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Do PFAS Precursors Induce Lipid Accumulation in the Hepatocytes?

Poly and Perfluoroalkyl substances are manmade chemicals commonly found in fire extinguishers and in Teflon^{TM} cookware such as nonstick pans. The main objective of my project was to test whether the newer PFAS precursors replacements of the original toxic PFAS compounds, are toxic or even more toxic than the original PFAS compounds. We hypothesized that the newer emerging PFAS precursors, have a capacity to induce lipid accumulation in our hepatocytes, than legacy PFAAs. In order to test the novel endpoint with emerging PFASs that are replacing the PFAAs subgroup, three different types of procedures were used in this experiment- hepatocyte treatments, nile red staining, and gene expression measurements.

The first run of the nile red experiment showed that the sodium palmate oleate (P/O) fold change values were not high enough to be significant. P/O is the positive control, which increases lipid accumulation in the hepatocytes allowing us to compare the PFAS precursors' values to the P/O. Doing so, informs us if the precursors cause lipid accumulation in our hepatocytes or not. This is why the values were supposed to be higher than what was calculated. Therefore, this experiment was counted as the preliminary data and the experiment was done again using the same plate design and procedures since we need the P/O to have high values showing that there was a significant amount of lipid accumulation in the hepatocytes. After the second run and the spectrometer data were read and graphed, it showed that the numbers were still too low and there was not much significance. However, once we looked closely as to why this was happening, it was realized that while vacuuming, the bottom of the wells were hit too many times damaging the cells. Therefore, their spectrometer data and their fold change numbers were inaccurate and images had to be taken to count DAPI's nuclei and find the mean gray values of the nile red images on Image J application to quantify the fluorescence intensity in order to get the accurate numbers for their fold change. Nevertheless, to save time, only the 25 μM concentrations were imaged, not including C14. However, the P/O fold changes were not as high as 1.5 or more to be significant, so the results were not as anticipated, but it did show that FOSA and MetFOSA precursors had significant fold changes. In addition, the data were actually very similar to the previous data that we obtained, which brings us to the conclusion that there might have been a problem with the application of the treatment, but not the treatment itself.

Therefore, our next step will be to redo this experiment with the treatment applied differently, and then gene expression will be conducted on plate 2 and analyzed.

As an undergraduate pharmaceutical sciences student, this project has been invaluable to me. I have learned and experienced how much energy and commitment it takes to create a lab project, supervise it, and present it. I have worked in previous lung cancer and neuroscience (Alzheimer's disease) related research, but never in a toxicology lab before, but I have always been interested in this field. I was able to apply my knowledge from these labs, into my project as well as learning more lab techniques and gaining confidence in my field of work such as being able to construct, conduct and lead a lab project while holding weekly lab meetings. I received certifications for mouse handling and care, as well as procedure planning. Over the course of this study, I dedicated a large amount of time analyzing data and learning how to use Image J and GraphPad Prism even more than before, which is a widely used program in this field. This experience has given me insight on the daily life of a PhD student and it has helped me to become a more competitive candidate for career and graduate opportunities. I hope that I will be able to apply my experience and knowledge that I have acquired from this project for my future endeavors in clinical research and graduate school programs in a PhD, MD, or MD/PhD.