

Expression of three synuclein genes following an embryonic atrazine exposure in the zebrafish. A decrease in expression in the 0.3 and 30 ppb treatment groups was observed for *sncga* (A) and *sncgb* (B). No changes in expression were observed for *sncb* (C). $N = 5$ (pools of 40 zebrafish each). Error bars represent standard deviation. * $p < 0.05$.

Zebrafish models have been used to study neurodegenerative diseases including Parkinson's disease (PD) due to having the same major brain structures and blood-brain barrier as humans. Alpha synuclein (aSyn) is a key pathogenetic target in PD and zebrafish have three synucleins (*sncga*, *sncgb*, *sncb*), of which *sncgb* is most similar to human aSyn. The zebrafish was used to test the hypothesis that an embryonic atrazine exposure alters expression of synuclein genes. AB adult zebrafish were bred and their embryos were collected in groups of 50 in petri dishes. Embryos were exposed to 0, 0.3, 3, or 30 parts per billion (ppb, $\mu\text{g/L}$) atrazine from 1 hour post fertilization (hpf) to the end of embryogenesis (72 hpf). Larvae were collected from each dish, RNA isolated, and

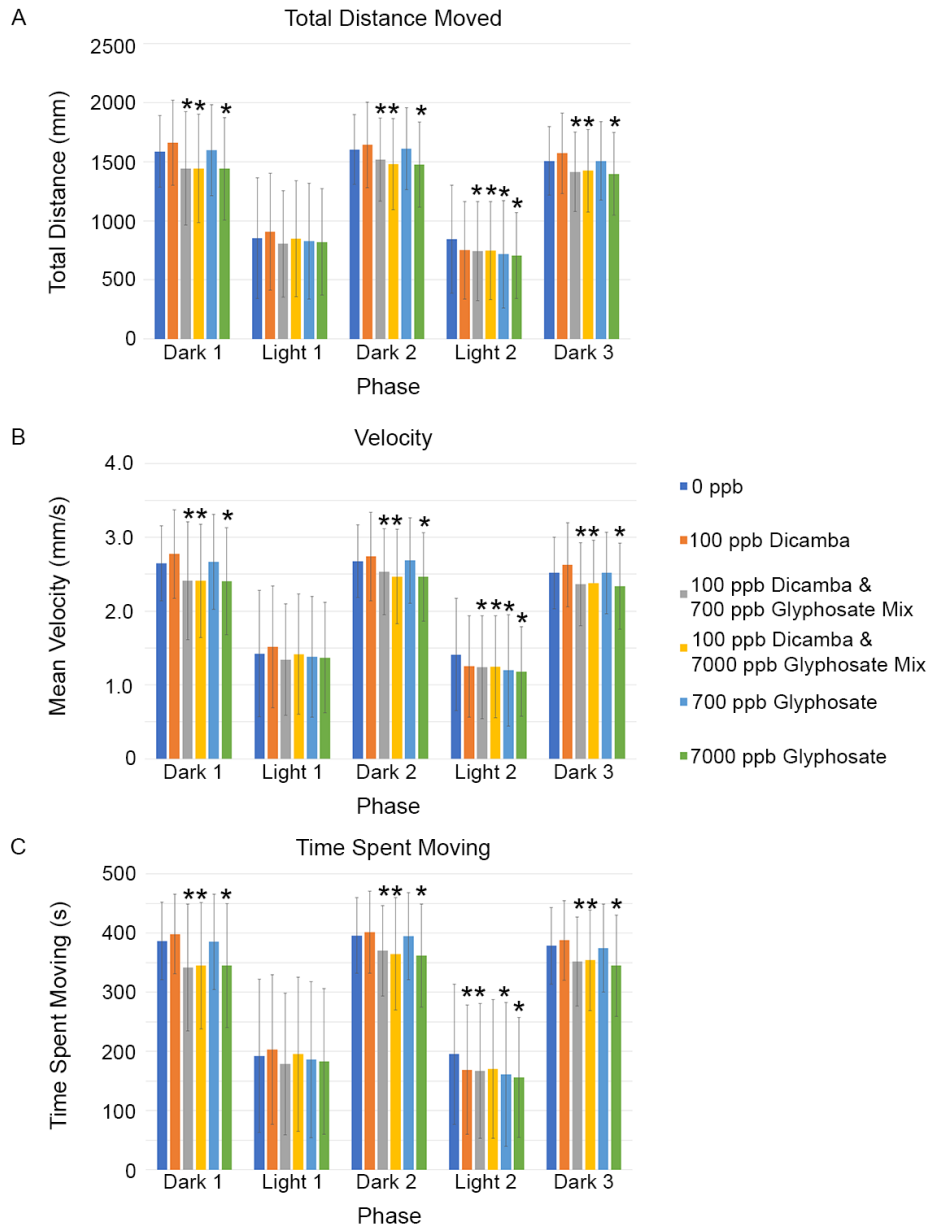
cDNA synthesized for qPCR analysis. qPCR compared the transcript level of each target gene among the atrazine treatment groups using β -actin as a reference gene, which was not changed with atrazine exposure. Statistical analysis was then conducted to determine significant changes using $\alpha = 0.05$. A decrease at 0.3 ppb and 30 ppb was detected for *sncga* and *sncgb*. These findings support our hypothesis that an embryonic atrazine exposure alters expression of the synuclein genes. Additional studies are needed to determine the impact of these gene changes on PD pathogenic phenotypes.

Research advisor Jennifer Freeman writes: "Isabelle's research project is evaluating if an embryonic atrazine exposure may alter genes in the synuclein family. Atrazine is a herbicide reported to alter some pathways related to Parkinson's disease but overall information is limited. Isabelle's findings support the need for further research in this direction."

Active Herbicide Ingredients in Roundup Ready Xtend Products, Glyphosate and Dicamba, Results in Hypoactivity in Zebrafish Larvae When Exposed During Development

Student researcher: Ryker Bond, Senior

Glyphosate (GLY) is the most used agricultural herbicide in the United States and the active ingredient in Roundup Ready products. GLY residues are detected in food products and the herbicide can move in the environment after field application, contaminating drinking water sources. Thus, there are multiple exposure routes in the general population in addition to occupational exposure risks to agricultural workers. Due to overapplication of GLY leading to weed resistance, a second generation of Roundup Ready products were developed that include the herbicide dicamba (Roundup Ready Xtend). Currently little is known on toxicity risks or if increased application will result in drinking water source contamination with dicamba. This study evaluated developmental neurotoxicity of GLY, dicamba, and binary mixtures with a visual motor response behavioral assay using the zebrafish model. Zebrafish were exposed from 1 to 120 hours post fertilization (hpf) encompassing embryogenesis and the early larval development period. GLY concentrations



*Behavioral alterations observed in zebrafish larvae following a developmental exposure (1–120 hours post fertilization) to glyphosate, dicamba, or a glyphosate/dicamba mixture as measured by total distance moved (A), mean velocity (B), and time spent moving (C) in the visual motor response assay. N = 8 with 16 subsamples per biological replicate to total 128 total fish per treatment group. Error bars are standard deviation. *p < 0.05.*

centered around the U.S. regulatory limit in drinking water (700 ppb; µg/L), with single treatment concentrations ranging from 0.7 to 7,000 ppb. With no current U.S. regulatory level for dicamba, treatment concentrations ranged from 0.01 to 1,000 ppb. Binary treatment concentrations were chosen based on results of the single chemical exposures. Hypoactivity was observed for both herbicides in total distance moved, velocity,

and time spent moving in groups exposed to 7,000 ppb GLY, at concentrations as low as 1 ppb dicamba, and in the mixtures of 700 ppb GLY/100 ppb dicamba and 7,000 ppb GLY/100 ppb dicamba. Overall, this is the first study to evaluate behavioral alterations for dicamba and glyphosate/dicamba mixtures. Further assessments into developmental neurotoxicity risks are needed.

Research advisor Jennifer Freeman writes: “Ryker’s research is investigating whether a developmental exposure to herbicides used in the midwestern United States may alter behavioral patterns. His research is important because his findings provide new toxicity information for the new herbicide mixture products containing glyphosate and dicamba.”

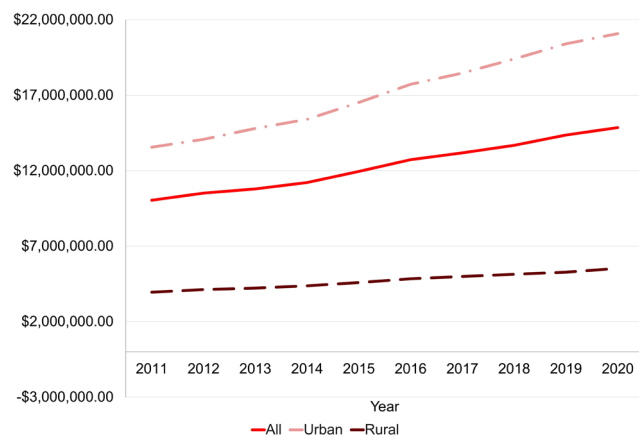
Analyzing Health Care Delivery Costs from 2011 to 2020 in the Emergency Departments and Overall Hospital Costs

Student researcher: Jacob A. Corey, Senior

The goal of this research was to analyze how COVID-19 affected the cost of health care within hospitals, specifically within the emergency department (ED) and overall hospital expenditures. It was hypothesized that both urban and rural hospitals would see a sharp increase in the costs for 2020, especially in emergency departments, due to the pandemic.

Hospital cost data for the years 2011–2020 from form CMS-2252-10 shared by the Centers for Medicare and Medicaid Services was utilized for this project. Inclusion criteria were based on whether hospitals had submitted cost results for EDs and total hospital cost results in at least 8 years of the 10-year time span. The total number of hospitals included in this study was $n = 3,141$, with 958 of these hospitals being rural and 2,183 being urban.

The average overall hospital cost increase from 2011 to 2019 was 4.48% per year, but it was 6.18% from 2019



Average emergency department costs from 2011 to 2020 for all, rural, and urban hospitals.

to 2020. The average urban total hospital cost increase from 2011 to 2019 was 4.76% per year, but it was 6.70% from 2019 to 2020. The average rural total hospital cost increase from 2011 to 2019 was 3.84% per year, but it was 6.12% from 2019 to 2020.

The average overall ED cost increase from 2011 to 2019 was 4.46% per year, but it was 3.43% from 2019 to 2020. The average urban ED cost increase from 2011 to 2019 was 5.03% per year, but it was 3.32% from 2019 to 2020. The average rural ED cost increase from 2011 to 2019 was 3.81% per year, but it was 4.72% from 2019 to 2020.

A major limitation of this study involves the inclusion criteria requiring 8 years of reports out of the 10 years of data. This caused around half of the hospitals in the CMS-2252-10 form to be excluded from this analysis.

Research advisor Cody Mullen writes: “When the COVID-19 pandemic started in 2020 our health care system quickly and effectively responded to the needs of the communities they serve. It is important to understand how hospital operations changed. This study starts to document the changes in hospital operations during the pandemic for both urban and rural facilities.”

Formulation of Preservation Solutions for Model Generation with In Vivo Tissue Morphology

Student researcher: Holly Pickett, Junior

Historically, standard tissue fixation methods present an unrealistic representation of in vivo tissue morphology. These resultant alterations, such as tissue hardening, not only hamper development of key connections between form and function in the anatomy classroom, but also the critical diagnostic skills necessary to attain clinical gains. The aim of this project is to develop a new tissue preservation method to bridge such gaps. This involves assessing the effects of a modified-release fixation component in solution containing physiological electrolytes. Porcine lungs were dissected into lobes and placed in two solutions with different concentrations of the fixation component. The lungs were observed using tactile manipulation for in vivo tissue resilience over the course of four months, also being attentive to bacterial growth that could lead to decay. Investigative results