Male Fathead Minnow Urine-Based Metabolomics for Assessing Impacts of Chemical Stressors

D. Ekman[1], Q. Teng[1], K. Jensen[2], M. Kahl[2], D. Martinovic[2], D. Villeneuve[2], G. Ankley[2], and T. Collette[1]
[1]U.S. EPA, NERL, Athens, GA 30605
2U.S. EPA, NHEERL, Duluth, MN 55804

We have developed the potential for profiling metabolites in urine from male fathead minnows (*Pimephales promelas*) to assess chemical exposures, using nuclear magnetic resonance (NMR) spectroscopy. Both one dimensional (1D) and two dimensional (2D) NMR spectroscopy was used for the assignment of metabolites in urine from unexposed fish. Because fathead minnow urine is dilute, we lyophilized these samples prior to analysis. Furthermore, 1D ^{1}H-NMR spectra of unlyophilized urine from unexposed male fathead minnow and Sprague-Dawley rat have been acquired to qualitatively compare rat and fish metabolite profiles and to provide an estimate of the total urinary metabolite pool concentration difference. As a small proof-of-concept study, lyophilized urine samples from male fathead minnows exposed to three different concentrations of the antiandrogen vinclozolin were analyzed by 1D ^{1}H-NMR to assess exposure-induced changes. Through a combination of principal components analysis (PCA) and measurements of ^{1}H-NMR peak intensities, several metabolites were identified as changing with statistical significance in response to exposure. Among those changes occurring in response to exposure to the highest concentration (450 μg/L) of vinclozolin were large increases in taurine, lactate, acetate, and formate. These increases coincided with a marked decrease in hippurate, a combination potentially indicative of hepatotoxicity. Subsequently, we have used the same methodology to study the impacts of a chemical mixture of a model androgen, trenbolone (TRB) and a model anti-androgen, cyproterone acetate (CA). Results demonstrate that, as expected, the presence of TRB (500 ng/L) decreases the impact that CA has on the male fish when the exposure level of CA is high (200 μg/L). But, interesting, the presence of TRB (500 ng/L) increases the impact of CA when the exposure to CA is below the level that causes decreased plasma testosterone concentrations (20 μg/L). The results of these investigations clearly demonstrate the utility of an NMR-based approach for assessing chemical exposures in male fathead minnow, using urine collected from individual fish.