Report as of FY2006 for 2006NJ101B: "Female Hormones in Surface Water of Central/Northern New Jersey: Impacts of Combined Sewer Overflows versus Treated Wastewater Discharge"

Publications

Project 2006NJ101B has resulted in no reported publications as of FY2006.

Report Follows

Project Summary:

Problem and Research Objectives

We proposed to detect and quantify female hormones — a major class of endocrine disrupting chemicals (EDCs) — in the surface water of Central/Northern New Jersey. This study is especially important for densely populated Central/Northern New Jersey where treated wastewater (TWW) is a major component of surface water and combined sewer overflows (CSO) have caused substantial problems in several watersheds. More importantly, the surface aquatic ecosystems are very precious in this populous area. Yet, they are not very healthy and the surface water quality has deteriorated due to heavy contamination from extreme urbanization and industrialization. Low but constant contamination with female hormones in surface water may adversely affect the reproductive behavior of animals such as fish, posing large ecological risks. Our study would provide data on the level and the source (TWW vs. CSO) of female hormone contamination in the watersheds of Central/Northern New Jersey. It could help determine whether future effort is needed, and which source — TWW or CSO — we should pay most attention to for reducing the ecological impact of the female hormones in these watersheds.

In this study, we planned to develop analytical methods for detection and quantification of three common female hormones [17β-estradiol (estradiol, or E2), estrone (E1), and 17α-ethinyl estradiol (EE2)] in surface water samples using either LC-MS/MS or GC-MS. After method development, we proposed to select two to three typical surface aquatic systems that are influenced variously by TWW and/or CSO. The goal of this study was to differentiate the contributions of TWW and CSO to the female hormones detected in these aquatic systems. The study would provide much needed information for regulating the emerging pollutants and for supporting future efforts to develop water quality models and Total Maximum Daily Loads (TMDLs) for these chemicals.

The specific **objectives** of this study are to:

1) collect water and colloid samples from two watersheds of North/Central New Jersey during and after major storms;
2) analyze the female hormones in the samples following published laboratory procedures and with liquid chromatography - mass spectrometry/mass spectrometry (LC-MS/MS) or gas chromatography-mass spectrometry (GC-MS);
3) quantify the loading of the hormones from different sources to the studied watersheds.

Methodology

<u>LC-MS/MS Method Development</u>

In the first five months of the project, we developed an LC-MS/MS method with collaboration of Dr. Zhiqiang Yu of Guangzhou Institute of Geochemistry, Chinese

Academy of Sciences. We used a liquid chromatograph with tandem mass spectrometric detection (LC-MS/MS) (Agilent). The liquid chromatographic separation was carried out at room temperature using a RP-C8 Hypersil MO5 phase (2.1x100 mm; 5 μm) from Agilent (Waldbronn, Germany) and an RP1 guard column (2x10 mm UltraSep ES; SEPSERV, Berlin, Germany). For the separation of the analytes, a programmed gradient was applied using acetonitrile and water as solvents. The initial composition of the mobile phase was 12% acetonitrile. This level was held for five minutes and then increased within 30 minutes to 25% and within another 15 minutes to 53%. The flow rate of the mobile phase was 0.2 mL/min. The addition of buffers (ammonium acetate or ammonium hydroxide at varying concentrations) to the mobile phase caused a decrease in the responses of the analytes due to lower ionization ratios.

Reference compounds (E1, E2, and EE2) and the deuterated surrogate standard compounds 2,4-d2-17β-estradiol (d2-E2) and 2,4,16,16-d4 Estrone (d4-E1) were used to prepare standard solutions in 2-propanol. The deuterated surrogates were used for both recovery efficiency and quantifying the concentrations of the target compounds. For method development, MiliQ water was spiked with the estrogen standards at levels of 0.1 to 50 μg/L.

Our results showed that the three estrogen compounds were separated with the instrumental conditions described above and the detection limits were about 0.5 μg/L. These detection limits suggested that these three estrogen compounds could be detected and quantified at ng/L levels for surface water samples collected from the field.

GC-MS/MS Method Development

Because the cost of LC-MS/MS analysis is very expensive due to limited availability of the instrument, we have also developed a GC-MS method with a derivatization procedure for quantification of the three estrogen compounds. The instrument used for method development was a Waters Quattro Micro tandem quadrupole GC-MS/MS. This instrument is available to us with no cost. It uses a 60 m by 0.25 mm i.d. DB-5 (5% diphenyl dimethyl polysiloxane) capillary column with a film thickness of 0.25 mm.

For method development, we used prefiltered miliQ water which was spiked to 100 ng/L with mesterolone, the surrogate standard, and the three estrogen compounds. Mesterolone has properties similar to the other steroids but is not commonly used in human therapy, and thus should not be detected in surface water samples. The spiked filtrate was extracted through a 47-mm C-18 solid-phase extraction disc. Prior extraction, the C-18 discs had been preconditioned by rinsing them twice with 25 ml methanol followed by two rinses with 50 ml of distilled water. After extraction, the discs were rinsed twice with 25 ml of a 60:40 (v/v) water:methanol solution to selectively elute polar organic matter from the SPE discs. After this washing step, the estrogen compounds were eluted from the discs with 20 ml of a 25:75 (v/v) water:methanol solution. The eluent was then completely dried under vacuum, re-dissolved in pure methanol, and the methanol solution was transferred to a 1-ml volumetric flask. The extract was dried once again under vacuum and re-dissolved in 200 μl of acetonitrile. Next, 50 μl of

heptafluorobutyric anhydride, the derivatizing agent, was added, and the volumetric flask was sealed and placed in a 55°C over for 1.5 h. After completion of the derivatization reaction, the flask was cooled to room temperature, and the solvent was evaporated under a nitrogen stream. The derivatized estrogen compounds were re-dissolved in 100 μl of iso-octane to which hexachlorobenzene (400 μg/L) was added as an internal standard. The samples were ready for instrumental analysis.

Our results showed that the three estrogen compounds were separated on the GC-MS/MS with detection limits of ng/L, which are comparable to the LC-MS/MS method. The recovery efficiencies were 65-91% for the spiked estrogen compounds.

Principal Findings and Future Work Plan

We have completed the analytical method development and the results showed that the three estrogen compounds can be separated and quantified with both LC-MS/MS and GC-MS/MS. The detection limits of both methods for the three chemicals are comparable and are on the order of ng/L. Tests using spiked water samples indicated that 65-91% of the chemicals spiked to pure water can be recovered.

With the no-cost extension of the project, we are in the process of testing field water samples. We will take water samples from the Raritan River of New Brunswick, New Jersey. Duplicate samples (5-10 liters) will be sampled, stored in cooler with ice bags, and transported back to the lab. They will be stored immediately at 4^{o}C for no more than 2 days. After suspended solid particles (SSP) have been settled, the supernatant (2−5 liters) will be filtered through 0.45 μm glass fiber filters, spiked with 2.5 ng of the surrogates d2-E2 and d4-E1 for quantifying both the recovering efficiency and the concentrations of the three compounds. For the water phase, sample extraction will be performed using SPE discs that will have been preconditioned with methanol and distilled water. The water samples will be filtered with an upper limit of flowrate at 5 mL/min. Then the discs will be sequentially washed and extracted following the procedures described above. The sample extract will be transferred to acetonitrile. One half of the acetonitrile solutions will be used for direct LC-MS/MS analysis, and the other half will be used for derivatization and subsequent GS-MS/MS analysis.

After validating the two methods with the field samples, we will conduct systematic water sampling and analyze the chemicals with either or both methods, depending upon the availability of the instruments. We expect to complete the proposed work by the end of the Fall 2007 semester. A complete progress report on the results will be provided in the Spring of 2008.