Meeting: SETAC2013, Nashville, Tennessee.

Presentation Type:
Platform Preferred

Track:
Aquatic Toxicology and Ecology

Session: A WET Conceptual Approach to Water Management Using Next Generation Bioactivity and MOA Bioassays

Abstract Title:
Coupling *in vitro* and *in vivo* neurochemical-based assessments of wastewater effluents from the Maumee River Area of Concern (AOC)

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Abstract:

Here we utilize *in vivo* and *in vitro* approaches to study whether real world effluents released in the Maumee River (Toledo, OH) Area of Concern (AOC) contain neuroactive substances that may impair fish reproduction and behavior. Our approaches help extend the concept of endocrine disruption beyond routine bioassays (ER, AR, TH) under the premise that toxicants may also interact with and disrupt the function of neurotransmitter receptors and enzymes that play critical roles in vertebrate reproduction and behavior. Cell-free methods were used to study such interactions, and to also compare the *in vivo* and *in vitro* responses. First, 288 fish (fathead minnow) were exposed in cages to river water at 8 different sites along the Maumee River, including several in close proximity to wastewater treatment plant (WWTP) discharges. After 4 d of *in situ* exposure, brains were sampled and analyzed for GABA, dopamine-2 and N-methyl-D-aspartate (NMDA) receptor binding, and monoamine oxidase (MAO) and glutamine synthetase enzyme activity. The preliminary work shows that fish caged downstream of a major WWTP had increased MAO activity (66% and 35% in males and females respectively), and that NMDA receptor binding was also significantly changed (30% decrease in females). Second, *in vitro* studies were performed on river water extracts (final concentration 5x) to see if they interfere with the aforementioned neurochemicals studied *in vivo*. This was performed in 5 model species of aquatic relevance (fathead minnow, rainbow trout, bald eagle, river otter, human). The initial *in vitro* results suggest that extracts optimized for recovery of alkylphenols significantly impacted the NMDA receptor binding (15% decrease), whereas those optimized for recovery of steroid hormones induced binding (up to 34%) in fish. In vitro responses for other organisms and endpoints will be presented. In summary our work thus far suggests that wastewater effluents discharged to the Maumee River AOC contain chemicals that may directly interact (and possibly interfere) with neurochemicals that are important in fish reproduction and behavior. In addition,

the work here (via a US EPA STAR grant) is taking next steps to identify key neurochemical indicators, resolve *in vitro* and *in vivo* responses, and compare responses across taxa.