

US EPA ARCHIVE DOCUMENT

GRO Summer Internship Final Report
Integrated Toxicological Approaches for Assessing the Ecological Risks of
Pharmaceuticals in the Environment
(Twelve Weeks Spent Learning in Lakeside)
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It was late afternoon on Monday, May 23, when my Mom and I drove over what we thought was Lake Superior. It had been so foggy on our drive through Superior, WI, that we couldn't tell if we were on a bridge hovering over water or not. Perhaps we were deep into a city of skyscrapers, perhaps a rain forest; there would be no way to tell with a ten-foot-field-of-vision fog surrounding us. And then, I saw it. What looked like the very tip top of a sailboat. After a grueling week of finals, sorrowful goodbyes at graduation, and a 13-hour drive to follow, I had finally arrived. Lakeside, as they call it, in Duluth, MN. where I would be spending the next 12 weeks interning at the Mid-Continent Ecology Division (MCED) working on, well, at that point I wasn't completely sure.

There was no time to waste settling in, because the very next day I started at the EPA facility. However, I was thoroughly relieved to meet the friendly face of Judy, the receptionist, who showed me the way to my mentor's office. Again, I was met by another friendly, welcoming face. My mentor, a post-doc from the University of Minnesota-Duluth, Carlie LaLone, immediately began showing me around and introducing me to everyone. I met my Project Advisor Dan Villeneuve, and several of the contracted students I would be working with this summer, among many others. After we had circled the building a few times meeting people, and getting my cool, retractable badge, we finally returned to Carlie's office to talk a little business.

For the next several weeks I would be working on a project titled, "Integrated Toxicological Approaches for Assessing the Ecological Risks of Pharmaceuticals in the Environment." Which, I'll admit, sounded like a mouthful at first, but after going over some details, the title wasn't so intimidating. Carlie explained that our experiment was just one part of a bigger pharmaceutical testing project at the EPA here in Duluth. According to recent studies, pharmaceutical residues have been detected in many environmental matrices worldwide, i.e., in waters, wastewaters, sediments, and sludge.^{1,2} More specifically, pharmaceuticals contaminating natural water systems are predominantly human and veterinary drugs. The most abundant sources of such contamination are households, wastewater treatment plants, hospitals, industrial units, and intensive animal-breeding farms. In fact, drugs are frequently detected in effluents at levels ranging from below 1 ng/L up to a few µg/L.³ Since many sewage treatment plants are currently not equipped to filter some of the drugs that return to these natural water habitats,⁴ the issue of drugs in the aquatic environment has raised increasing concern. Recent toxicological studies have even revealed that many drugs prove harmful to aquatic organisms,⁵ and that some compounds in the aquatic environment can alter reproductive sexual characteristics.⁶

¹ Babu, B. Ramesh , Venkatesan, P. , Kanimozhi, R. and Basha, C. Ahmed (2009), *Journal of Environmental Science and Health, Part A*, 44: 10, 985-994.

² Karl Fent , Anna A. Weston, Daniel Caminada. (2006), *Aquatic Toxicology*, 76, 122-159

³ Babu, et al. 2009.

⁴ Sander C. Van Der Liden, Minne B. Heringa Hai-Yen Man, Edwin Sonneveld, Leo M. Puijker, Abraham Brouwer, and Bart Van Der Burg. (2008) *Environ. Sci. Technol.*, 42, 5814-5820.

⁵ Marina DellaGreca, Antonio Fiorentino, Marina Isidori, Margherita Lavorgna, Lucio Previtiera, Maria Rubino, Fabio Temussi. (2004), *Chemosphere* 54 629-637

⁶ Van Der Liden, et al. 2008.

Our experiment began by exposing a group of fathead minnows (FHM, *Pimephales promelas*), for three weeks to a single pharmaceutical, dexamethasone (DEX), at varying concentrations. Following the exposure was a day of tissue sampling, a few weeks of molecular assays on those tissues, and finally the analysis of all the data output. Our methods of data collection and assays focused on our determining whether DEX affected the reproductive function of the FHMs.

I don't know what was making my head spin more, all this information that was thrown at me on the first day, or the number of times I walked around the circle-like facility trying to find my way. It was a lot to take in, but after the first few weeks had gone by, I felt a little more grounded and definitely knew my way around. However, I can't give myself all the credit for making progress. Much of the credit is owed to my very patient mentor and all the student contractors I worked with on a daily basis. I had countless questions about everything from how devices operated, what was happening chemically during an assay, and even where things were. But through it all, everyone was very friendly and always willing to help answer questions to the best of their ability. I believe that's what I like most about MCED, the teamwork. Everyone has their specialties, and helps where they can. This is very different from the competitive academic environment I am used to. The sense of being on a larger team working towards a common goal is something you can't get in a classroom setting.

A more specific example of the type of teamwork is actually best described through one of the challenges we had during the 3-week DEX exposure. Carlie and I were regularly coming in over the weekends to count fish eggs. Part of the reproduction study includes keeping a record of eggs from each female to later compare the fish exposed to high DEX concentrations to the controls. Everything was going smoothly until we realized one of the pumps responsible for dispensing the pharmaceutical wasn't working. We spent hours looking over and cleaning what seemed like miles of tubing, but to no avail. We left that Saturday knowing our pump still wasn't working. However, another EPA employee, one who specializes in the pumps' upkeep, came in later that day, giving up part of his Saturday, to fix it. Needless to say he resolved the problem in a matter of minutes, but still it was definitely a team effort.

As I mentioned before, I wasn't quite sure what to expect before I started here in Duluth. But, one thing is for sure, from the very beginning I have been learning, and not just about the scientific methods, but about myself as well. Before this summer I had never worked with live animals in a laboratory setting, nor did I know how much work goes into keeping live animals, well, alive. I've learned everything from FHM life cycles to portions of their metabolic pathways. My biological background definitely has been strengthened, and I put my chemically-oriented studies to good use when I actually personally conducted some of the chemical/molecular assays that have been merely lectured upon in my curriculum back at DePauw.

And that brings me to my second point, the "learning about myself part." Going into my undergraduate career I wanted to go into a biomedical research field to contribute to society, to find a cure, much like the rest of my freshman biochemistry peers. But after my experience with the EPA, I realize there is more to the realm of research than creating the newest antibiotic. Not that there is anything wrong with biomedical research for those who aspire to work in that field, it just isn't my cup of tea. My experience here, along with a very thought-provoking class I took last spring with a respected professor, has slightly diverted my academic and career goals from their original path. Instead of *production* of new drugs or consumer products to help society, I would rather *preserve* what we have already. I can't say that I'm a minimalist, or that I only use recycled goods, but do I feel very passionate about where this new focus could take me. And since passion is a key ingredient to this line of work, I'm just that much closer.

As for our dexamethasone study, most of the statistical analysis has been completed with only a few leads to follow-up testing. In summary, our results thus far suggest DEX, at any environmentally relevant concentration, doesn't affect FHMs' reproductive functions. There are still a few loose ends, but my 12 weeks are nearing their end, and a great 12 weeks they have been.

It will be sad to leave the wonderful Lakeside weather and all the friendly folks I have had the pleasure to meet and work with in Duluth, but one thing I won't have to leave is the experience that has made me who I am at the end of a short 12 weeks.