

# Report as of FY2006 for 2005MA47B: "Monitoring Estrogenic Hormones Undesired Fish Contraceptives, and "

## Publications

- Articles in Refereed Scientific Journals:
  - Zuo Yuegang; Yuejuan Lin, 2007, Solvent effects on the silylation-gas chromatography-mass spectrometric determination of natural and synthetic estrogenic steroid hormones, *Chemosphere*,(in press)
  - (2) Zuo Yuegang; Kai Zhang, Yuejuan Lin, 2007, Microwave-accelerated derivatization for the simultaneous gas chromatography-mass spectrometric analysis of natural and synthetic estrogenic steroid hormones. *J. Chromatography A* 1148, 211-218.
  - Zuo Yuegang; Kai Zhang, Yiwei Deng, 2006, Occurrence and photochemical degradation of  $\alpha$ -ethinylestradiol in Acushnet river estuary. *Chemosphere* 63, 1583-1590.
  - Zuo Yuegang; Kai Zhang, 2005, Discussion: Suitability of N,O-bis(trimethylsilyl)trifluoroacetamide as derivatization reagent for the determination of the estrogens estrone and 17  $\alpha$ -ethinylestradiol by gas chromatography-mass spectrometry. *J. of Chromatogr. A* 1095, 201-202.
  - Zhang Kai; Yuegang Zuo, 2005, Pitfalls and solution for simultaneous determination of estrone and  $\alpha$ -ethinylestradiol by gas chromatography mass spectrometry after derivatization with N,O-bis(trimethylsilyl)trifluoroacetamide. *Anal. Chim. Acta* 554, 190-196.
- Other Publications:
  - Lin Yuejuan; Yuegang Zuo, 2007, Ion-pair HPLC Determination of estrogens and their Conjugates in Water Samples. 7th Csaba Horvath Medal Award Symposium, April 19-20, 2007, Hartford Convention Center, Hartford, Connecticut.
  - Zuo Yuegang, 2007, Estrogenic Steroid Hormones in Aquatic Ecosystems, Their Effects on Fish Population and Their Environmental Fate. The Department of Chemistry Seminar at Kansas State University, Feb. 1, 2007 (invited).
  - Lin Yuejuan; Yuegang Zuo, 2007, Determination of estrogens and their Conjugates by Ion-pair HPLC. University of Massachusetts Dartmouth Thirteenth Annual Sigma Xi Research Exhibit, April 24-25, 2007, North Dartmouth, MA.
  - Wang Chengjun; Jiping Zhou, Vanessa Ruelos, Amita Sachdeva, Yuegang Zuo, 2007, Simultaneous Determination of Creatinine and Uric Acid in Human Urine Samples by High Performance Liquid Chromatography. University of Massachusetts Dartmouth Thirteenth Annual Sigma Xi Research Exhibit, April 24-25, 2007, North Dartmouth, MA
  - Zuo Yuegang, 2007, Incorporation of authentic chemical separation research projects into analytical chemistry curriculum. 233rd ACS National Meeting, Chicago, IL, March 25-29, 2007.
  - Zhang Kai; Yuegang Zuo, 2006, Occurrence, Microbial and Photochemical Degradation of Endocrine Disrupting Estrogens in Surface Waters. University of Massachusetts Lowell, Dept. of Chemistry Seminar Series, Oct. 18, 2007.
  - Zuo Yuegang, 2006, Bioeffects and fate of estrogenic steroid hormones in fresh and coastal marine waters. International Workshop on water contaminants and Health Effects. Edmonton, Alberta, Canada, July 5-9, 2006 (invited).
  - Zuo Yuegang, 2006, Improved gas chromatography-mass spectrometry determination of estrogenic hormone steroids in aquatic environments. 37th ACS Central Regional Meeting, May

16-20, 2006. Frankenmuth, MI.

- Wu Jingping; Yuegang Zuo, 2006, GC determination of phthalate esters and their photodegradation in natural water. University of Massachusetts Dartmouth Twelfth Annual Sigma Xi Research Exhibit, April 25-26, 2006, North Dartmouth, MA.
- Zhang Kai ; Yuegang Zuo, 2006, Pitfalls and solution for simultaneous determination of estrone and 17alpha-ethinyestradiol by GC-MS after derivatization with N.O-Bis(trimethylsilyl) trifluoroacetamide. University of Massachusetts Dartmouth Twelfth Annual Sigma Xi Research Exhibit, April 25-26, 2006, North Dartmouth, MA
- Lin Yuejuan; Yuegang Zuo, 2006, Analysis of free-form estrogen steroid hormones and their conjugates in urine and natural water using HPLC. University of Massachusetts Dartmouth Twelfth Annual Sigma Xi Research Exhibit, April 25-26, 2006, North Dartmouth, MA.
- Zuo Yuegang; Kai Zhang, 2006 Pitfalls and solution for simultaneous determination of estrogenic steroids using silylation-gas chromatography-mass spectrometry. The 231st ACS National Meeting and Exposition, Atlanta, GA March 26-30, 2006.

## **Report Follows**

The occurrence of estrogenic compounds in aquatic environment has become of increasing concern during the past decade due to their endocrine disruption potential. Among these estrogenic chemicals, a group of synthetic steroids, such as 17 $\alpha$ -ethinylestradiol, is of particular concern. This concern arises in part from the increasing use of birth-control pills, formulated with exogenous estrogenic and progestational chemicals that show high physiological activity at very low concentrations and have been associated to certain alarming effects on reproduction and developmental processes such as feminization, decreased fertility or hermaphroditism. The Buzzards Bay receives stormwater runoff, effluents from wastewater treatment facility of New Bedford, Fairhaven, Fall River and other surrounded towns. This leads to direct input of many different classes of pollutants, including endocrine-disrupting estrogenic hormones, through the sewage effluents and industrial wastewater. The combination of these estrogenic compounds and other pollutants can adversely affect plankton, and fish, and could be related to the declines in lobster abundance in Buzzards Bay. The objectives of this research project are (1) to develop an SPE-GC-MS analytical method for the separation and quantitation of estrogenic hormones: estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -ethinylestradiol and mestranol; (2) to employ the analytical methods developed in this project to monitor estrogenic hormones: estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -ethinylestradiol and mestranol in New Bedford Harbor and Buzzards Bay Water; (3) to assess the microbial and photochemical fate of estrogenic hormones in the Buzzards Bay; (4) To train graduate and undergraduate students to use the techniques developed in this project to monitor and protect our aquatic environment.

In the first stage of this project, our research had been focused on developing a Solid-Phase Extraction (SPE) GC-MS analytical method for the separation and quantitation of estrogenic hormones and other endocrine disrupting compounds. These included developing a new silylation solution to prevent the formation of undesired multiple derivatization products and conversion of trimethylsilyl derivatives of EE2 formed to their respective E1 derivatives reported in previous studies, and integrating an SPE method into GC-MS analysis, as well as the effects of solvent, temperature, and reaction time on the derivatization of EE2. To shorten the derivatization time of estrogenic steroids, we have developed a microwave-accelerated derivatization method for the simultaneous gas chromatography-mass spectrometric analysis of natural and synthetic estrogenic steroid hormones. We have also validated an HPLC method for the simultaneous determination of free and conjugate steroid hormones.

With the newly developed analytical techniques, we have determined the estrogenic hormones in seawater around Acushnet river estuary in Buzzards Bay and examined their possible sources, effluents from wastewater treatment plants. We have also carried out some preliminary studies on the microbial and photochemical degradation of estrogenic steroid hormones in Buzzards Bay seawater.

We presented our research results obtained in the project in professional conferences, published five papers in peer-reviewed international scientific journals, and are preparing two more manuscripts for publication soon.

For the educational component, I supervised five graduate students (one supported by this grant and the others by other UMass Dartmouth funds as the match) under this environmental estrogenic steroid research project. One of them, Kai Zhang, has completed his Ph.D. dissertation defense. Another, Yuejuan Lin, is planning to have

her M.S. thesis defense in August, 2007. The third student, Chengjun Wang, is working on his dissertation research.

## 9. Project Findings:

1. The determination of estrogenic steroids, particularly in natural water systems, have been an analytic challenge for chemists due to the extremely low concentration of estrogenic steroids and interference from the sample matrices. Many immunoassay, gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography (LC), and liquid chromatography-mass spectrometry (LC-MS) techniques have been developed for the determination of estrogenic steroid hormones in aquatic environments. GC-MS has been a preferred approach for simultaneous analysis of both synthetic and natural estrogenic steroids because of its superior separation and identification capabilities. In order to employ high-resolution GC for the analysis of estrogenic steroids, derivatization is required to increase analyte volatility and thermal stability and thus improve chromatographic separation. Many reagents are available for this purpose. Trimethylsilyl (TMS) derivatives are probably the most widely employed. The combination of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMCS) is amongst the most popular silylating reagents used for the identification and quantification of estrogenic steroid hormones in water samples. However, several research groups reported on the formation of different derivatization products of EE2 with this silylating reagent [Shareef et al., 2004; Zuo and Zhang, 2005, Zhang and Zuo, 2005]. And thus suggested that derivatization with BSTFA + TMCS might not be suitable for the determination of EE2 by GC-MS under the previously reported conditions. In this project, we have developed a new silylation mixture (BSTFA:TMCS:pyridine = 49.5:05:50 (v/v/v)), overcome these pitfalls and generated a single product of di-TMS derivative of EE2 (Zuo and Zhang, 2005; Zhang and Zuo, 2005). We have also established a microwave-accelerated derivatization method for the simultaneous gas chromatography-mass spectrometric analysis of natural and synthetic estrogenic steroid hormones (Zuo et al., 2007). We have also validated an HPLC method for the simultaneous determination of free and conjugate steroid hormones (Lin et al. 2006).

2. We have successfully applied our developed analytical procedure in the simultaneous determination of both natural and synthetic estrogenic steroids (estrone and 17 $\alpha$ -ethynylestradiol) in Acushnet River estuarine seawater (Zuo et al., 2004, 2006). Our results have shown that the concentration of three common estrogenic hormones, 17 $\alpha$ -ethynylestradiol, estrone and 17 $\beta$ -estradiol, could be over 4.7, 1.2 and 0.83 ng/L, respectively, during the summer, which can certainly cause fish feminization in the Bay and may responsible for the significant decline in lobster population in Buzzards Bay. To further identify the sources of estrogenic hormones in the Bay, we have examined both influents and effluents of New Bedford Wastewater Treatment Plant with the GC-MS and HPLC methods developed in this projects and found significant amount of conjugate and trace free steroid hormones.

3. Our preliminary study has shown that EE2 can undergo a rapid photodegradation in estuarine seawater under natural sunlight irradiation, with a half-life of less than 1.5 days in spring sunny days. Our studies have also shown that natural estrogenic compound E2 can be oxidized to E1 by microorganisms in natural river water

with half-lives of 0.2-9 days at 20 °C, and E1 is then further degraded at rates consistent with previous investigations. Compared to E2, synthetic EE2 is much more resistant to biodegradation in natural water. Although EE2 is relatively resistant to microbial degradation, EE2, like other estrogenic steroids, contains a phenolic functional group, which is susceptible to photodegradation. To study the photochemical degradation of EE2 in seawater, EE2 was dissolved into seawater collected from Buzzards Bay and Acushnet River Estuary and irradiated under natural sunlight or simulated solar source in cylindrical quartz tubes (20 cm long x 1.4 cm i.d.). The results obtained indicate that the photochemical transformation represents a major fate of estrogenic steroids in natural surface water.

4. Five graduate students have trained in this project to use the techniques developed to monitor and protect our aquatic environment from estrogenic pollutants.