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Use of Chemical Mixtures to Differentiate Mechanisms of Endocrine Action in a Small Fish Model. Jensen, K.M.*, Kahl, M.D., Durhan, E.J., Makynen, E.A., Cavallin, J.E., Martinović, D., Wehmas, L., Villeneuve, D.L., and Ankley, G.T., Mid-Continent Ecology Division, U.S. Environmental Protection Agency, Duluth, MN, USA.

Various assays with adult fish have been developed to identify potential endocrine-disrupting chemicals (EDCs) which may cause toxicity via alterations in the hypothalamic-pituitary-gonadal (HPG) axis via different mechanisms/modes of action (MOA). These assays can be sensitive and highly diagnostic for key MOAs such as agonism of the estrogen and androgen receptors (ER, AR) and inhibition of steroid synthesis. However, most of the tests do not unambiguously identify AR antagonists. The purpose of this work was to explore the utility of a mixture test design with the fathead minnow (*Pimephales promelas*) for detecting different classes of EDCs including AR antagonists. The basis of the approach lies in evaluating the ability of test chemicals to block occurrence of an in vivo response mediated through the AR. Studies in our lab and elsewhere have shown that the synthetic androgen, 17β-trenbolone (TB), binds with high affinity to fish AR(s) and masculinizes female fathead minnows causing the development of craniofacial nuptial tubercles, external structures which can be visually detected and easily quantified. Adults of both sexes were exposed via the water to EDCs with diverse MOA in the absence or presence of TB. Exposure to several model AR antagonists (flutamide, vinclozolin, cyproterone acetate) in the presence of TB effectively decreased expression of nuptial tubercles in female fathead minnows. Mixture studies with TB and the model ER agonists, 17α-ethinylestradiol and bisphenol A, also showed inhibition of tubercle formation in the females, but unlike the AR antagonists, the estrogens markedly induced synthesis of

vitellogenin (VTG), particularly in males. The ER agonists also offset TB-induced depressions in plasma VTG concentrations in female fish. Additional mixture experiments were conducted with TB and triclocarban, an anti-microbial reported to enhance AR-mediated responses, or ammonia, a "negative control" with no known direct effects on HPG function. Neither chemical affected VTG status in males or females in the absence or presence of TB; however, both slightly enhanced TB-induced tubercle formation in females. Based on these results with the fathead minnow, a TB co-exposure assay appears to be an effective approach for clearly identifying AR antagonists as well as potential EDCs with other relevant MOA. *This abstract does not necessarily reflect EPA policy*.