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Effects of Xenoestrogen and Androgen Mixtures on Ovarian Transcriptome of the Fathead Minnow

Authors:

Carlie A. LaLone¹, Gerald T. Ankley¹, Elizabeth J. Durhan¹, Natalia Garcia-Reyero², Kathleen M. Jensen¹, Michael D. Kahl¹, Elizabeth A. Makynen¹, Edward J. Perkins³, Daniel L. Villeneuve¹

¹US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Laboratory, Mid-Continent Ecology Division, Duluth, MN, USA.

²Jackson State University, Department of Chemistry, Jackson, MS, USA.

³US Army, Engineer Research And Development Center, Environmental Laboratory, Vicksburg, MS, USA.

Abstract:

Endocrine disrupting chemicals (EDCs), such as the estrogens ethinylestradiol (EE2) and bisphenol A (BPA), and androgens like 17 β -trenbolone (TRB) can occur as mixtures in aquatic environments. To date, however, most studies with EDCs in fish have focused on their effects as individual pollutants. In this study we employed a 15,000 gene oligonucleotide microarray to examine transcriptional responses in ovaries of mature (5-6 month old) female fathead minnows (*Pimephales promelas*) exposed to mixtures of TRB and EE2 or BPA. Water-borne exposures were conducted in a flow through system (with no carrier solvents) for 14 days. Concentrations of the test chemicals were analytically verified over the course of the exposure. For each mixture study there were six treatments: a control, TRB alone (500 ng/L), low and high concentrations of each xenoestrogen individually (1 and 10 ng/L of EE2 or 10 and 100 μ g/L of BPA), and each xenoestrogen concentration in combination with the TRB. Analysis of the BPA/TRB microarray data using one-way ANOVA ($p \leq 0.01$) with no multiple comparison correction identified 679 differentially expressed genes. Using a t-test ($p \leq 0.01$) to compare each treatment to controls identified approximately twice as many differentially expressed genes with the BPA alone treatments as with those from the BPA/TRB mixture or TRB alone. Principle component analysis of the 679 genes differentially expressed in the BPA/TRB experiment, indicated general similarity between the gene expression profiles associated with BPA 10 and 100 μ g/L treatments, and similarity between those associated with either concentration of BPA in combination with TRB. The gene expression profiles between TRB alone and 10 μ g BPA alone /L were the most divergent. Overall, gene expression profiles for both TRB-BPA mixtures appeared more similar to that of TRB than either BPA concentration alone. A similar analysis of data from the EE2/TRB study revealed some differences from the BPA/TRB study, including a greater number of differentially expressed genes (3608) overall, and a greater number of differentially expressed genes in fish exposed to a mixture of EE2/TRB as compared to

EE2 alone. Data from this study demonstrate the types of variability in transcriptional data that could be expected when attempting to interpret mixture effects, for example, in a field setting and provides insights into mechanistic similarities and differences in these chemicals' effects on the fish ovary.