HEALTH & HUMAN SCIENCES

Investigating the Role of the Hedgehog Pathway in Connection to Atrazine Endocrine Disruption

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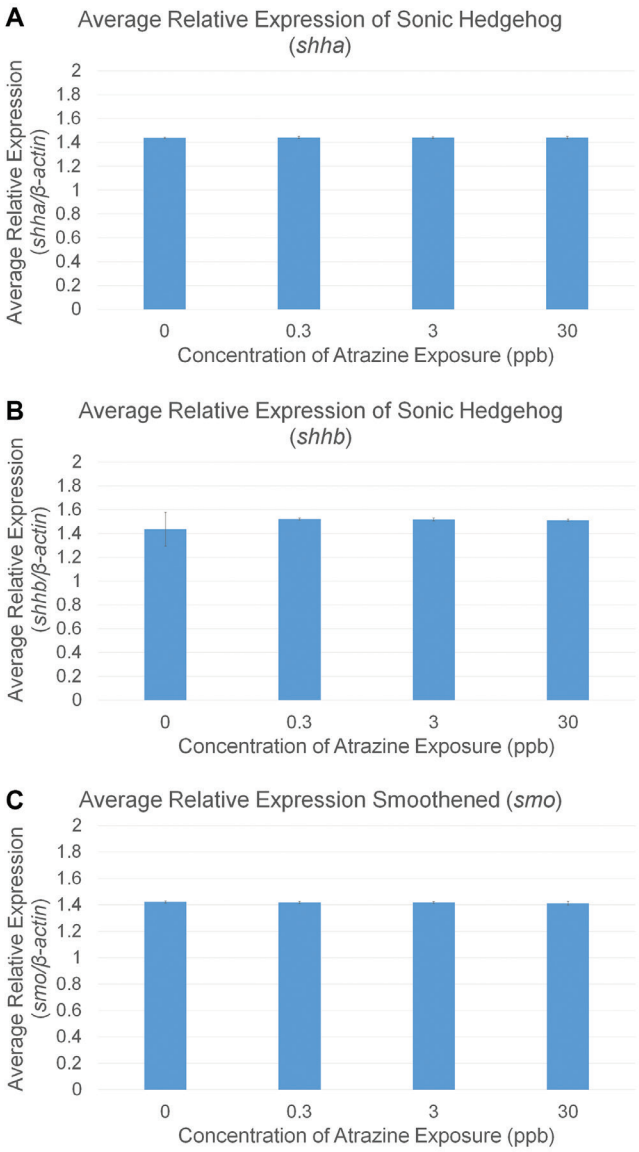
Atrazine is an agricultural herbicide commonly used in the midwestern United States where exposure commonly occurs from contaminated drinking water supplies. Additionally, atrazine has been classified as an endocrine-disrupting chemical (EDC), which can alter biological pathways by disrupting hormones such as aromatase, resulting in adverse health effects. Past studies have shown that atrazine targets the neuroendocrine system. There is current speculation about atrazine supporting the stimulation of the estrogenic pathway, including alterations with aromatase and progesterone in multiple animal models. One of the enzymes in the hedgehog pathway also utilizes aromatase, which is indicated to be activated by atrazine exposure. This hedgehog pathway (Hh) is an important signaling pathway for tissue repair and stem cell maintenance. Overexpression of the signaling mediators have been associated with neuroendocrine tumors and cancers. The goal of this study was to determine the influence of exposure to the agricultural herbicide atrazine on connections between aromatase, three genes of the hedgehog pathway (sonic hedgehog [*shha*, *shhb*] and smoothened [*smo*]), and neuroendocrine disruption. Zebrafish were used as a biomedical model in this study and were exposed to 0, 0.3, 3, or 30 ppb (µg/L) atrazine from 1 to 72 hours post-fertilization (hpf) (the embryonic period). At 72 hpf, zebrafish were collected, RNA isolated, cDNA synthesized, and quantitative PCR completed to determine if atrazine exposure altered gene expression. The results showed no alterations in the tested genes of the hedgehog pathway following the embryonic atrazine exposure ($p > 0.05$). Further investigation of additional genes in this pathway is needed.

Research advisor Jennifer Freeman writes: "Anusha's research project is contributing to our overall understanding of the pathways that may be altered following an early developmental exposure to the agricultural herbicide atrazine. These findings are important as atrazine is the most common agricultural contaminant in drinking water in the United States."



Quantitative polymerase chain reaction (qPCR) analysis to determine gene expression alterations following embryonic atrazine exposure. Following the embryonic atrazine exposure from 1 to 72 hours post fertilization (hpf), RNA was isolated, cDNA was synthesized, and qPCR analysis was completed following the Freeman laboratory protocol and MIQE guidelines. The main targets that were studied were the sonic hedgehog genes, (A) shha and (B) shhb, along with (C) smoothened (smo). β-actin was included as the reference gene to determine relative expression and was confirmed to have consistent expression under these atrazine treatment conditions. Data was analyzed with an ANOVA ($\alpha = 0.05$; N = 6 replicates of 45 pooled larvae). The results showed no alterations in the tested genes of the hedgehog pathway at 72 hpf following the embryonic atrazine exposure ($p > 0.05$). Error bars represent standard deviation.

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