Examining the Negative Effects of Camp Fire Toxins on The Immune System

The Camp Fire burned thousands of acres of land in Paradise, CA in November 2018. Classified as the most destructive fire in California's history, a vast majority of the town's infrastructure was burned, completely devastating the community and contaminating the area. Drs. Jackson Webster (in Engineering) and Sandrine Matiasek (in Geology) conducted a study of the surrounding waterways of Paradise and identified a plethora of harmful compounds. The concentrations of many of these chemicals are considerably higher than acceptable human levels. Other chemicals detected have not been well defined for their effects on humans yet. Aluminum was identified in this analysis.

I have been investigating the detrimental effects of aluminum nitrate on embryonic development in zebrafish (*Danio rerio*), and have observed significant differences in embryos that have been exposed to this toxin. Zebrafish are a popular model system in toxicology research as they develop externally, and embryos are transparent allowing for observation of blood development in real time. Visualization of certain blood cell types is made possible with transgenic zebrafish, which are genetically engineered to have fluorescent blood when looked at under a microscope. In addition, zebrafish lay hundreds of eggs every time they spawn, allowing us to study numerous embryos at once. Zebrafish share many genes and developmental pathways with humans, having similar circulatory and immune systems. These similarities allow us to not only observe the potential deleterious effects of these chemicals on the development of fish, but also extrapolate these effects to humans. Because thousands of people were affected by the Camp Fire and many were exposed to chemical contaminants, it is vital we research this topic. Defining safe levels and the long-term effects of exposure for the immune system is imperative to understanding and mitigating the negative outcomes of these chemicals. Importantly, as climate change threatens to increase the numbers and severity of these fires, this information will be useful to others around the world.

I have already begun conducting experiments involving exposing zebrafish embryos to aluminum nitrate. Although aluminum particles were found in the waterways surrounding the areas affected by the Camp Fire, I am using aluminum nitrate nonahydrate to conduct these experiments because this compound is soluble in water and can easily be added to the embryos in their water. My current work on aluminum nitrate in the Stachura laboratory suggests that aluminum nitrate has negative impacts on the development of zebrafish embryos. I am subjecting embryos at various stages in development to increasing concentrations of aluminum nitrate to investigate the extent of developmental deficits and abnormalities, and if they have any correlation to when the embryos were exposed to the chemical. In exposing zebrafish embryos to aluminum nitrate, I have observed a significant decrease in survivorship of these embryos, as well as abnormal behaviors in embryos that were exposed to aluminum nitrate. These behavioral abnormalities include rapid, burst-like swimming movement in a circular pattern followed by periods of inactivity as well as swimming along the walls of the petri dishes. These behaviors have previously been closely linked with seizures. Based on these initial trials, I will more thoroughly study animals at these dilutions.

I will conduct several trials a week, in which I will expose embryos to varying concentrations of aluminum nitrate and record survivorship at different stages of development. I will measure the length of the animals, potential curvature of the spine, size of the eyes and optic vesicles, edema, and any gross malformations. All animals will be imaged and pictures will be saved for future reference. Additionally, I will observe the development of blood cells with fluorescent transgenic animals. Our zebrafish have blood cells that fluoresce when observed under a microscope, which allows us to observe red blood cells, neutrophils, T cells, and macrophages, all of which are vital to survival. The fluorescent markers in the cells allow us to quantitate how many are present in each individual embryo. These data will be examined to see if numbers are statistically different between the control and chemical-treated fish. Additionally, I will monitor the behavior of the control and chemical-treated fish because of the abnormalities observed in the early trials of this experiment. I will record the duration of swimming, length of swimming, and the number of individual swimming events that occur. These metrics are common tools for quantitating issues with fish locomotion and neurological defects.

This research will help enhance my analytical and problem-solving skills. The responsibility of developing an experimental approach and collecting and organizing data in a timely manner will help me become more confident in my individual decision-making. I will also benefit from this research by gaining experience in public speaking by presenting the research in poster sessions and local meetings. With the help of Dr. Stachura, I will publish these studies in a peer-reviewed journal describing my findings. Completing this research as an undergraduate will enhance my application for medical school.

Dr. Stachura is always present to help me tackle obstacles that arise. We work together in the laboratory, and have weekly meetings of our progress and findings. Dr. Stachura ensures that I understand the conceptual basis of laboratory techniques, and has helped me learn skills that will be useful in my academic career ahead. Funding provided by the COAST award that I received will help purchase the necessary supplies to continue my research which includes zebrafish husbandry ($250), buffers, chemicals, and reagents for experiments ($250), microscopy supplies ($100), tissue culture supplies ($500), and enzymes ($250). We also would like to purchase a video software package to visualize and quantitate the fish's defective locomotion ($850), which would be funded by the SARC proposal. I am excited to see how these experiments will help inform the dangers of wildfires on human health.