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# Title:

Assessment of wastewater treatment plant effluent on fish reproduction utilizing the adverse outcome pathway conceptual framework

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# Abstract:

Wastewater treatment plant (WWTP) effluents are a known contributor of chemical mixture inputs into the environment. Whole effluent testing guidelines were developed to screen these complex mixtures for acute toxicity. However, efficient and cost-effective approaches for screening effluents or surface waters for more subtle, sublethal effects of emerging contaminants (e.g., endocrine disrupting compounds, pharmaceuticals, and personal care products) are lacking. Effects-based monitoring using pathway-based endpoints (e.g., gene expression, in vitro bioassays, biomarkers) holds promise in this regard. However, the challenge of discriminating adaptive biological change from early warning of potential adverse effects remains. The present research employed a case study approach to probe this question. In previous years, exposure of adult fathead minnows to the case study effluent (50%) has resulted in vitellogenin induction and elevated 17β-estradiol concentrations in males. Estrogenic activity was detected in both the effluent and surface water proximal to the point of discharge using the T47D-kBluc cell bioassay, although significant induction of vitellogenin was not detected in male fathead minnows caged in the receiving water. For the present work, a 21 d reproduction study using adult fathead minnows was conducted in a novel flow-through testing system at the WWTP. Breeding pairs of male and female fathead minnows were exposed to control Lake Superior water and three dilutions of final treated effluent (5%, 20%, and 100%) with 12 males and 12 females per treatment. A variety of molecular and biochemical endpoints representing key events along established adverse outcome pathways linking estrogen receptor activation and other

relevant endocrine molecular initiating events to reproductive impairment in fish were examined. Additionally, in order to directly compare observed biological effects with the chemical composition and in vitro estrogenic activity of the effluent, several 7 d composite effluent samples were collected throughout the exposure for chemical and in vitro bioassay analyses. Results of the study provide both further characterization of the case study effluent with respect to its chemical composition and biological activities and insights into the significance of pathway-based effects with regard to predicting adverse reproductive outcomes. *The contents of this presentation do not constitute official EPA policy*.

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