Developing an Analytical Method for Measuring Atrazine in Zebrafish Embryos
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Abstract
Atrazine is a common herbicide used in agriculture to kill broadleaf weeds. This synthetic chemical is the second most commonly used herbicide in the United States with volumes of 76 million pounds per year being used. Currently, there is not a routine protocol in place to detect low doses of atrazine in tissue of zebrafish embryos. Due to the extensive use of this toxicant in the environment, there is a need for a diagnostic protocol for detecting low doses of atrazine in tissue. This study highlights the attempted uses of LC-MS and GC-MS to detect atrazine in the embryonic tissue of zebrafish. The LC-MS protocol briefly involves the following: 1) homogenize the tissue in 0.01% acetic acid, 2) vortex and put the samples on ice, 3) centrifuge and separate the supernatant, 4) dry down the supernatant and reconstitute with 0.05% acetic acid in ethanol, and 5) run samples using LC/MS. The unique peak for atrazine using LC-MS has a molecular weight 215.8. After testing this protocol on zebrafish embryos exposed to atrazine concentrations of 3 ppb, 1000 ppb, or 10000 ppb, it was found that 1000 ppb was the lowest concentration that was detectable using this protocol. Further experiments are now focused on GC-MS testing. Standards of 1000 ppb, 500 ppb, 300 ppb, 100 ppb, 50 ppb, 30 ppb, and 3 ppb atrazine in ethanol have been tested utilizing GC-MS. It has been concluded that atrazine is detectable at all concentrations but only measurable down to 30 ppb.

Introduction
- Atrazine is “One of the most widely used agricultural pesticides in the U.S.” according to the EPA.
- Atrazine is an agricultural pesticide that is used to control weeds by inhibiting photosynthesis in broad-leaf plants.
- Atrazine is applied to fields both before and after planting.
- As shown below in figure 1, atrazine is highly utilized in the Midwest.
- The FDA has set the MCP of atrazine in drinking water at 3 ppb.
- Atrazine is indicated to cause numerous negative health effects including endocrine disruption, reproductive issues, and cancer.

Discussion
- Each sample at each concentration consisted of the tissue from roughly 40 zebrafish embryos.
- Figure 2 shows a peak at 215.8 (atrazine’s molecular weight) indicating the presence of atrazine.
- Figure 3 shows no such peak which was expected for exposure at 0 ppb atrazine.
- Figure 4 shows a strong peak at 215.9, indicating that atrazine can be detected in tissue exposed to 1000 ppb atrazine.
- Figure 5 shows a fairly strong peak at 217.2 suspected to be atrazine. This indicates that atrazine can be detected in the embryonic tissue exposed to 1000 ppb atrazine.
- Figure 6 shows no peak at 215.8, indicating that atrazine cannot be detected in the embryonic tissue exposed to 3 ppb atrazine.
- The concentration of 3 ppb is necessary to label this protocol and method of detection a success because a concentration of 3 ppb atrazine is the current Maximum Contaminant Level (MCL) allowed in drinking water in the U.S.
- Each standard consisted only of atrazine in ethanol, and embryonic tissue has not yet been employed in this procedure.
- Figure 7-12 show the results for 6 of the 7 concentrations. All of these figures show a peak of 215.8 showing that atrazine is detected. The graphs also show quantifiable data down to 30 ppb in ethanol.
- Figure 13 shows the peak at 215.8 but not any quantifiable data. This result indicates that GC-MS cannot be used to measure atrazine in ethanol at 3 ppb.
- If 3 ppb cannot be measured in ethanol, it will not be measured in tissue.

Conclusions
The final conclusion of this phase of this project is that the LC-MS and GC-MS methods of detection is not adequate of detecting levels of atrazine that are relevant to the concentrations of the pesticide that would be concentrations humans are exposed to through water.

Future Directions
After reviewing the results from the LC-MS protocol and GC-MS protocol, it was concluded that these methods could not be used to detect atrazine at levels that make the research relevant to human exposure. The next step of this project is to troubleshoot the GC-MS method. We are currently rerunning our standards to verify our results.

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