

**DEVELOPMENT OF A REAL-TIME QUANTITATIVE PCR (TaqMan)  
ASSAY TO ASSESS THE EFFECTS OF ENDOCRINE DISRUPTING  
CHEMICALS ON SHEEPSHEAD MINNOW (*Cyprinodon variegatus*)  
GROWTH**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

**Introduction**

The threat of widespread distribution and persistence of endocrine-disrupting contaminants (EDCs) in the environment and the potential for serious effects in human, fish and wildlife populations has warranted a Federal research strategy (National Science and Technology Council report, 1996). Considerable research effort has been given to pollutants that may mimic estrogen ( Colborn *et al.*, 1996) little attention has been directed toward other equally important endocrine systems which may also be affected by anthropogenic chemicals.

Increasing evidence also implicates gonadal steroids in somatic growth regulation. Chronic exposures to estrogen have been shown to inhibit somatic growth (Borski *et al.*, 1996; Murphy and Friesen, 1988 ), while chronic exposure to dihydrotestosterone enhanced growth (Borski *et al.*, 1996) in ovariectomized,

hypophysectomized female rats. Growth regulation by estradiol in rats appears to involve inhibition of growth hormone (GH)-dependent hepatic insulin-like growth factor (IGF)-I gene expression. Estrogens have also been shown to impair growth in several fish species (Bulkley, 1972 ; Donaldson *et al.*, 1979) , however possible mechanisms for growth inhibition were not examined.

Some pesticides have also been shown to inhibit growth in fish. For example, chlorpyrifos (Dursban), a commonly used pesticide for mosquito control and garden use, reduced growth in the sheepshead minnow (*Cyprinodon variegatus*) after 28 days of exposure to concentrations as low as < 3.0 ug/L (Cripe *et al.*, 1986 ). Chlorpyrifos (an organo-phosphate pesticide) inhibits acetylcholinesterase; however the mechanism for growth inhibition in fish has not been established. Chlorpyrifos has been shown to affect hypothalamic GnRH gene expression, cell survival, and neurite outgrowth in vitro. In in vivo experiments, t chlorpyrifoscaused significant alterations in GnRH mRNA levels in female. These findings suggest that chlorpyrifos may act as an EDC (Gore, 2001).

Alterations in somatotropic activity in response to endogenous and xenobiotic chemicals can be evaluated by measuring insulin-like growth factor (IGF) synthesis in response to chemical exposure. Insulin-like growth factors are anabolic peptide hormones, which play a crucial role in growth and development. Hepatic IGF-I synthesis is controlled by growth hormone (GH) and may also be influenced by other hormones (T<sub>3</sub>, estradiol). We have developed an assay for to measure IGF-I mRNA in Sheepshead minnow livers by quantitative real-time PCR (TaqMan). TaqMan analysis is a highly sensitive method to measure specific sequences from a small amount of total RNA using a fluorescent probe and specific primer pairs. Measuring induction of IGF-I will allow us to determine whether these measurements have predictive value for growth enhancement or inhibition due to chemical exposure.

We conducted an 18-week aquatic exposure of newly hatched sheepshead minnows (*Cyprinodon variegatus*) to E<sub>2</sub> and chlorpyrifos. The fish were exposed to two measured concentrations of E<sub>2</sub> (21 and 112.5 ng/L) and to three measured concentrations (6.5, 13.3 and 24.3 ug/L) of chlorpyrifos. Fish exposed to the highest dose of E<sub>2</sub> grew larger than controls only during the last week of the experiment. Fish exposed to the lower dose of E<sub>2</sub> were not significantly different from controls. The fish exposed to all doses of chlorpyrifos had significantly reduced growth in a dose-dependent manner compared to controls. Hepatic IGF-I mRNA levels were measured using TaqMan technology, but no

significant differences were found in hepatic IGF-I mRNA levels in any treatments. These results were in contrast to other research done in our lab in which fish were injected with 5 ug/g body weight E<sub>2</sub> had significantly reduced hepatic IGF-I mRNA up to 12 h after injection (data not shown).

In conclusion, aquatic exposure to environmentally relevant concentrations of E<sub>2</sub> did not affect hepatic IGF-I mRNA levels in the SHM. Aquatic exposure to chlorpyrifos inhibited growth in a dose-dependent manner, but the mechanism of action is still unclear. Hepatic IGF-I mRNA levels in chlorpyrifos treated fish were no different than control fish. The TaqMan method is a useful tool to screen for physiological effects of both anthropogenic chemicals and endogenous hormones on somatic growth.

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