

## **Investigating the Impact of Organic Mixtures on Cardiac Development of the Killifish**

Superfund sites are often contaminated with complex mixtures of organic and inorganic substances. Because data on the toxicological consequences of multiple chemical exposures are limited, it is difficult to assess risks to humans and wildlife exposed to such mixtures. Dr. Richard Di Giulio at the Duke University Superfund Basic Research Program is conducting studies to explore the effects of real-world contaminant mixtures on development in the estuarine fish, *Fundulus heteroclitus*, or killifish. The findings of these mechanistic studies will likely have both human health and ecological relevance.

Dr. Di Giulio leads a team of researchers studying a population of killifish that inhabits an estuary in the Elizabeth River, Virginia, a tributary of the James River just below the Chesapeake Bay. The study site is adjacent to the Atlantic Wood Industries, Inc. Superfund site where a wood treating facility operated for over 50 years until its closure in 1992. The Superfund site was polluted by a complex mixture of chemicals, dominated by creosote that was used as a wood preservative. Due to runoff, sediments in the Elizabeth River study area are contaminated with polycyclic aromatic hydrocarbons (PAHs), various nitrogen-, sulfur- and oxygen-substituted PAHs, and the wood preservative pentachlorophenol.

Previous research determined that older individuals in the killifish population inhabiting the study site exhibit elevated rates of cancer, particularly liver cancer. The Duke team learned that killifish from the Elizabeth River study area are resistant to the acute toxicity (i.e., lethality) of the sediments at the site. The researchers have identified cardiovascular effects in developing embryos, such as edema in the yolk sac and “tube heart”, as the most sensitive effects underlying acute toxicities. By exposing laboratory-reared offspring to extracts of Elizabeth River sediments, they found that second through fourth generation offspring of adults captured in the Elizabeth River are far more resistant to acute toxicity and developmental anomalies than offspring from Kings Creek, a nearby relatively unpolluted site. They also demonstrated these adaptations for resistance to acute toxicity have come at a price, as these resistant fish are more sensitive to natural stressors such as low dissolved oxygen and ultraviolet light. Thus, this population provides an interesting case study in “evolutionary ecotoxicology”.

The Duke University researchers are investigating the mechanisms that underlie both the effects of the Elizabeth River contaminant mixture on cardiovascular development and the molecular and biochemical mechanisms that underlie the adaptations providing resistance to the acute toxicity of Elizabeth River sediment contaminants. At least part of this resistance is heritable for multiple generations in the laboratory in clean conditions and is likely the result of evolution, but part also appears to be based on non-heritable physiological acclimation.

Because many of the chemicals present in Elizabeth River sediments share a common ability to exert toxicity in part via oxidative stress, Dr. Di Giulio's team focused on the hypothesis that fish inhabiting the Elizabeth River have more highly developed antioxidant defense systems than fish from unpolluted sites. Results to date support this hypothesis as the researchers have observed elevated levels of antioxidant enzymes (such as superoxide dismutase) and nonenzymatic antioxidants (such as glutathione) in tissues of Elizabeth River killifish versus killifish from Kings Creek. Also, Elizabeth River killifish are far more resistant to the toxicity of a simple oxidant (butyl peroxide) than Kings Creek killifish. The researchers suggest that the Elizabeth River killifish may be able to survive in their contaminated environment, at least in part, because of antioxidant defenses that are upregulated via both short-term physiological acclimation and heritable mechanisms.

The discovery of the marked sensitivity of cardiovascular development to Elizabeth River sediment extracts led Dr. Di Giulio's team into explorations of this phenomenon that have ramifications for both ecological and human health. Results to date suggest important interactions between aryl hydrocarbon receptor (AHR) agonists such as

benzo(a)pyrene (BaP) and cytochrome P450 (CYP1A) enzyme inhibitors such as flouranthene (FL). Agonists are compounds that bind to a receptor to form a complex which elicit a full biological response, such as increased production of the enzyme CYP1A in the case of AHR; inhibitors block the activity of specific enzymes, such as CYP1A in the case of FL.

Both BaP and FL are found in sediments at the study site and the interactions observed by Di Giulio's team could not be predicted from typical single compound studies. Initial findings were obtained using dose response studies with varying dilutions of sediment extracts. These studies employed a novel assay for measuring CYP1A enzyme activity, in living embryos without harm to these organisms. The scientists observed that co-exposure to AHR agonists and CYP1A inhibitors greatly synergized (100 to 1000-fold) the cardiovascular toxicity of these compounds when applied singly. Interestingly, this effect of CYP1A inhibitors is the opposite of their effect in combination with chlorinated AHR agonists such as dioxin (2,3,7,8-TCDD) and PCB 126. The basis for this difference is currently under investigation, as well as relationships among AHR agonism, CYP1A inhibition, oxidative stress, and cardiovascular development. Cardiovascular deformities are a very sensitive endpoint with these mixtures, and also represent a human health outcome for which there is great concern. Thus, these mechanistic studies with the killifish model will likely have both human health and ecological relevance.

A mechanistic understanding of the contaminant-induced changes in gene expression in these fish populations promises to improve our understanding of the long-term impact of sublethal exposure to pollutants on wild populations, an area of increasing concern for environmental scientists.

**For More Information Contact:**

Richard T. Di Giulio, Ph.D.  
Duke University  
Nicholas School of the Environment and Earth Sciences  
Box 90328  
Durham, NC 27708-0328  
Tel: 919-613-8024  
Fax: 919-668-1799  
Email: richd@duke.edu

**To learn more about this research, please refer to:**

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