

## **Final Report Executive Summary**

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

**Date of Report:** March 30, 2004

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

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**Institution:** University of South Alabama

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

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

**Rationale for this ACES-Sponsored Research Project:** Within the past several years, the Alabama Department of Public Health (ADPH) has closed numerous shellfish resources to the taking of shellfish due to elevated bacteriological levels. These areas include complete closure of the upper Mobile Bay north and east of the E. Fowl River from the mid-channel marker to Daphne (including Fairhope beach and the Grand Hotel beach; ADPH Area IV), and conditional closure south and west of Daphne to the mid-channel marker west of Mullet Point. Other closed or prohibited waters include Perdido Bay, Oyster Bay, the Bon Secour River and Little Lagoon in the east, as well as the Mississippi Sound west of Bayou la Batre to the Alabama state line (ADPH Area II). In addition, numerous small shellfish beds (*e.g.*, Weeks Bay) remain closed due to unacceptable levels of fecal coliform bacteria. Often, closure is a result of either overloaded point sources (sewage treatment plant) during a storm event, or non-point source pollution due to septic failure, illicit storm drain connections, etc. Besides the obvious health-related impact due to potential disease vectors, concomitant increased nutrient loading promotes eutrophication and diminished water quality. Further, closure of shellfishing areas negatively impacts a viable and valuable economic resource.

One of the main problems associated in determining the cause (human/nonhuman) and prevention of fecal coliform bacteria contamination is the inability to precisely determine the main source of contamination. The use of fecal coliform bacteria as an indicator species of pollution as the *sole* basis for water quality assessment should be limited, because in many environments high levels of naturally occurring fecal coliform (for example, from waterfowl) may be present. A newer technique utilized to help identify the source of pollution attributed to human vs. animal activity is spectrofluorometry.

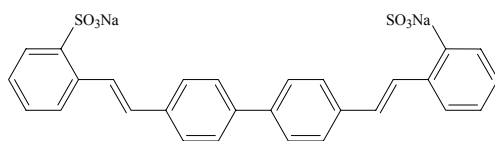
Detergents are used in very large quantities, and contribute a significant portion of the load of anthropogenic chemicals to the aquatic environment. Fluorescent whitening agents (FWAs; also referred to as “brightening agents”) contribute only 0.15% of the total mass of most laundry detergents and may be discharged to a municipal facility, a septic system (Individual Sewage Disposal System or ISDS), or to a stormwater drainage system (through an illegal connection). These moderately water soluble, fluorescent organic compounds have a high affinity for cellulosic material. When bound to fabrics, the intense blue fluorescence of FWAs compensates for the slight yellowish cast of cotton; thus bound FWAs improve or restore whiteness during washing. Although these FWAs are partially bound to fabrics during the washing process, a considerable fraction (5-80%) remains in the washing liquor and is discharged. In general, FWAs do not readily break down in the environment, remaining either in solution or slowly adsorbing onto sediment particles. Since all FWAs absorb UV light at approximately 350nm, with a molar extinction coefficient of over 50,000 M<sup>-1</sup>cm<sup>-1</sup>, and emit visible blue light at a maximum of 430nm (with high fluorescence), they may readily be detected with a high-resolution spectrofluorometer at a high sensitivity (<10 parts per trillion). Since FWAs are light-sensitive chemicals, a reversible *E-Z* photoisomerization of the stilbene moiety is possible and individual FWA classes may potentially be further elucidated using nuclear magnetic resonance spectroscopy (NMR). The recovery and identification of FWA's in the Mobile Bay watershed is selectively indicative of human anthropogenic activity.

**Objective(s) of the Research Project:** The objectives of this research project were to: (a) investigate the use of fluorescent whitening agents (FWAs) as anthropogenic markers of municipal, 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 FWAs; (c) investigate new selective field sampling techniques; (d) attempt to identify the specific classes of FWAs using high-field  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy.

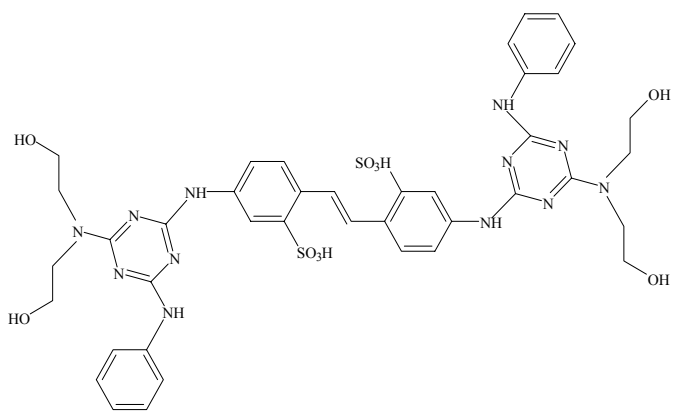
### Summary of Findings

The major accomplishments in this project have been to: (1) identify the best available solid-phase extraction (SPE) technologies for successful isolation and quantitation of fluorescent whitening agents (FWAs); (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 FWAs prior to laboratory quantitation. The use of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy for identification of specific FWAs (and their respective photo-initiated *E/Z* isomeric ratios) both in authentic and field sample at low analyte concentrations has been very limitedly successful (*vide supra*).

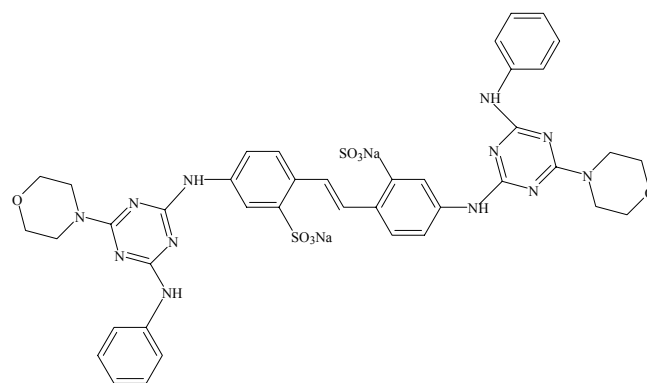
The most important classes of FWAs are sold under the tradenames DAS, DSBP, and BLS (diaminostilbene, distrylbiphenyl, and bleachable, respectively). The DAS and DSBP classes are found in commercial domestic detergents; the BLS class is common to large-scale laundry facilities (*e.g.*, hospitals). FWAs of the distrylbiphenyl type (tradename DSBP-1) and of the diaminostilbene type (tradenames DAS-2, DAS-3, DAS-4) were investigated in this study (Figure 1) and are considered the most common FWA's found in detergent products worldwide.



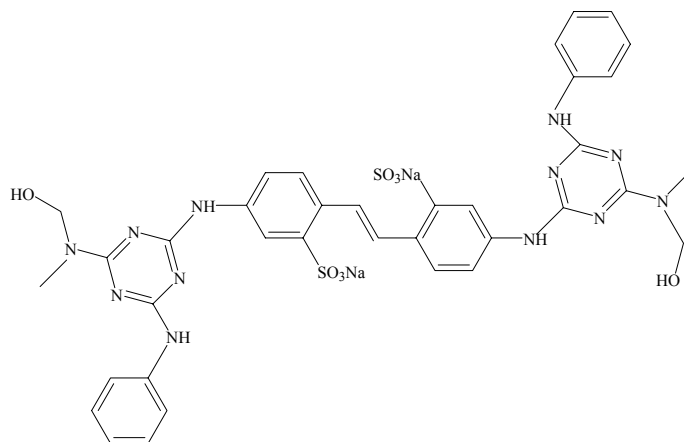
DSBP



DAS-2



DAS-3



DAS-4

Figure 1. Structures of FWA's Included in This Study

#### A. 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>. A comparison of the performance of five commercially available SPE cartridge brands using a synthetic mixture of FWAs prepared at three dilutions (1ppb; 25 ppb; 100ppb) are shown in Table 1.

## B. Optimization of the Ion-Pairing Extraction Protocol

To minimize the possibility of fluorescent interferences, all controls, blanks, samples, and dilutions were made using "spectra-grade" reagents. Ultrapure water was prepared at  $>18\text{M}\Omega$  purity.

Of a series of ion-pairing reagents examined, the most selective and efficient ion-pairing reagent was found to be 0.05 M tetra-n-butyl ammonium hydrogen sulfate (TBA- $\text{HSO}_4$ ) in methanol (MeOH). All final dilutions were made in methanol (MeOH).

## C. Extraction Protocol (for both Standards and Field samples)

1. 2 ml 0.05M TBA- $\text{HSO}_4$  in methanol added to SPE cartridge for pre-rinse/conditioning
2. Rinse w/ 10 ml MeOH.
3. Rinse w/ 10ml ultrapure  $\text{H}_2\text{O}$
4. Add 100 ml mixed standards or sample
5. Rinse w/ 10ml ultrapure  $\text{H}_2\text{O}$
6. Vacuum dry for approx. 5 minutes
7. Elute FWA's w/ 4 ml 0.05M TBA- $\text{HSO}_4$  in methanol into 10 ml volumetric flask
8. Repeat Step #7 elution into same 10 ml volumetric flask
9. Dilute volumetric flask to final volume with MeOH.
10. Analyze extracts using high-resolution fluorescence spectroscopy (*vide infra*).

Note: SPE cartridge flow rates should average 1 drop/sec.  
SPE cartridges should not be allowed to go dry between steps #1- #6.

## D. 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 ( $\lambda_{\text{ex}} = 350\text{nm}$ ;  $\lambda_{\text{em}} = 397\text{nm}$ ). A standard curve at four dilutions, comprising an equal mixture of the four commercially-available FWA's in MeOH, was constructed for quantitation purposes. The FWA's found ranged from 6 ppb - 368 ppb. The locations of the environmental samples and respective concentrations calculated are shown in Table 2. It was found that FWA's are relatively ubiquitous in the Mobile Bay watershed, with apparent differences at differing locations. This study has successfully shown that FWA's may be isolated and quantitated from environmental samples, although the source of entry into the watershed is presently unclear.

## E. $^1\text{H}$ and $^{13}\text{C}$ Nuclear Magnetic Resonance (NMR) Spectroscopy

Individual fluorescent whitening agents (FWAs) and FWA mixtures were examined using both  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopy. Pure standards at concentrations above 10 mg/mL in afforded good  $^1\text{H}$  NMR spectra in a reasonable amount of time. The  $^{13}\text{C}$  NMR spectra required overnight runs to give adequate S/N for peak identification. At concentrations below approximately 1 mg/mL ( $<1000$  ppb), S/N was poor in the  $^1\text{H}$  NMR spectra, requiring overnight acquisitions; the  $^{13}\text{C}$  NMR signals could not be accurately identified due to unacceptable S/N ratios. Preliminary  $^1\text{H}$  NMR experiments at lower analyte concentrations ( $<0.5$  mg/mL;  $<500$  ppb) were limitedly feasible using an inverse-detection probe and long acquisition times; indirect-detection experiments for  $^{13}\text{C}$  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 conducted using a large-scale sampling protocol. In addition, derivatization of isolated analytes and subsequent capillary gas-chromatography/ mass spectroscopy may provide insight into potential point-source discharges. Further investigation into the spectral resolution of E/Z FWA isomers via  $^1\text{H}$  and indirect-detected  $^{13}\text{C}$  NMR spectroscopy will be attempted at higher field ( $> 500$  MHz; S/N 2-3 orders of magnitude higher). 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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## ATTACHMENTS

**Table 1**

### **Comparison of Commercial SPE Cartridges for FWA analysis**

<b><u>Cartridge brand</u></b>	<b><u>FWA conc.</u></b> <b><u>(ppb)</u></b>	<b><u>Emission intensity</u></b> <b><u>(397nm)</u></b>	<b><u>Excitation intensity</u></b> <b><u>(350nm)</u></b>
All-Tech C-18	1	6883	5936
	25	19998	19910
	100	80058	75654
All-Tech Prevail <sup>®</sup>	1	4361	3124
	25	32522	10860
	100	110600	83472
Chromabond Easy <sup>®</sup>	1	12053	11085
	25	22812	14074
	100	112564	83526
Chromabond Xtri <sup>®</sup>	1	<1000	<1000
	25	4273	4196
	100	105075	82744
Strata-X	1	2954	2654
	25	20885	17920
	100	65156	63286

**Table 2****Mobile Bay Environmental Samples Collected and Analyzed**

<b><u>Sample #</u></b>	<b><u>Latitude</u></b>	<b><u>Longitude</u></b>	<b><u>Approx. location*</u></b>	<b><u>Tide stage</u></b>	<b><u>Em. intensity</u></b>	<b><u>FWA (ppb)</u></b>
1	30°32.00	088°07.40	Under AL.193- S.side TIC	Neap	112129	362
2	30°32.04	088°07.40	Seawall/AL 193- N. side TIS	Neap	25109	27
3	30°31.08	088°06.44	Marsh bridge- lower AL 163	Neap	113617	368
4	30°31.09	088°06.43	Stream east of AL 163 bridge	Neap	92149	285
5	30°31.92	088°06.50	Lower AL 163- N. side TSC	Neap	19709	6
6	30°33.88	088°05.30	Dog Rvr. Bridge- Marina fish. pier	Neap	82597	249
7	30°34.08	088°05.07	Mobile Y.Club - Dog Rvr. Fish pier	Neap	87873	269
8	30°34.09	088°05.14	Mobile Y.Club - wave breaker pillar	Neap	28872	42
9	30°24.90	087°49.56	Pier behind restaurant	Neap	81489	244

Location abbreviations: N = North  
S = South  
Rvr. = River  
TIS = Theodore Industrial Seaway  
TSC = Theodore Shipping Channel