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**Abstract Title:**

Unsupervised Analysis of the Effects of a Wastewater Treatment Plant Effluent on the Fathead Minnow Ovarian Transcriptome

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**Abstract:**

Wastewater treatment plant (WWTP) effluents contain complex mixtures of chemicals, potentially including endocrine active chemicals (EACs), pharmaceuticals, and other contaminants of emerging concern (CECs). Due to the complex and variable nature of effluents, biological monitoring has often been used to provide an integrated evaluation of effluent quality. However, biologically-based monitoring that relies solely on apical endpoints like survival or a relative handful well characterized biomarkers may not be well equipped to detect exposure to and/or impacts of many CECs. Microarray technology provides one potential means to broaden the scope of biological monitoring by allowing for an unsupervised analysis of wide scale gene expression changes. In this study we utilized a 15,000 gene oligonucleotide microarray to examine the gene expression profiles in ovary tissue from mature female fathead minnows (*Pimephales promelas*) exposed to 0%, 10%, 50% and 100% WWTP effluent for 48 h. Changes in gene expression were linked to physiological endpoints such as steroid production and specific biological pathways and functions (i.e., gene ontology [GO] categories) through statistical analysis. Among the 15,000 features on the microarray, 170 were identified as differentially expressed ( $p < 0.01$ ) among the effluent treatments. Principal component analysis (PCA) of the data revealed that the treatments cluster into distinct groups with the 50% effluent treatment being the most distinct from the other treatments. Correspondingly, only the 50% effluent treatment had significantly elevated estradiol and testosterone production when compared to the control. Functional analysis identified several GO terms that were statistically over-represented in the differentially expressed gene list, compared to their overall representation on the array. The most notable were related to ribosomal proteins and genes associated with eye development. The data from this experiment will help identify new genes as potential biomarkers of effluent exposure with potential utility for in situ monitoring and provide new avenues of investigation regarding biological pathways affected by effluent exposure. *The contents of this abstract neither constitute nor necessarily reflect official US EPA policy.*