

**Abstract Title:**

A time-course analysis of effects of the steroidogenesis inhibitor ketoconazole on components of the hypothalamic-pituitary-gonadal axis of fathead minnows

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**Abstract:**

The objective of this study was to evaluate temporal effects of the model steroidogenesis inhibitor ketoconazole (KTC) on aspects of reproductive endocrine function controlled by the hypothalamic-pituitary-gonadal (HPG) axis in the fathead minnow (*Pimephales promelas*). Ketoconazole inhibits the activity of two cytochrome P450s (CYPs) key to sex steroid production in vertebrates, CYP11a and CYP17. In these studies, sexually-mature fish were exposed to KTC (30 or 300 µg/L) for up to 8 d, following which animals were allowed to recover in clean water. Samples were collected after 1, 4 and 8 d of exposure, and after 1, 8 and 16 d of recovery. A shorter-term time-course experiment was conducted in which females were sampled on seven occasions during a 12-h KTC exposure. Ketoconazole depressed *ex vivo* gonadal synthesis of testosterone (T) in both sexes, and 17β-estradiol (E2) in females during exposure and recovery phases of the time-course studies. Effects on *ex vivo* steroidogenesis in females occurred within as little as 1 h of exposure. Plasma concentrations of T in males and E2 in females also were depressed early in the KTC exposure, but those decreases did not persist to the same degree as observed for the *ex vivo* effects. In females, after decreases within 12 h, plasma E2 concentrations were similar to (or greater than) controls at 24 h of exposure, while in males, plasma T returned to levels comparable to controls within 1 d of cessation of KTC exposure. The discrepancy between the *ex vivo* and *in vivo* data at later stages in the test is consistent with some type of compensatory response to KTC in fish. However, we were unable to ascertain the mechanistic basis for such a response. Although a number of genes related to steroid synthesis were up-regulated in male and female gonads during the exposure and early recovery phases of the experiment, this did not seem to account for the resurgence in plasma steroid concentrations in KTC-exposed fish. Further studies focused on metabolism and clearance of steroids might lend insights as to the effects of KTC on plasma steroid concentrations. Overall, our results demonstrate the complex, temporally-dynamic nature of the vertebrate HPG system in response to chemical stressors.

**Keywords:** Steroid Synthesis, Fungicide, Toxicity, Fish

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