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Gene Expression Consistent During Zebrafish Embryonic Development for *rnf14* and *ttc3* with Expression Altered with Atrazine Exposure at 60 hpf for *ttc3*

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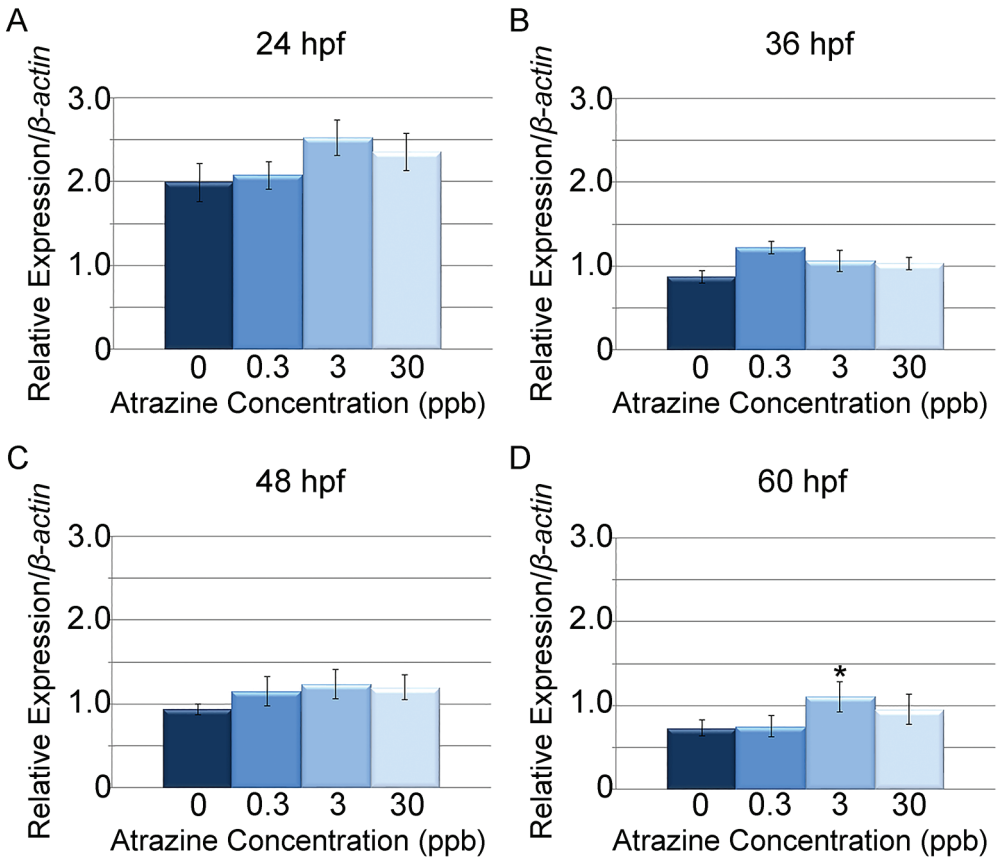
Atrazine is an herbicide commonly applied to crops in the Midwest part of the United States. The chemical moves into drinking water sources after rainfall events, which increases the risk of human exposure. The United States Environmental Protection Agency set the maximum contaminant level at 3 parts per billion (ppb) in drinking water, but even these levels are suspected to cause adverse health effects. Developmental exposure to atrazine is reported to increase birth defects and result in endocrine disruption.

Previous studies in our laboratory have shown that genes related to the neuroendocrine and reproductive system, cell cycle, and carcinogenesis have altered expression in zebrafish after an embryonic exposure to atrazine (1–72 hours post-fertilization (hpf)). Two of these genes, ring finger protein 14 (*rnf14*) and tetratricopeptide repeat domain 3 (*ttc3*), were further tested in this study. The gene *rnf14* interacts with the androgen receptor. When expression of *rnf14* is increased, it is expected to cause abnormal cell growth and lead to carcinogenesis. *ttc3* is expected to be involved with neuronal proliferation and differentiation when expression is increased. Overexpression of *ttc3* leads to strong inhibition of neurite extension.

The purpose of this experiment was first to determine how gene expression changed over the zebrafish embryonic developmental time course at 24, 36, 48, 60, and 72 hpf. Then, gene expression was assessed following atrazine exposure at 0, 0.3, 3, or 30 ppb at multiple developmental time points. Expression of *rnf14* was consistent throughout the developmental time course and was not impacted by atrazine exposure ($p > 0.05$). In addition, expression of *ttc3* was consistent throughout the developmental time course ($p > 0.05$), but was significantly increased at 60 hpf with atrazine exposure ($p = 0.0099$; $n = 6$), which was similar to the previous findings at 72 hpf. The data shows that the *rnf14* and *ttc3* expression is steady throughout embryogenesis and that changes caused by atrazine exposure only occur at the time points of 60 and 72 hpf for *ttc3* and only at 72 hpf for *rnf14*.

Research advisor Jennifer Freeman writes: "Leeah has completed her research project as part of the School of Health Sciences Undergraduate Research Honors Program. Her work is furthering our understanding on the dynamic molecular changes occurring during an embryonic exposure to the most common pesticide contaminant in U.S. potable water supplies, the agricultural herbicide atrazine."

Reidenbach, L. S. (2018). Gene expression consistent during zebrafish embryonic development for *rnf14* and *ttc3* with expression altered with atrazine exposure at 60 hpf for *ttc3*. *Journal of Purdue Undergraduate Research, 8,* 70–71. https://doi.org/10.5703/1288284316749



The time course of *ttc3* relative expression after developmental atrazine exposure. No significant differences in relative expression were observed between treatment groups at 24 hpf (A), 36 hpf (B), or at 48 hpf (C). At 60 hpf, the 3 ppb treatment had increased relative expression as compared to the controls (D). n = 6, qPCR run in triplicate, error bars represent standard deviation, * = $p < 0.05$. (hpf: hours post-fertilization)

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