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QUANTITATIVE BIOMARKERS OF ESTROGENIC EXPOSURE IN FISH: GENE EXPRESSION BIOASSAYS IN TWO MODEL SPECIES

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ABSTRACT: Endocrine disrupting compounds have been detected in waterways across the country. Specifically, concern exists regarding the impacts of compounds that exhibit estrogenic activity because of their potential feminizing effects on male organisms. Municipal wastewater treatment effluent, agricultural runoff, and industrial wastes all contribute to the load of estrogenic chemicals found in rivers and streams which often includes human and animal hormones and pharmaceuticals. Reliable, accurate biomarkers of estrogenic exposure are important tools in detecting the presence of these contaminants in water and rapid, sensitive, and inexpensive bioassays are needed to enhance the efficiency of detection. In this study, we demonstrate that quantifying changes in the expression of reproductively relevant genes in tissues of male fish is a robust indicator of estrogenic exposure. Male Japanese medaka (Oryzias latipes) were exposed to 1 femtomolar, 1 picomolar, or 1 nanomolar concentrations of the endogenous hormone 17β--Estradiol (E2) (or vehicle control) over a time course ending at 96 hours. Similarly, male fathead minnows (Pimephales promelas) were exposed to the same concentrations of E2 for 96 hours. High-quality mRNA was isolated from liver and gonad tissues and used in real-time RT-PCR to quantify changes in the expression of vitellogenin (an egg-yolk precursor) and a steroidogenic enzyme (11β-hydroxylase in medaka and 17α-hydroxylase in fathead minnow). Medaka and fathead minnow exposed to 1 nanomolar E2 showed levels of vitellogenin that were induced 1,000 fold and 10,000 fold (respectively) over controls. Induction was seen in fish exposed to lower concentrations of E2, but it was statistically non-significant. Significant vitellogenin induction occurred in medaka as early as 24 hours. Expression levels of the androgenic enzymes measured in each species were down-regulated at the highest exposure concentrations, but the response was not as robust as that seen in the induction of vitellogenin. These data demonstrate that vitellogenin induction is a robust biomarker of estrogenic exposure that can be assayed in many fish species in a rapid and reliable manner.

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