

# Annual Report Summary

**Period Covered by the Report:** April 2, 2001- May 30, 2003

**Date of Report:** June 30, 2003

**Title:** Fluorescent Whitening Agents as Facile Pollution Markers in Shellfishing Waters

**Investigators:** Eugene A. Cioffi

**Institution:** University of South Alabama

**Research Category:** Small Grants for Exploratory Research (SGER)

**Project Period:** August 23, 1999 - December 31, 2003

**Objective(s) of the Research Project:** The objective of this research project is to: (a) investigate the use of fluorescent whitening agents (FWA's) as anthropogenic markers of ISDS pollution and potential storm-drain contamination of the Mobile Bay estuarine system; (b) evaluate commercially available solid phase extraction (SPE) supports for selective isolation and quantitation of FWA's; (c) investigate new selective field sampling techniques; (d) attempt to identify the specific classes of FWA's using high-field  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy.

## **Progress Summary/Accomplishments:**

The major accomplishments in this project to date have been to: (1) identify the best available solid-phase extraction (SPE) technologies for successful isolation and quantitation of fluorescent whitening agents (FWA's); (2) optimize the ion-pairing extraction protocol; (3) to screen numerous potential sites within the Mobile Bay watershed for the presence of these anthropogenic materials. New field sampling techniques have been optimized for the pre-isolation and clean up of FWA's prior to laboratory quantitation. The use of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy for identification of specific FWA's (and their respective photo-initiated *E/Z* isomeric ratios) both in authentic and field sample at low analyte concentrations has been limitedly successful (*vide supra*).

## FWA Detection in the Mobile Bay Watershed:

Nine representative sites in Mobile bay were chosen for sample collection. All sites were sampled from shoreline points accessible to the public. All site coordinates were accurately recorded using a portable GPS device. One-liter (1L) samples were collected in pre-cleaned HDPE bottles and chilled in ice during transport to the laboratory. FWA analyses were conducted using the All-Tech C-18 High Capacity SPE cartridges, eluted with TBA- $\text{HSO}_4$  in MeOH, and quantitated using high-resolution fluorescence spectroscopy ( $\lambda_{\text{ex}} = 350\text{nM}$ ;  $\lambda_{\text{em}} = 397\text{nM}$ ). The FWA's found ranged from 30ppb - 145ppb.

## Solid-Phase Extraction technologies:

A series of commercially available C-18 Solid Phase Extraction (SPE) cartridges were evaluated for the isolation and quantification of the four classes of FWAs. These included the following: All-Tech C-18 High Flow; All-Tech C-18 High Capacity; All-Tech Prevail<sup>®</sup>; Chromabond XTR; Chromabond Easy<sup>®</sup>; Strata-X. All cartridges were compared with regards to analyte recovery, limit

of detection, and ease of use. With the exception of Chromabond XTR, all SPE cartridges performed well, with the best analyte reproducibility found using either the All-Tech C-18 High Capacity or the All-Tech Prevail<sup>®</sup>. Of a series of ion-pairing reagents examined, the most selective and efficient ion-pairing reagent was found to be tetra-n-butyl ammonium hydrogen sulfate (TBA-HSO<sub>4</sub>).

#### <sup>1</sup>H and <sup>13</sup>C Nuclear Magnetic Resonance (NMR) Spectroscopy

Individual fluorescent whitening agents (FWAs) and FWA mixtures were examined using both <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopy. Pure standards at concentrations above 10mg/mL in afforded good <sup>1</sup>H NMR spectra in a reasonable amount of time. The <sup>13</sup>C NMR spectra required overnight runs to give adequate S/N for peak identification. At concentrations below approximately 1mg/mL, S/N was poor in the <sup>1</sup>H NMR spectra, requiring overnight acquisitions; the <sup>13</sup>C NMR signals could not be accurately identified due to unacceptable S/N ratios. Preliminary <sup>1</sup>H NMR experiments at lower analyte concentrations (< 0.5 mg/mL) were limitedly feasible using an inverse-detection probe and long acquisition times; indirect-detection experiments for <sup>13</sup>C NMR signals were not successful.

**Publications/Presentations:** B. Wallace and **E.A. Cioffi**; "Fluorescent Whitening Agents as Facile Pollution Markers in Shellfishing Waters"; Dauphin Island Sea Laboratory, August 2001.

**Future Activities:** Assessment and quantification of both the individual and mixed FWA's isolated from all Mobile Bay field samples using the optimal identified SPE technology will be concluded using high-resolution fluorescence spectroscopy. Additional investigation into the spectral resolution of E/Z FWA isomers via <sup>1</sup>H and indirect-detected <sup>13</sup>C NMR spectroscopy will be attempted at higher field (> 500 MHz). These analyses will be conducted at the National High Field Magnetic Laboratory (NHFML), Florida State University, Tallahassee, Florida.

**Supplemental Keywords:** Fluorescent Whitening Agents, FWA's, Fluorescence Spectroscopy, Solid-Phase Extraction, NMR Spectroscopy

**Relevant Web Sites:** None

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