

Report as of FY2006 for 2005IL63B: "Occurrence and Ecological Effects of Pharmaceutical Chemicals in Chicago Metropolitan Area Streams"

Publications

Project 2005IL63B has resulted in no reported publications as of FY2006.

Report Follows

Occurrence and effects of pharmaceutical chemicals in Chicago metropolitan area streams

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Problem and Research Objectives

Since the 1970's, aquatic ecologists and toxicologists have become increasingly concerned with the presence of human associated contaminants in the environment. In recent years, the occurrence and effects of pharmaceuticals and personal care products (PPCP's) in our rivers and lakes have received increased attention (Kolpin et al. 2002). Significant amounts of PPCP's such as hormones, antibiotics, caffeine and antacids persist after their intended use and enter wastewater treatment facilities. Treatment facilities are not designed to remove these chemicals so they are discharged into our nation's rivers. Currently the presence of PPCP's has been detected (Kolpin et al. 2002); however, the ecological effects of these novel contaminants in surface waters have not been measured.

Metropolitan areas affect the aquatic ecosystems draining them in numerous ways, including altered hydrology, increased nutrient loading and increased exposure to contaminants such as pesticides, trace metals and organic contaminants (Paul and Meyer 2002). Recently pharmaceutical chemicals have been detected in surface waters receiving wastewater treatment (WWT) effluent in highly urbanized watersheds (Kolpin et al. 2002, Gross et al. 2004, and others). The US Geological Survey conducted a nationwide survey of surface waters and detected numerous PPCP's in Illinois streams (including hormones, caffeine, and painkillers) (Barnes et al. 2002). Given the large amounts of permitted wastewater discharged into streams draining the Chicago area, the prevalence and ecological significance of PPCP's in these streams may be high.

The goals of this project were to measure the concentration of pharmaceutical compounds in the Chicago area and measure the effects of PPCP's on four major components of stream ecosystems (algae, detritus, grazers and shredders). Per advice from reviewers, we focused our attention on measuring the effects of PCPPs in a laboratory setting, rather than measuring concentrations extensively in the field.

Our first major task was to narrow down the list PCPPs present in surface waters to a few that might affect aquatic taxa. A group of commonly used PPCP's are H₂ histamine receptor antagonists. Histamine is a neuroactive amine found in the nervous systems of animals from diverse phyla (Hashemzadeh-Gargari and Freschi 1992). In humans, H₂ histamine antagonists are commonly used for the treatment of acid related gastrointestinal conditions. The H₂ antagonist reduces acid stimulation by competitively binding to parietal cells in the stomach. Since the introduction of the first H₂ antagonist in 1967 the effects of H₂ antagonists on mammals has been well documented. Cimetidine HCl (Tagamet[®]) continues to be one of the most commonly used H₂ antagonists today. Consequently, an estimated maximum concentration of cimetidine found in 84 samples from U.S. streams was 0.58µg/L (Kolpin et al. 2002). Although invertebrate histamine receptors have not been widely studied, research has shown histamine to be a regulator of invertebrate functions. Histamine stimulates pyloric rhythm and gastric mill rhythm in the stomatogastric nervous system of the crab (*Cancer borealis*) and these actions were blocked by doses of cimetidine (Christie et al. 2004). This research has provided valuable knowledge into the neurological effects of cimetidine on invertebrates. Research on the ecotoxicological effects of Cimetidine was one of the major accomplishments of this grant.

In addition to Cimetidine, we have also been examining the effects of the generalized anti-microbial agent, triclosan, a popular antimicrobial and antifungal agent found in consumer products ranging from dish soap to toothpaste to makeup. Triclosan is classified as a specific biocide; it functions by a physical-chemical mechanism that targets a specific biochemical pathway in bacterial and fungal cells. Triclosan was found to have a concentration of $0.14 \mu\text{g L}^{-1}$ in a recent US Geological Survey (Kolpin et al 1999). Triclosan targets the fabI protein Enoyl-acyl protein reductase, blocking the synthesis of lipids in cells (McMurry 1999). As lipid synthesis is critical for the integrity of the cell membrane and for cell replication, Triclosan ultimately causes lysis of cells (McMurry 1999). We have examined the effects of triclosan on microbial function and have established that sediments below waste water treatment plants contain triclosan resistant bacteria. We are continuing to investigate this in our laboratory using internal funds.

The third compound we have been exploring are the effects of caffeine. Caffeine is a very common PPCP and was found in 70% of water samples ($0.1 \mu\text{g/l}$ median concentration) collected in a nationwide survey (Kolpin et al. 2002, Audenkampfe 2006) and has been used as a tracer of human activity and indicator of water quality (Ferreira 2005, Buerge 200). Caffeine occurs in plants where they presumably are natural pesticide and reduce herbivory. Caffeine is a purine alkaloid and as a result affects adenosine receptors on the cell surface and phosphodiesterase inside cells. Caffeine usually acts as an adenosine antagonist, accounting for its stimulating effects. As adenosine is a very common transmitter and secondary messenger, caffeine may affect ecosystem constituents. As caffeine is very common in surface waters, chronic exposure to ecologically relevant doses of caffeine may have integrated consequences on ecosystem functions. An graduate student, Antoine Aubeneau has received internal funding from Loyola University Chicago to conduct this research using methods we developed for our examination of cimetidine, worked funded by this grant.

Methodology

In order to adequately assess exposure concentrations to contaminants in a laboratory experiment, effective analytical chemistry to measure compounds is essential. Using grant funds, we successfully develop high-performance liquid chromatography (HPLC) methods to extract and detect low concentrations of specific pharmaceutical compounds in water samples (cimetidine and caffeine methods are complete and successful). The input of PPCP's is continual via WWT effluent, but it is not known how long these compounds persist in aquatic ecosystems. We are currently employing these analytical techniques to measure the concentrations of compounds in the artificial streams and are analyze Chicago area surface waters for cimetidine to put our laboratory findings in context.

Instantaneous growth and mortality experiments have been performed using the pharmaceutically active compound cimetidine. Chironomids were collected and then exposed to $0.07 \mu\text{g/L}$, [x10], [x100], [x1000] and [0]. Organisms were placed in an incubator for 1 week under controlled conditions, and then growth and mortality were analyzed. Microbial communities have been used to measure the effects of Triclosan, another PPCP found in Illinois Rivers. To determine the ecological impact of Triclosan, we incubated leaves (source of microbes) with concentrations of $0.14 \mu\text{g/L}$, [x10], [x100], [x1000] and [0]. Microbes were incubated in LB agar with the same concentrations.

The artificial stream facility (48 recirculating streams of 4 meter length) has been an invaluable match from Loyola University Chicago in this research. Each stream contained unglazed clay tiles to measure algal growth, leaf packs to measure decomposition, and three species of aquatic invertebrates. The streams were used to look at the effects of cimetidine on

stream ecosystems. The results of this experiment will be published in peer reviewed journals and will be the work of Paul Hoppe who has been conducting this research for his MS thesis. Currently, a similar experiment will be started looking at the effects of caffeine in stream ecosystems using internal funds. The combination of these two experiments will be invaluable for obtaining additional external funds.

We are also conducting fieldwork in the Chicago area, mainly to collect specimens for our laboratory experiments. In addition, we are striving to put our laboratory experiments in context, i.e., to determine if the endpoints we observe in the artificial streams are occurring in the field. The sampling sites focus on proximity to three major waste water treatment facilities in the Chicago area including Stickney, North Side and the Kirie Water Reclamation Plants. 10 established sampling sites have varying degrees of urbanization and inputs of waste water treatment effluent. At these sites, a number of Loyola University Chicago supported undergraduate research projects are being conducted in conjunction with this research.

Principal Findings and Significance

The results of our experimental stream experiment demonstrated that cimetidine did not affect basal resources, such as algae and leaves, but increasing concentrations of cimetidine (at concentrations measured in US surface waters) did have a significant affect on invertebrate growth, mortality and population dynamics. The results of this experiment will be submitted for publication in a peer-reviewed journal by Dec. 2007. These results suggest that continual input of cimetidine and other PPCP's could affect mortality of and growth rates aquatic invertebrates in surface waters.

The triclosan study showed that increasing triclosan concentrations lead to increased variation in microbial respiration. The concentrations of triclosan measured in US surface waters did not affect microbial respiration or microbial growth rates; however, this research is ongoing. As mentioned above we did find significant microbial resistance to triclosan. We are continuing this line of research in collaboration with Dr. John Kelly (a microbial ecologist, Loyola University Chicago) and plan to examine if there is an effect of triclosan on microbial community composition.

In laboratory incubations, we found that caffeine affected *Helicopsyche* growth rates but not *Gammarus* growth. This research is ongoing with additional support provided by Loyola University Chicago.

Related and Seed Projects

A graduate student received funding (\$24000 tuition and \$32000 stipend) from Loyola University Chicago Biology Department to continue working on this project. Undergraduates received funding (\$10,500) from the Mulchahey Scholarship program and (\$6000) from Loyola University Chicago Center for Urban Environmental Research and Policy (CUERP). An undergraduate received funding (\$4000) from CUERP, work-study from Loyola University Chicago (\$4000) and a Mulchahey Scholarship (\$2000) to conduct research that contributes to this project. Although funds from this grant are exhausted, we continue to conduct research in this area. We have 2 undergraduates, one graduate, student and a high school student continuing to work on the effects of pharmaceuticals in streams, using internal funds.

