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Title:
Effects of a real-time exposure to an estrogenic effluent on reproduction in the fathead minnow (*Pimephales promelas*)

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Abstract:

Wastewater treatment plant (WWTP) effluents are a well-established point of convergence through which anthropogenic chemicals enter surface waters. Whole effluent testing guidelines were developed to screen these complex mixtures for acute toxicity. However, effects-based approaches for evaluating effluents or surface waters for more subtle, sublethal effects of contaminants are lacking. Of particular concern are contaminants such as pharmaceuticals and some pesticides that could affect endocrine function through action on the estrogen or androgen receptors or inhibition of sex steroid synthesis. The present study employed an effects-based approach using a historically estrogenic WWTP effluent to assess potential chronic effects of contaminants on different biological pathways. Specifically, a 21 d reproduction study using adult fathead minnows was conducted on-site at a WWTP using a continuous flow-through testing system that delivered final treated effluent in real-time. Breeding pairs of male and female fathead minnows were exposed to control water and three effluent dilutions (5%, 20%, and 100%). A variety of molecular and biochemical endpoints representing key events along established adverse outcome pathways linking estrogen receptor (ER) activation, and other relevant endocrine molecular initiating events, to reproductive impairment in fish were examined. In order to directly compare observed biological effects with

the chemical composition and in vitro estrogenic activity of the effluent, 7 d composite effluent samples were collected for chemical and in vitro bioassay analyses. Chemistry results were used to construct a chemical-gene interaction network to aid in targeted gene expression analyses. Cumulative fecundity was significantly reduced in fish exposed to 100% effluent and significantly increased in those exposed to 20% effluent. The most potent estrogen detected in the effluent was estrone, and estrone concentration trends aligned with in vitro ER activation. Male vitellogenin mRNA transcript abundance and plasma protein concentrations increased in a dose-dependent manner with effluent concentration. Although predictable biological responses to ER agonism were observed, given the nature of chemical mixtures, additional pathways were evaluated. Results of the study provide 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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