Title:
A Time-Course Study with the Androgen Receptor Antagonist Flutamide in Fish

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Abstract:
Flutamide, a drug registered to treat some types of prostate cancer in humans, has been used for many years as a model androgen receptor (AR) antagonist in studies aimed at characterizing disruption of the vertebrate hypothalamic-pituitary-gonadal (HPG) axis. Various studies have shown that flutamide affects HPG-mediated reproductive and developmental processes in fish, but nothing is known about time-dependent, mechanistic changes in the HPG axis of exposed animals. This information is important to selection of diagnostic biomarkers of exposure/effects of AR antagonists in fish in lab and field studies. In these studies, male and female fathead minnow (*Pimephales promelas*) were exposed for 8 days in a flow-through system to 50 or 500 µg flutamide/L, followed by an 8 day depuration/recovery phase in clean water. Fish were sampled on days 1, 2, 4 and 8 during both the exposure and recovery portions of the test. Several endpoints indicative of reproductive function were measured in the fish, including ex vivo gonadal production of steroids (testosterone [T], 17β-estradiol [E2]), plasma concentrations of vitellogenin, T and E2, and expression (via QPCR) of several HPG axis-related genes. While significant effects on various endpoints were observed, responses were generally transient and varied with concentration. For example, 500 µg flutamide/L caused significant reductions in ex vivo T (males, females) and E2 production (females) at several (but not all) points during the exposure/recovery phases of the test. The lower concentration of flutamide either had no effect (females), or caused a significant increase in ex vivo T production over the latter half of the exposure period (males). Changes in male ex vivo steroid production were accompanied by significant alterations in transcription of several genes including those coding for 11β-hydroxysteroid dehydrogenase, androgen receptor, cytochrome P45011a, and follicle stimulating hormone receptor. No effects on plasma vitellogenin concentrations in males or females were detected. Compared to past experiments in our lab with other classes of HPG-active chemicals, mechanistic responses of fathead minnows to an AR antagonist were less clear in terms of direct impacts or compensation.