

## **The effect of anthropogenically produced ligands on the bioavailability of mercury in aquatic systems**

Mercury is considered one of the most hazardous contaminants in aquatic environments, and it has caused the most fresh water fish advisories in the U.S. out of any other anthropogenically-released pollutant. Among the various species of mercury that can exist in aquatic environments, the organic compound and neurotoxin methylmercury ( $\text{CH}_3\text{Hg}^+$ ) is the most toxic because it is easily absorbed in the gastrointestinal tract and able to cross the blood-brain barrier. The U.S. Environmental Protection Agency has stated that consumption of fish and shellfish contaminated with methylmercury is the primary route of human exposure to mercury<sup>2</sup>. Over 95% of the methylmercury found in aquatic environments is converted from inorganic mercury by microorganisms in anoxic sediments, and methylation can only occur when mercury has passed through the cell membrane and entered the cell<sup>3</sup>. Thus, it is essential to understand the factors that influence the bioavailability of mercury, defined as the fraction of mercury able to be taken up by a cell. Our previous experiments have shown that the bio-uptake of mercury can be enhanced by the presence of various ligands – compounds that bind mercury in solution. The objective of my research is to experimentally measure the effect of anthropogenically produced ligands and inorganic mercury speciation on mercury bioavailability.

In 2006, a global estimate of 2480 tons of mercury was released into the environment as a result of human activities, and 65% of that total was emitted into the atmosphere by stationary fuel combusting sources<sup>8</sup>. Mercury is transported through the atmosphere and deposited into aquatic environments, where it can exist in many different forms<sup>1</sup> (Fig 1). The bioavailability of mercury, and hence the production of methylmercury, is mainly controlled by the chemical speciation of inorganic mercury in aqueous solutions. With the release of anthropogenically produced chelating ligands into the environment like EDTA, NTA and SHMP found in food preservatives and laundry detergent, the speciation of mercury can be altered to create mercury ligand complexes. The effects of these anthropogenically produced ligands on the bioavailability of mercury are relatively unknown. Current bioavailability models like the Free Ion Activity Model and Biotic Ligand Model predict that metals bound to strong chelates will not be bioavailable, but many exceptions to these models exist<sup>5</sup>.

In the spring and summer of 2011, I worked with a PhD student under the supervision of Prof. Jean-François Gaillard to determine the bioavailability of mercury in the presence of four chelating ligands (EDTA, cysteine, glutathione, and penicillamine) using a genetically modified *E. coli* as a whole-cell biosensor. Recent developments in biosensor technology have allowed our lab to experimentally measure the bioavailability of mercury using a biological process, whereas previous methods were only capable of estimating bioavailability through chemical methods. The operating principle of the biosensor is that mercury able to enter the cell will bind to a promoter region on the chromosome that has been fused with a reporter gene that induces bioluminescence<sup>6</sup> (Fig 2). We have shown that the intensity of the bioluminescence signal is proportional to the concentration of bioavailable mercury in the solution. Using a luminescence microplate reader, the light emitted by the biosensor can be detected and quantified. In performing a series of assays, it is possible to probe the bioavailability of mercury in the presence of

many different ligands of various concentrations. We predicted the mercury speciation in solution with the chemical equilibrium software ChemEQL, which allowed us to directly compare the speciation and bioavailability of mercury.

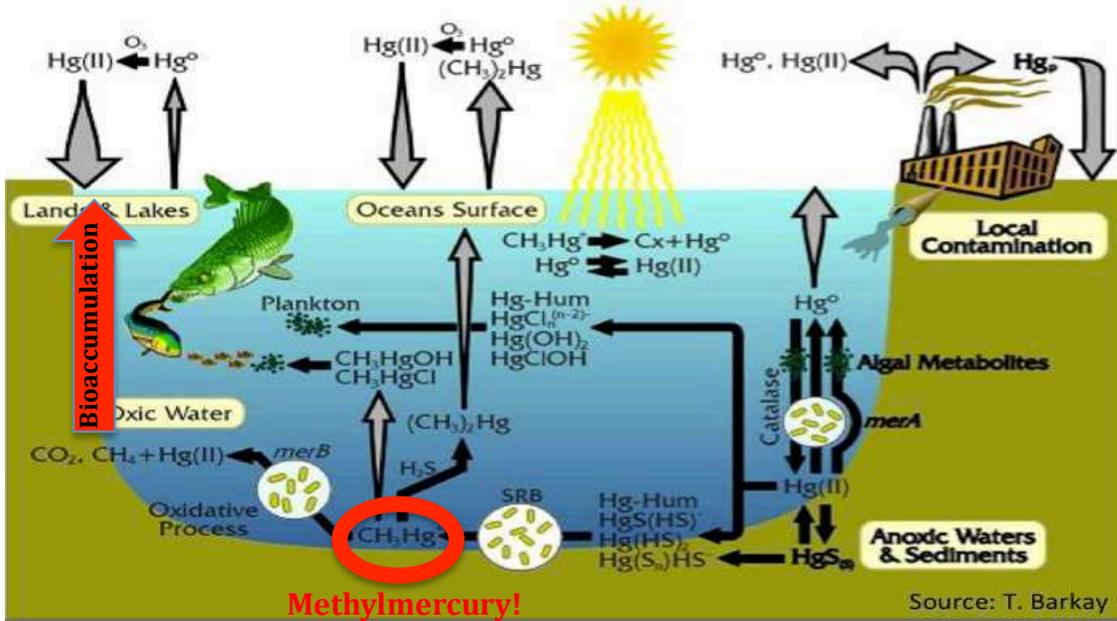
Contrary to current bioavailability models, we discovered that certain mercury-ligand complexes enhanced the bioavailability of mercury instead of inhibiting it. EDTA is especially of interest to me because it is the only anthropogenically produced ligand out of the four, and it caused an increase in bioavailability at both the high ( $10^{-3}$ M EDTA) and low ( $10^{-6}$ M EDTA) concentrations. EDTA is known to bind to divalent cations present on the outer membrane of gram-negative bacteria, disorganizing the membrane and potentially permeabilizing it<sup>9</sup>. *E. coli* is gram-negative; therefore, my hypothesis is that in the presence of certain chelating ligands like EDTA, the membrane of the biosensor becomes more permeable, allowing mercury to be more readily internalized. Many types of mercury methylating bacteria are also gram-negative, meaning the permeating ability of chelating ligands could be a cause for increased methylmercury production in the environment. My research aims to relate mercury bioavailability with membrane integrity of the biosensor in the presence of various anthropogenically produced chelating ligands like EDTA, NTA and SHMP. The goal is to define the conditions under which the transport of mercury across microbial cell membranes is facilitated by the presence of ligands.

One method of probing the outer membrane of a microorganism is to use fluorescence spectrophotometry. In my research I plan to use the LIVE/DEAD BacLight bacterial viability kit in order to analyze membrane integrity of *E. coli* (the biosensor) in the presence of EDTA, NTA, and SHMP. The viability kit is comprised of two nucleic acid stains: SYTO9, which is able to pass through damaged and undamaged cell membranes, and propidium iodide (PI), which can only enter cells with damaged membranes. When excited, cells that contain only SYTO9 will fluoresce at green wavelengths (530nm), while cells containing both SYTO9 and PI fluoresce at red wavelengths (630nm). Therefore, using the fluorescence reader in the High Throughput Analysis lab at Northwestern University, the ratio of green to red fluorescence can be measured, which is proportional to the number of bacterial cells with intact membranes. The bioavailability of mercury in the presence of these ligands can be measured with the biosensor, and the mercury speciation in solution can be calculated at equilibrium using ChemEQL. If successful, this research should suggest conditions under which anthropogenic ligands could be released into the environment without causing unintended negative impacts.

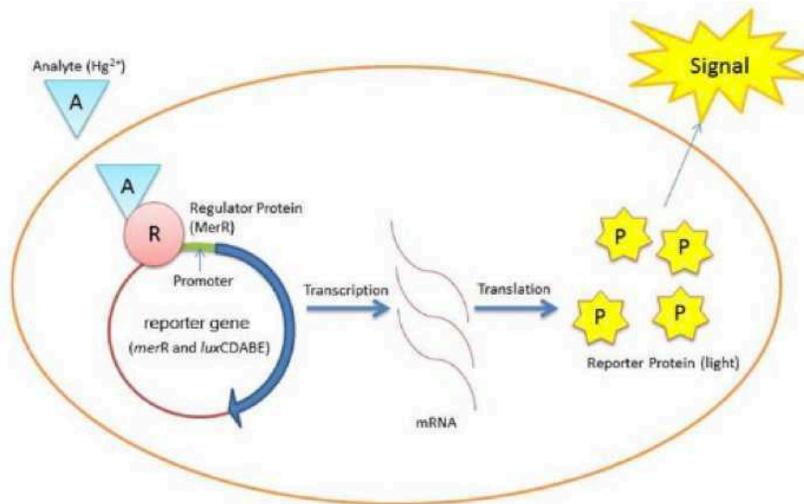
I will receive a Civ\_Env 399 credit for this research project to begin in winter 2012, and it will comprise the second half of my senior honors thesis in combination with the independent study I completed in spring 2011. Currently, I am within 4 credits of finishing my bachelor's in Environmental Engineering, and I am just beginning my Master's in the same discipline. I am very comfortable working with the topic of metal speciation in aquatic environments; I have taken a class on aquatic chemistry as well as a graduate level class focusing solely on metal speciation. I began working in Prof. Gaillard's lab in spring 2011, and I gained experience using the equipment at Northwestern's HTA lab and the viability kit during summer 2011. In the future I hope to publish my research in a scientific journal, and I am considering pursuing a PhD in Environmental Engineering.

Figures

### Mercury Cycle in Surface Waters



**Figure 1:** This image depicts the geochemical cycle of mercury in the environment with a focus on mercury speciation in aquatic systems. The neurotoxin methylmercury is produced by anaerobic bacteria in the sediments, which then bioaccumulates up the food chain. According to the U.S. EPA, the consumption of fish and shellfish contaminated with methylmercury ( $\text{CH}_3\text{Hg}^+$ ) is the primary route of human exposure to mercury<sup>1</sup>.



### Whole Cell Biosensor: E coli ARL1

**Figure 2:** A schematic of the principle of the biosensor<sup>6</sup>. When mercury enters the cell, it binds to the promoter region (regulator protein MerR), which is fused to the luxCDABE cassette. This binding causes the production of a bioluminescent protein. The overall intensity of light emitted is proportional to the bioavailable mercury in solution.

## References

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