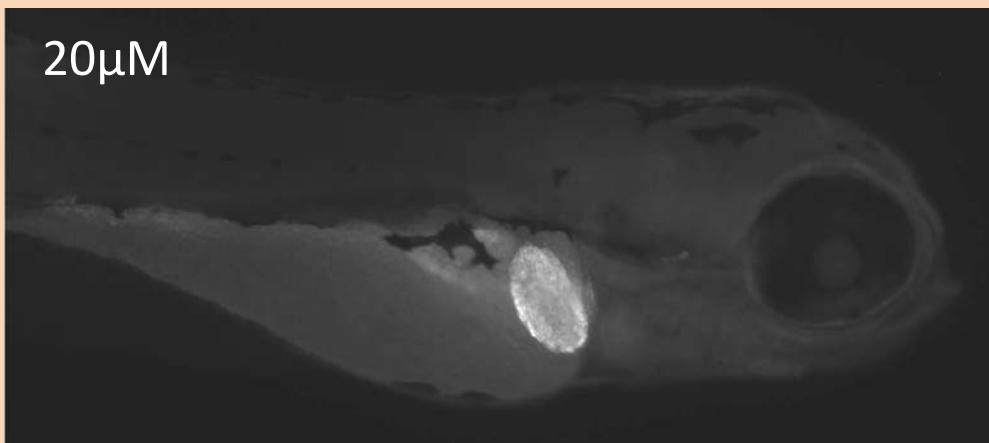
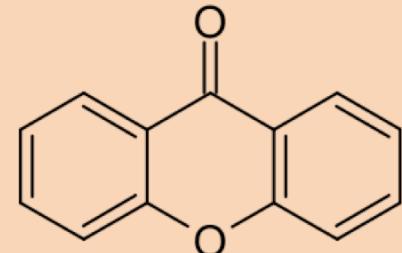
9,10-Anthraquinone

- $C_{14}H_8O_2$
- Quinone



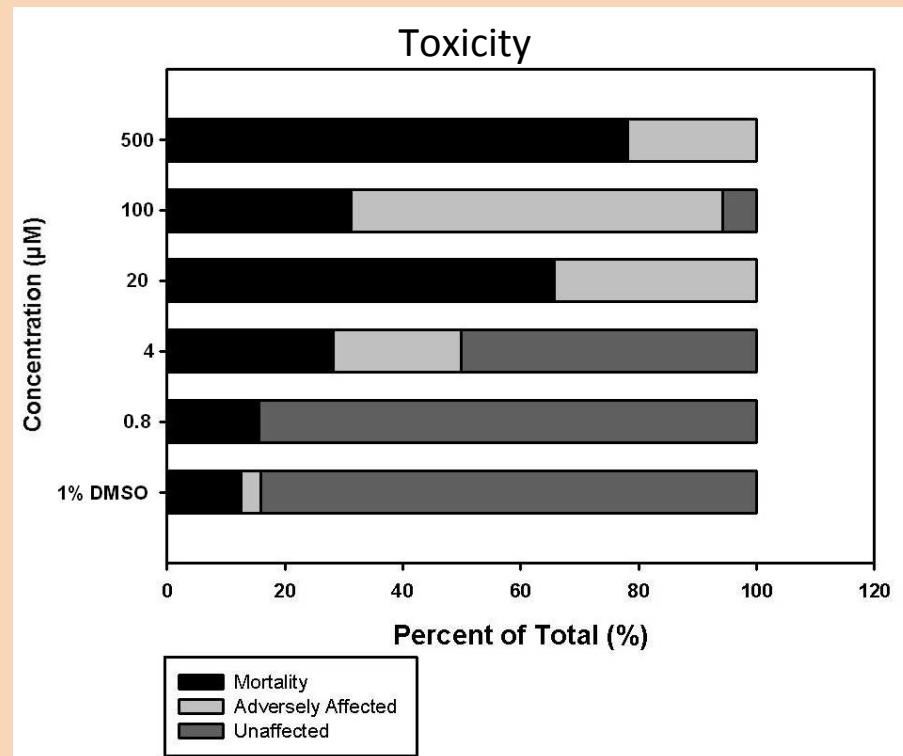
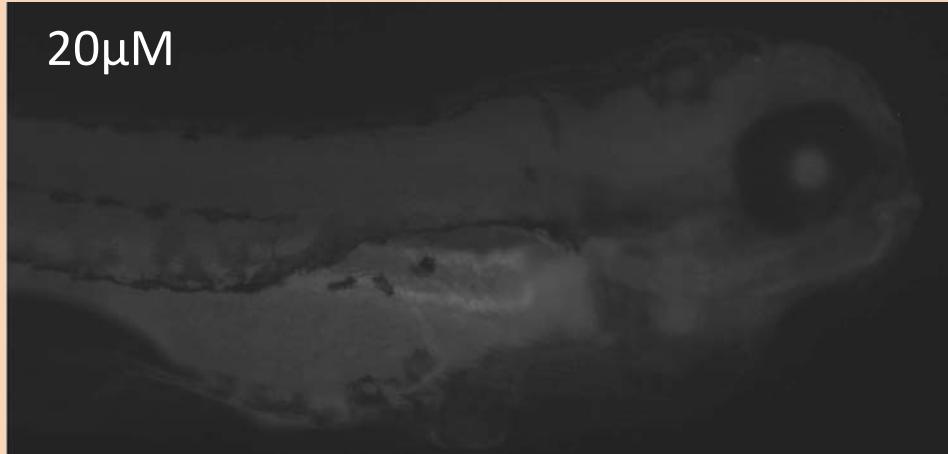
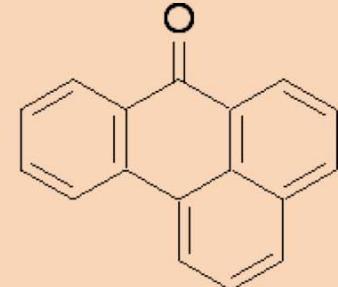
Xanthone

- $C_{13}H_8O_2$
- Very little information known



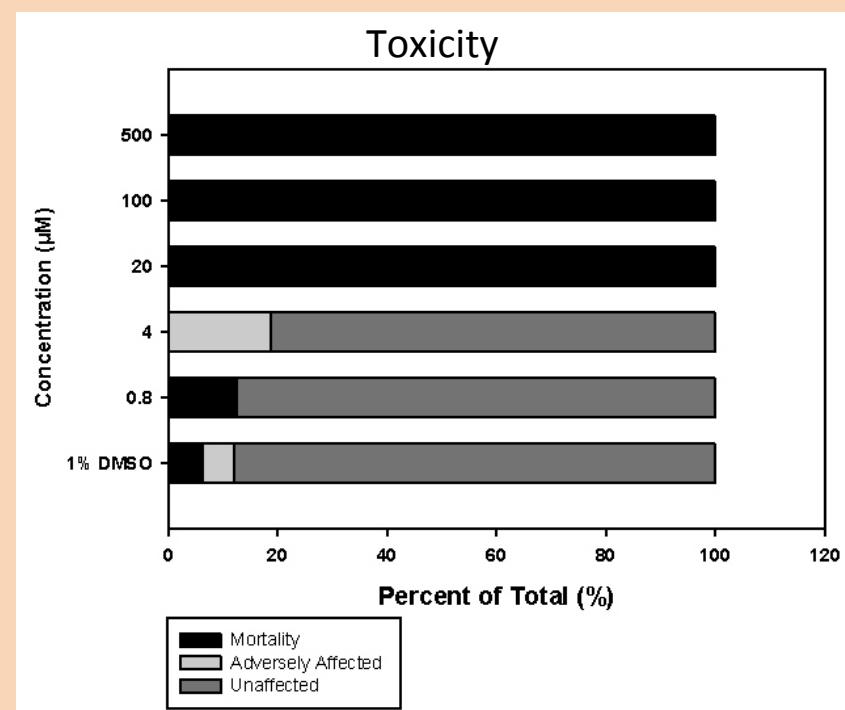
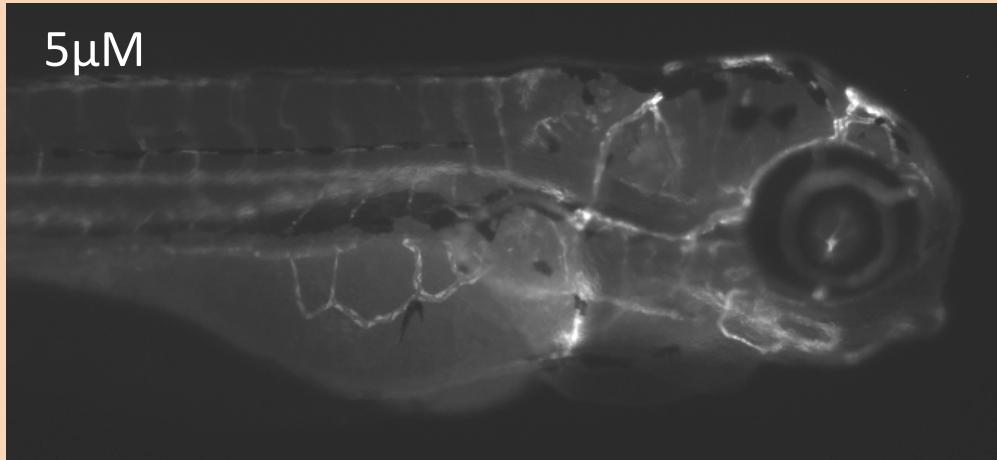
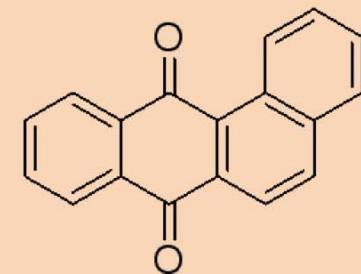
1,9-Benz-10-anthrone (BAO)

- $C_{17}H_{10}O$
- Very little information known



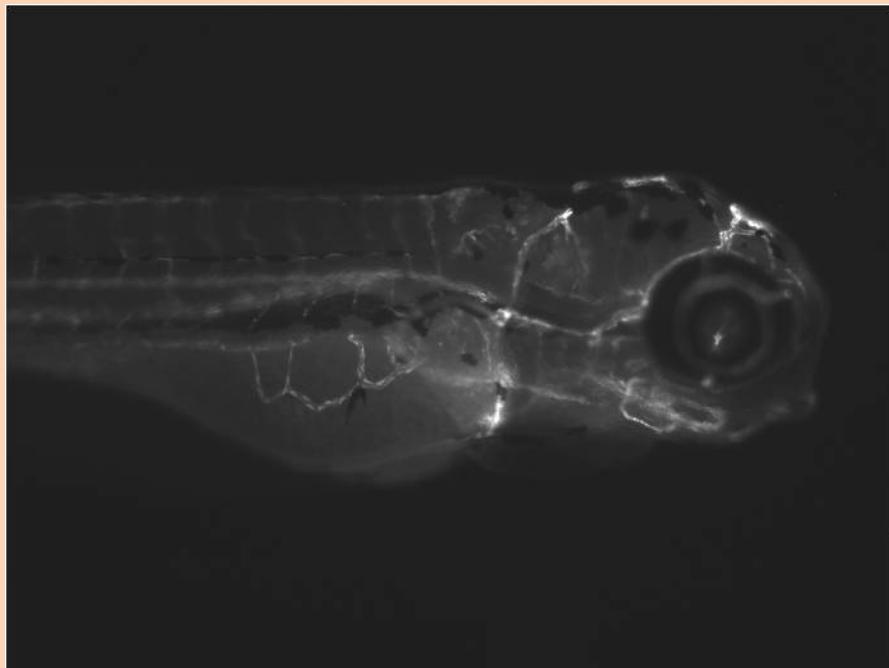
Benz[a]anthracene-7,12-dione (BADO)

- $C_{18}H_{10}O_2$
- Quinone

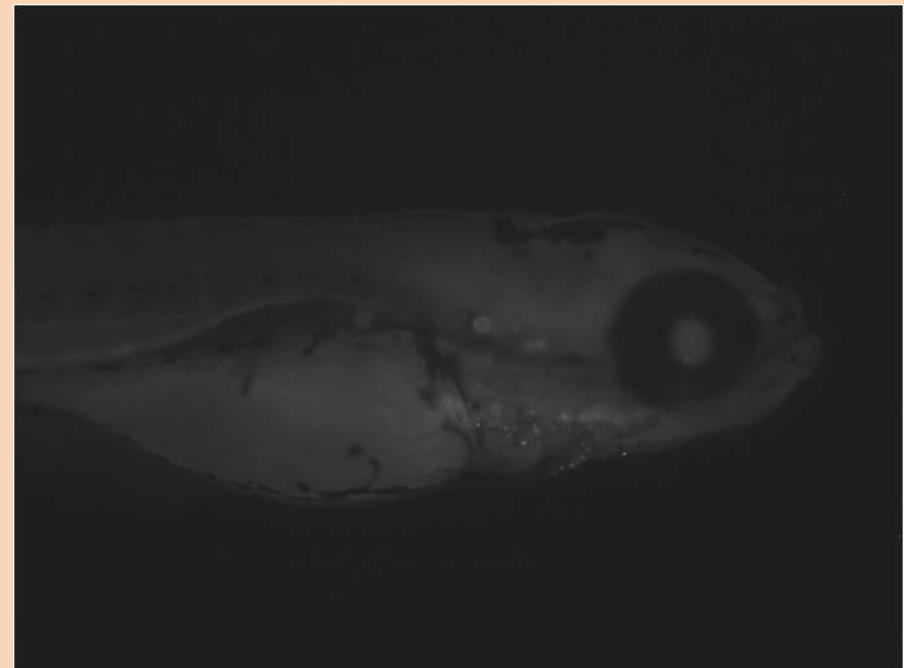


AHR2 Knockout

- 5 μ M Benz[a]anthracene-7,12-dione (BADO)



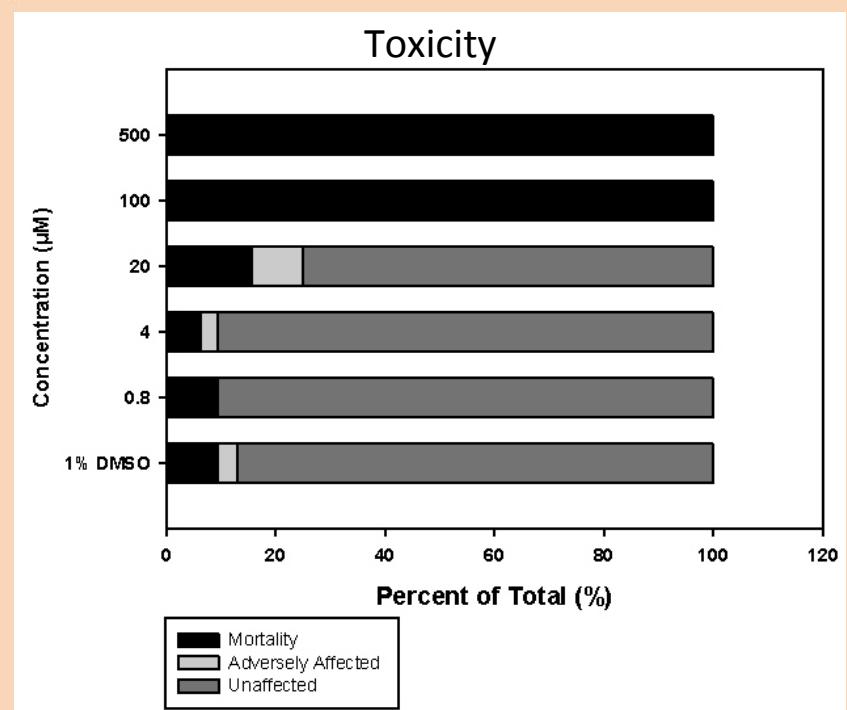
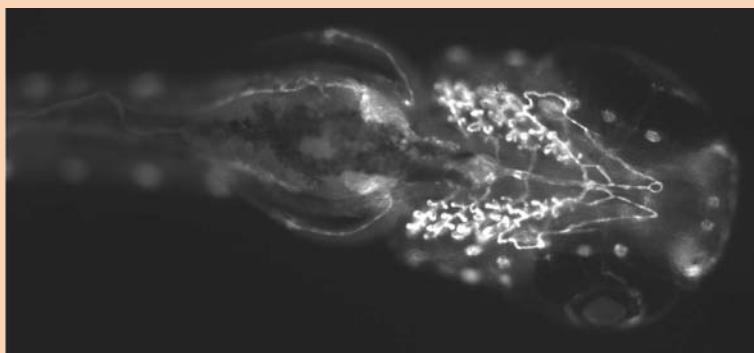
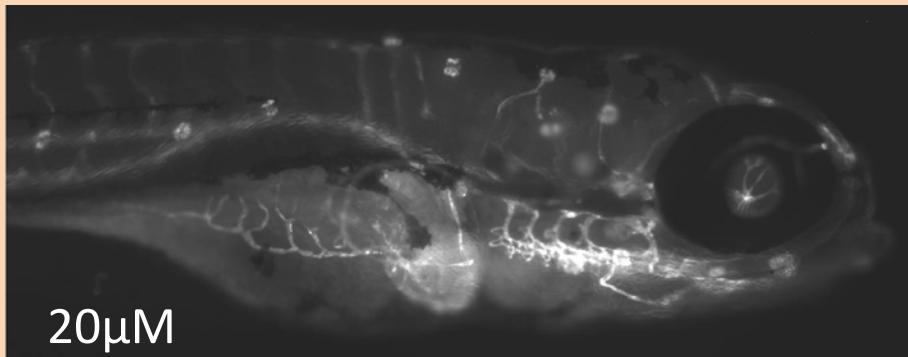
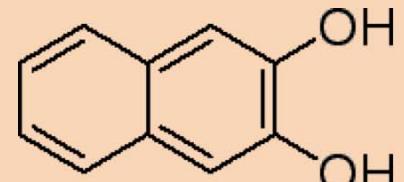
Wild-type Fish



AHR2 Knockout Fish

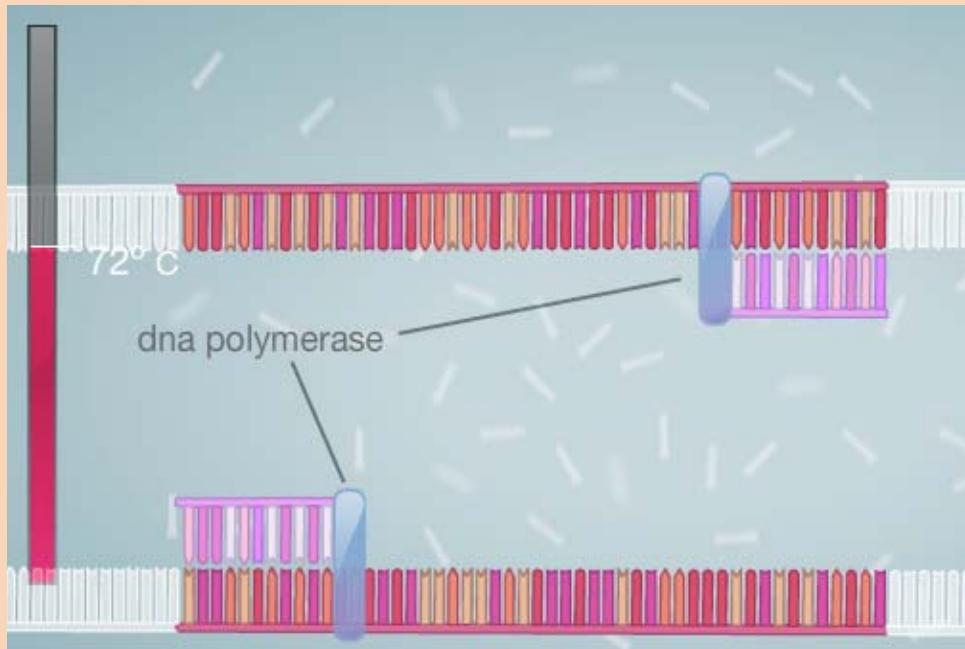
2,3-Dihydroxynaphthalene

- $C_{10}H_8O_2$
- 2-ring, more volatile
- Strong unique CYP1A expression



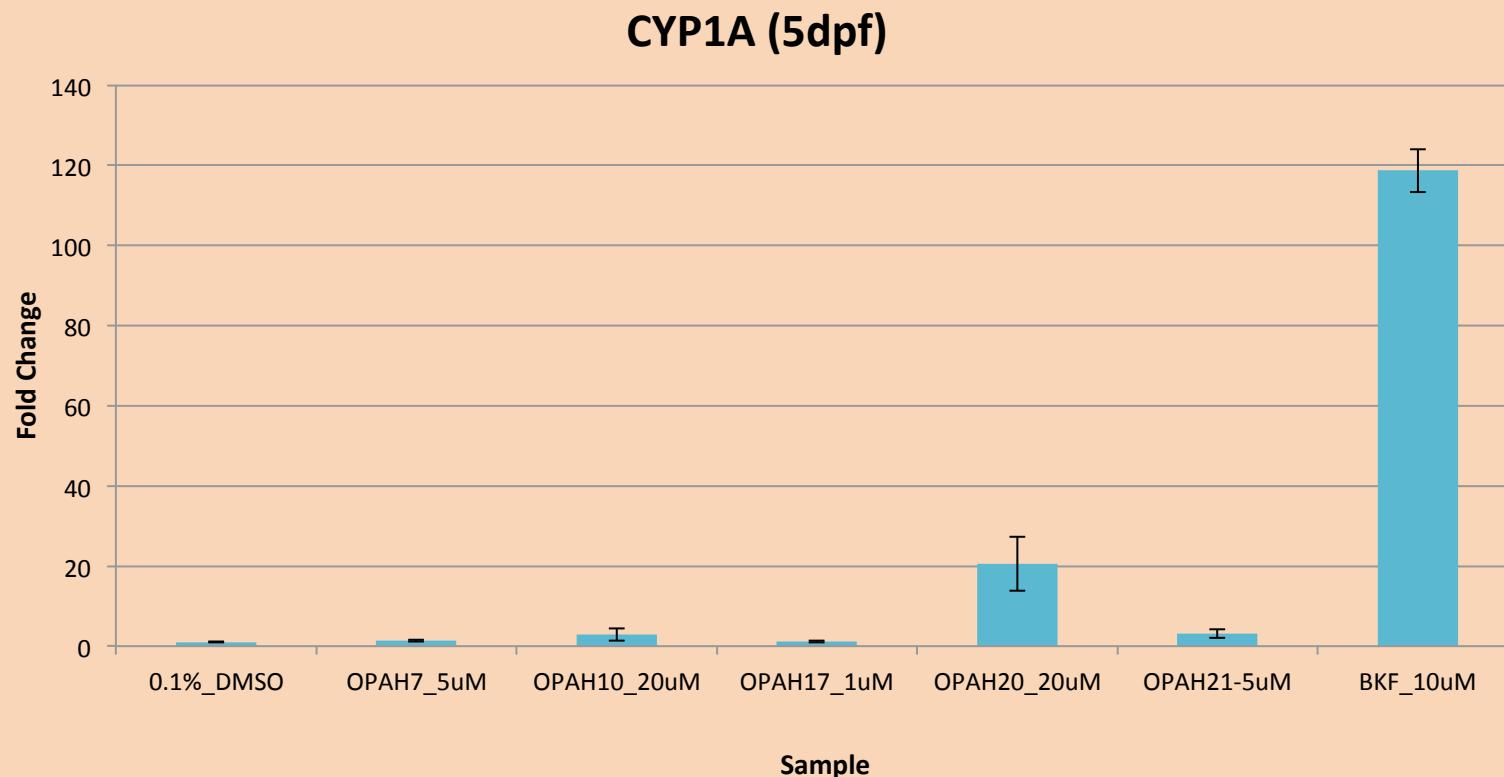
Experiment

- Polymerase Chain Reaction
 - (PCR)
 - Gene expression changes
 - qPCR

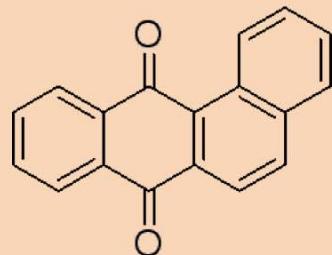


Experiment

- Quantitative PCR
 - Gene expression differences between products



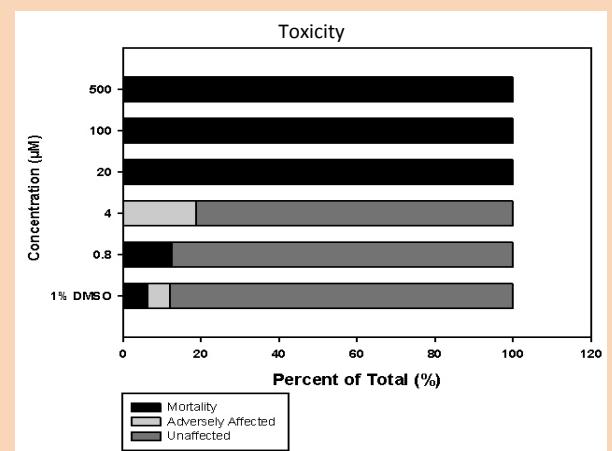
Structural Dependence of OPAH Toxicity



Benz[a]anthracene-7,12-dione (BADO)



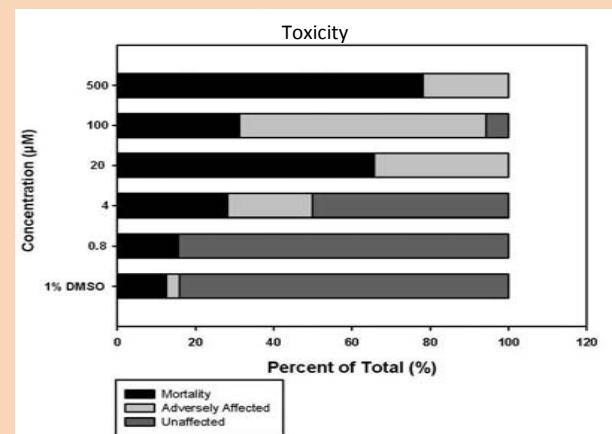
5 μ M

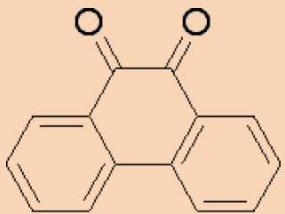


1,9-Benz-10-anthrone (BAO)



10 μ M

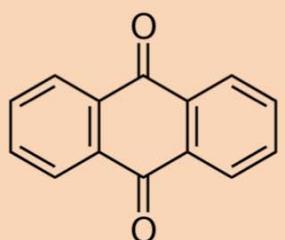
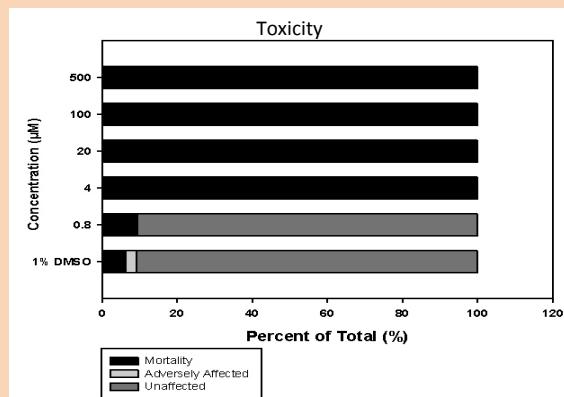




9,10-Phenanthrenequinone (PQO)



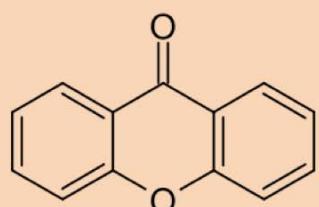
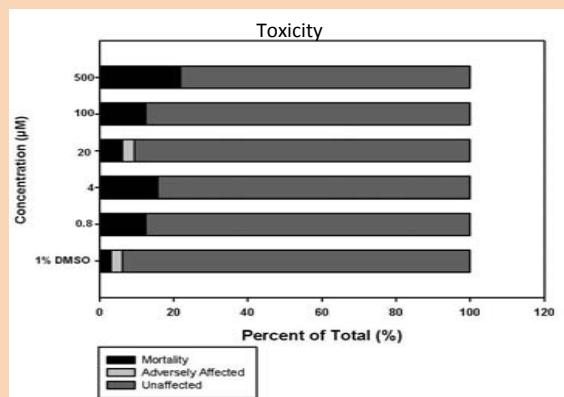
2 μ M



9,10-Anthraquinone



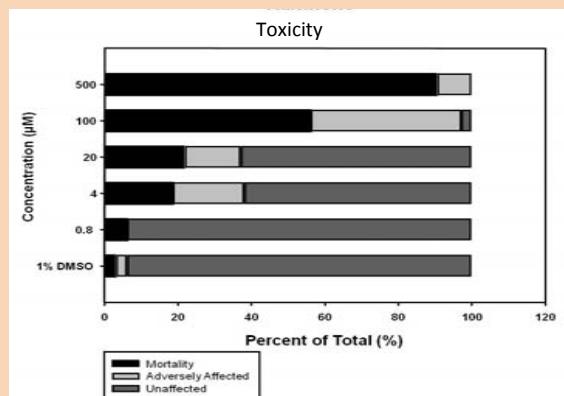
20 μ M



Xanthone



20 μ M



Conclusion

- Oxygenated polycyclic aromatic hydrocarbons
 - Toxic compounds
 - Structural relationships
 - Present in environment
- Future
 - 2-ring OPAHs
 - Injections into chorion



Acknowledgements

- Howard Hughes Medical Institute
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- Dr. Kevin Ahern

