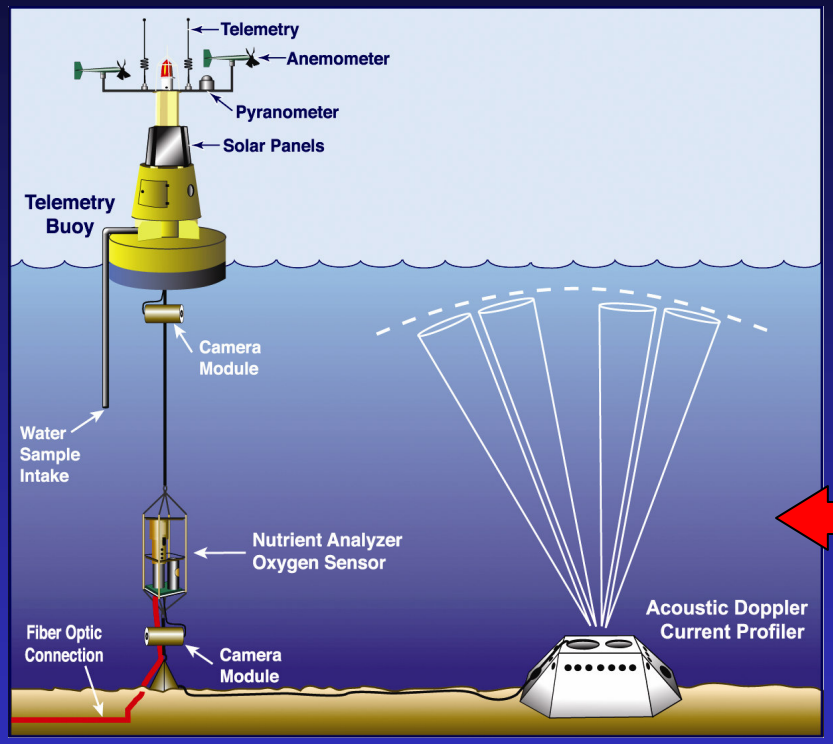
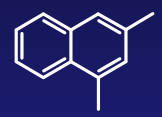
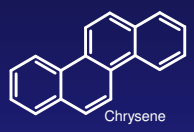
Antibody-based Chemical Sensor Development at VIMS



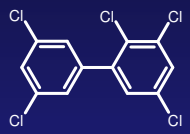
There is a need for "real-time" analysis of toxic chemicals in the environment



1,3-Dimethyl-naphthalene



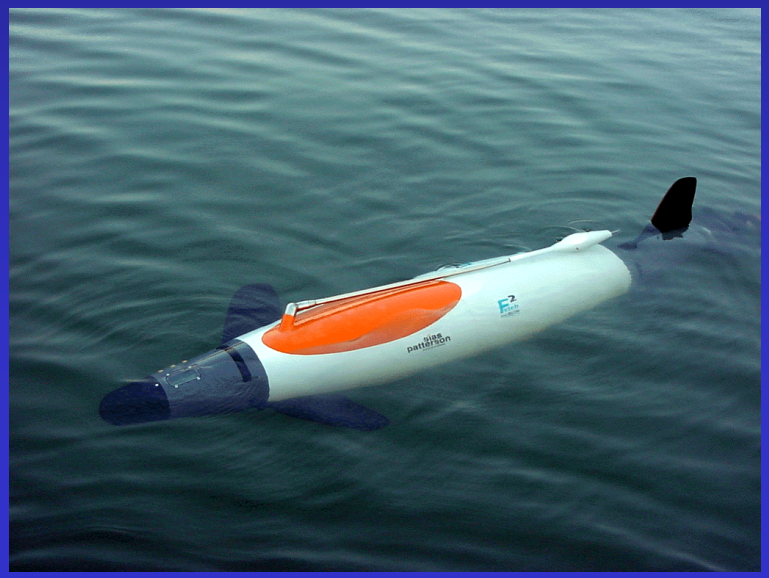
Chrysene



2,3,5,3',5'-Pentachloro-biphenyl (PCB)

Current technology focuses on physical conditions and inorganic chemicals

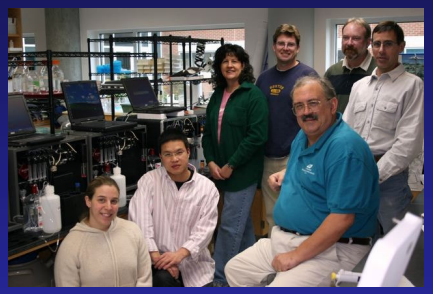
Two remote platforms



Potential uses:
Pollution Monitoring
Public Health
Homeland Defense
Industrial Processes/Outfalls

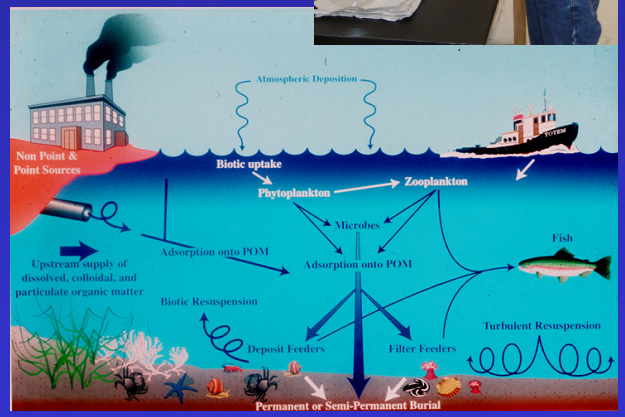
Interdisciplinary Effort of Immunologists & Environmental Chemists

The laboratories of Dr. Steve Kaattari and Dr. Mike Unger

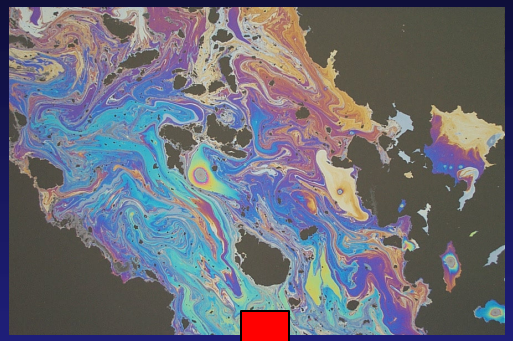


Team

Dr. Erin Bromage
Dr. Steve Kaattari
Dr. Mike Unger
Dr. Tom Harris
Ms. Candace Spier
Ms. Mary Ann Vogelbein
Mr. Jianmin Ye
Mr. George Vadas
Ms. Ellen Harvey
Sapidyne Instruments Inc



We need a method that is REMOTE, RAPID, and **RELIABLE**



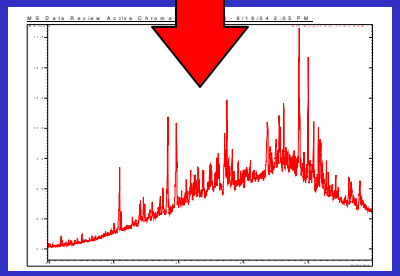
Environmental samples are extremely complex: 100,000's of compounds

The "Black Box"

VIMS Research Focus

Compound specific monoclonal antibodies coupled to electronic detection

Progress?



Electronic output of compatible data

Antibody-based Chemical Sensor Development at VIMS

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BIOSENSORS & BIOELECTRONICS
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The development of a real-time biosensor for the detection of trace levels of trinitrotoluene (TNT) in aquatic environments

E.S. Bromage [a], T. Lackie [b], M.A. Unger [a], J. Ye [a], S.L. Kaattari [a,*]

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[b] *Sapidyne Instruments Inc, Boise, ID, USA*

Received 17 May 2006; received in revised form 13 September 2006; accepted 3 October 2006
Available online 7 November 2006

Abstract

This paper describes the development of a highly sensitive TNT immunosensor consisting of a highly specific monoclonal antibody coupled with a prototype fluorescence-based detector system (KinExA Inline Biosensor, Sapidyne Instrument Inc). The antibody developed possesses a high affinity for TNT (association constant, a*K* 8.2) with minimal cross reactivity with other compounds such as tetryl, 2,4-dinitrotoluene and 2-amino-4,6-dinitrotoluene. This system provides sample assessment within 160 s from source acquisition and possesses sensitivity for TNT of 0.05 μg/L in ground water. The sensor can be regenerated in 8 min, allows a minimum of 40 repeated readings, and has a standard error of 0.1–0.4% between repeat readings. The fluidics and software allow samples to be obtained from up to eight different sources allowing the user to examine the stratification of the pollutant in the water column. We believe that this immunosensor can be used to rapidly assess trace levels of TNT in environmental water samples.

Keywords: Biosensor; TNT; Monoclonal antibody; Fluorescence

1. Introduction

The introduction of toxic pollutants into coastal waterways can have a dramatic impact on the health of aquatic ecosystems (Colborn and Liroff, 1990; Patty et al., 1999). Accidental releases of chemicals into aquatic systems, such as those occurring with oil spills are often highly visible and measurements can be taken to immediately monitor and control the spread of the spill. However, most chemicals will have a biological impact on aquatic species at concentrations to low to be visually observed (Anderson et al., 1997). Thus the ability to rapidly detect and quantify trace concentrations of pollutants in aquatic systems has long been the goal of resource managers to protect these sensitive systems.

The focus of this project was the development and assessment of a biosensor to detect the environmental pollutant trinitrotoluene (TNT) in aquatic systems. Environmental contamination with TNT has occurred particularly at military sites throughout the United States from the manufacturing, storage and handling of munitions. The reported concentration of TNT in soils at these sites can be as high as 3400 mg/kg. This contamination occurs through surface water runoff or ground water inflow, resulting in the accumulation of TNT in aquatic sediments (Richter-Torres et al., 1995). TNT contamination has been demonstrated to have detrimental effects on benthic invertebrates (Conder et al., 2004) and fish (Ek et al., 2005), and has been shown to accumulate in plants and fish (Belden et al., 2005; Ownby et al., 2005).

Although numerous techniques have been described for the assessment of TNT pollution (Rabbany et al., 2000; Green et al., 2002; Goldman et al., 2003a,b), the standard method employs high pressure liquid chromatography (HPLC) coupled with UV detection (EPA method 8330, www.epa.gov/SW-846/pdfs/8330.pdf). This technique allows the quantification of TNT and its breakdown products to low μgL^{-1} levels; however, while the technique is sensitive, it is also expensive, laborious and incapable of real or near-time assessments at low concentrations. More recently the use of immunochemical methods has gained widespread acceptance for measuring environmental pollutants (for review see Marquette and Blum, 2006), including TNT.

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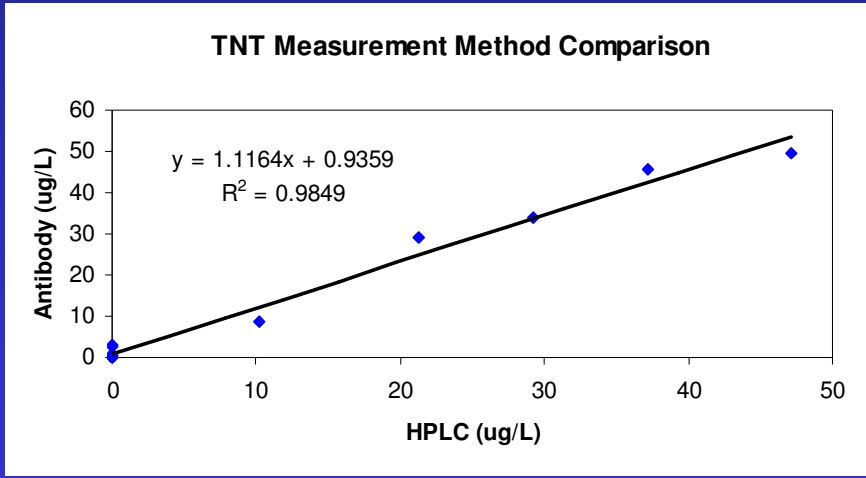
Biosensors and Bioelectronics

Published May 2007

Demonstration of the VIMS antibody/Sapidyne Inline as a viable technique to the sensor community



Antibody-based Chemical Sensor Development at VIMS

Validation of an Antibody-Based Biosensor for Rapid Quantification of 2,4,6-Trinitrotoluene (TNT) Contamination in Ground Water and River Water

ERIN S. BROMAGE, GEORGE G. VADAS, ELLEN HARVEY, MICHAEL A. UNGER, AND STEPHEN L. KAATTARI*

Department of Environmental and Aquatic Animal Health, Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, Virginia 23062

Nitroaromatics are common pollutants of soil and groundwater at military installations because of their manufacture, storage, and use at these sites. Long-term monitoring of these pollutants comprise a significant percentage of restoration costs. Further, remediation activities often have to be delayed, while the samples are processed via traditional chemical assessment protocols. Here we describe a rapid (<5 min), cost-effective, accurate method using a KinExA Inline Biosensor for monitoring of 2,4,6-trinitrotoluene (TNT) in field water samples. The biosensor, which is based on KinExA technology, accurately estimated the concentration of TNT in double-blind comparisons with similar accuracy to traditional high-performance liquid chromatography (HPLC). In the assessment of field samples, the biosensor accurately predicted the concentration of TNT over the range of 1—30 000 μg/L when compared to either HPLC or quantitative gas chromatography—mass spectrometry (GC-MS). Various pre-assessment techniques were explored to examine whether field samples could be assessed untreated, without the removal of particulates or the use of solvents. In most cases, the KinExA Inline Biosensor gave a uniform assessment of TNT concentration independent of pretreatment method. This indicates that this sensor possesses significant promise for rapid, on-site assessment of TNT pollution in environmental water samples.

Introduction

The manufacture and storage of munitions at military and civilian sites has led to considerable environmental contamination in the surrounding ecosystem (*1–3*). Often high levels of trinitrotoluenes (TNT), dinitrotoluenes (DNTs), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) can be found in soils around such sites (*4*). The disturbance of soils through redevelopment or rainfall can mobilize these contaminants into streams and groundwater. Thus, the contaminants can travel considerable distances away from the point source and have an impact on water quality and animal health at remote locations.

Military installations have placed considerable emphasis on the remediation and monitoring of contaminated sites. The Naval Weapons Station (WPNSTA) in Yorktown, Virginia, has been subject to considerable remediation efforts, performed by removal or treatment of soils containing nitroaromatics, nitramines, and other contaminants. The ongoing remediation efforts require continued monitoring of soil, sediment, surface water, and groundwater for the presence of these contaminants. In the most recent public health assessment by the Agency for Toxic Substances and Disease Registry (ATSDR), TNT was detected at concentrations up to 3400 mg/kg in soil samples, and water samples were as high as 19 μg/L (*5*). Such values are close to, or exceed, the minimum levels established for environmental health-based exposure (20 ppm). The standard method, EPA 8330, for analyzing environmental samples containing nitroaromatics is high-pressure liquid chromatography with ultraviolet detection (HPLC-UV), although other detection systems such as immunosensors have been employed (*6–10*).

Recently, we employed a fast, highly sensitive, automated system (KInExA Inline BioSensor (*11*), Sapidyne Instruments) to serve as a biosensor for the detection of trinitrotoluene in aquatic systems which demonstrated considerable potential for on-site measurement (*12*). The next phase of development was to ensure that the system would work with field-acquired samples and their associated problems such as matrix effects and particulates. This paper describes the development and validation of the KinExA Inline Biosensor for the monitoring of TNT in samples obtained from groundwater and surface water, previously known to be contaminated with munition constituents.

Methods

Sample Collection. Groundwater and surface water samples were collected from the Naval Weapons Station Yorktown (WPNSTA) on July 25, 2006. Sampling sites and wells were selected on the basis of previous monitoring results that showed elevated TNT concentrations (*5*). The sites chosen for sampling have been remediated following contamination during the loading of munitions. Site 9 encompassed the untreated wastewater drainage system from Ammo Loading Plant #1 from the 1930s to 1975 which included Lee Pond (*13*). Site 7 was an unlined drainage ditch for untreated wastewater discharge from Ammo Loading Plant #3 from 1945 through 1975 (*14*). Site 6 was a flume and unlined impoundment for untreated explosives reclamation and weapons loading wastewater from 1942 through 1975 (*14*). Groundwater samples were collected using a peristaltic pump after purging at least one volume of standing water from the well. Surface water samples were collected with a pole sampler containing a 4 L bottle. Water samples were placed into pre-cleaned 4 L amber bottles and stored on ice for transport to the laboratory.

HPLC Analysis. A 1.0 mL aliquot of each sample and a laboratory blank were added to an autosampler vial containing the internal standard 1,2-dinitrobenzene (Ultra Scientific, USA) and injected on the HPLC. Any remaining sample in the four liter bottle was acidified with hydrochloric acid (HCl) to a pH < 2 and refrigerated. Any samples that exceeded the range of the calibration curve were diluted with deionized water and reinjected on the HPLC.

For low-level analysis (<10 μg/mL), one liter of each acidified sample was liquid–liquid extracted with 25 mL of dichloromethane three times for 3 min in a pre-cleaned 2 L separatory funnel. A laboratory blank and a TNT fortified sample were analyzed with each batch of environmental samples. Combined extracts were drained into a pre-cleaned 60 mL tube and frozen overnight. Excess water was decanted,

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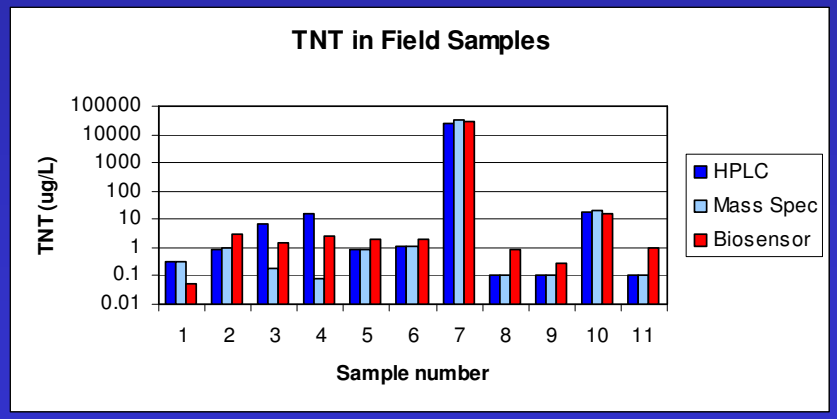
Environmental Science and Technology

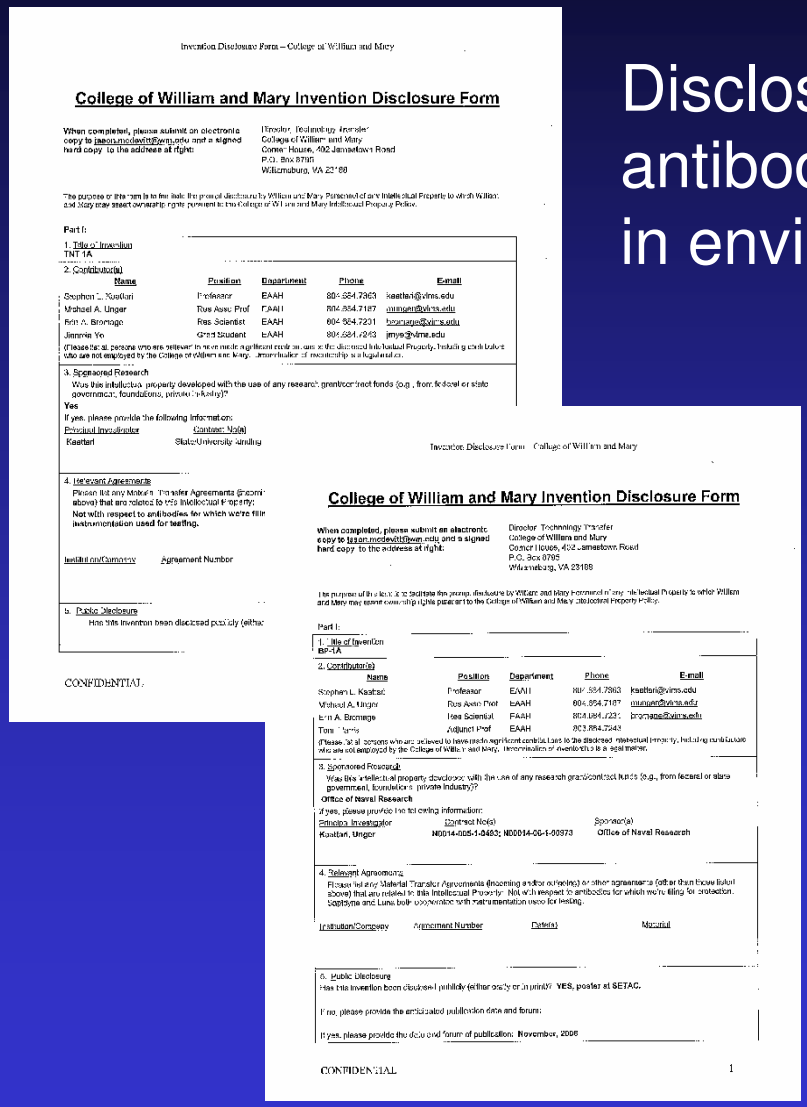
(in press for 2007)

Demonstrate the utility with field samples

Exposure for our biosensor development to the environmental monitoring community



Sensor Development & Validation: Basic and Applied Research



Invention Disclosure Form – College of William and Mary

College of William and Mary Invention Disclosure Form

When completed, please submit an electronic copy to jason.medewitt@wm.edu and a signed hard copy to the address at right:

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College of William and Mary
Corner House, 402 Jamestown Road
P.O. Box 8795
Williamsburg, VA 23188

The purpose of this form is to facilitate the prompt disclosure by William and Mary Personnel of any Intellectual Property to which William and Mary may assert ownership rights pursuant to the College of William and Mary Intellectual Property Policy.

Part I:

1. Title of Invention
TNT 1A

2. Contributor(s)

Name	Position	Department	Phone	E-mail
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Michael A. Unger	Res Assc Prof	EAAH	804.684.7187	munger@vims.edu
Erin A. Bromage	Res Scientist	EAAH	804.684.7231	bromage@vims.edu
Jinmrie Ye	Grad Student	EAAH	804.684.7243	jmye@vims.edu

(Please list all persons who are believed to have made significant contributions to the disclosed Intellectual Property, including individuals who are not employed by the College of William and Mary. Inventorship is a legal issue.)

3. Sponsored Research
Was this intellectual property developed with the use of any research grant/contract funds (e.g., from federal or state government, foundations, private industry)?
Yes
If yes, please provide the following information:

Principal Investigator	Contract No(s)	Sponsor(s)
Kaattari	State/University funding	

4. Relevant Agreements
Please list any Material Transfer Agreements (incoming and/or outgoing) or other agreements (other than those listed above) that are related to this Intellectual Property:
Not with respect to antibodies for which we're filing instrumentation used for testing.

Institution/Company	Agreement Number

5. Public Disclosure
Has this invention been disclosed publicly (either

CONFIDENTIAL

Invention Disclosure Form – College of William and Mary

College of William and Mary Invention Disclosure Form

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Name	Position	Department	Phone	E-mail
Stephen L. Kaattari	Professor	EAAH	804.684.7363	kaattari@vims.edu
Michael A. Unger	Res Assc Prof	EAAH	804.684.7187	munger@vims.edu
Erin A. Bromage	Res Scientist	EAAH	804.684.7231	bromage@vims.edu
Tom Harris	Adjunct Prof	EAAH	804.684.7243	

(Please list all persons who are believed to have made significant contributions to the disclosed Intellectual Property, including individuals who are not employed by the College of William and Mary. Inventorship is a legal issue.)

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Office of Naval Research
If yes, please provide the following information:

Principal Investigator	Contract No(s)	Sponsor(s)
Kaattari, Unger	N00014-05B-1-0483; N00014-06-1-00973	Office of Naval Research

4. Relevant Agreements
Please list any Material Transfer Agreements (incoming and/or outgoing) or other agreements (other than those listed above) that are related to this Intellectual Property: Not with respect to antibodies for which we're filing for protection. Sepsityne and Luna bulb: cooperation with instrumentation used for testing.

Institution/Company	Agreement Number	Date(s)	Material

5. Public Disclosure
Has this invention been disclosed publicly (either orally or in print)? **YES, poster at SETAC.**

If no, please provide the anticipated publication date and forum:

If yes, please provide the date and forum of publication: **November, 2006**

CONFIDENTIAL 1

Disclosure Statements filed for antibodies to detect TNT and biphenyl in environmental samples

Next step will be disclosure and publication on an antibody to detect thiophenes (oil)

Antibody-based Chemical Sensor Development at VIMS

The Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET)
FY 2007 Full Proposal Application
Environmental Technology Development Program

Project Title: Validation of a Real-Time, Field Deployable Biosensor for the Detection and Quantification of Polycyclic Aromatic Hydrocarbon (PAHs) in Aquatic Systems

Project Duration: 1st June 2007 31st May 2009

FY 2007 CICEET Funds Requested: Year 1:$199,196 Year 2:$197,858 Total. $397,054

Principal Investigator(s):
Name: Dr. Eric S. Bromage

Position and Institutional Affiliation: Research Assistant Scientist, Virginia Institute of Marine Science College of William and Mary

Address: PO Box 1346 Gloucester Point, VA 23062

Phone:804 684 7231 Fax:804 684 7186 e-mail: Bromage@vims.edu

Additional Investigator(s):
Dr. Stephen J. Kaattari, Virginia Institute of Marine Science (VIMS), kaattari@vims.edu
Dr. Mike A. Unger (VIMS), munger@vims.edu

NERR site(s): Goodwin Island

Lead Principal Investigator's Signature: _____
Date: 12/13/2006_____

Please Note: The Contents of this proposal are CONFIDENTIAL!

All information contained herein is considered confidential, this information shall not be duplicated, used, or disclosed in whole or in part for any purpose other than the evaluation of this application.

Next Steps:

NOAA CICEET funded research
Sept 2007-2009
Bromage, U Mass
Kaattari & Unger, VIMS

CICEET

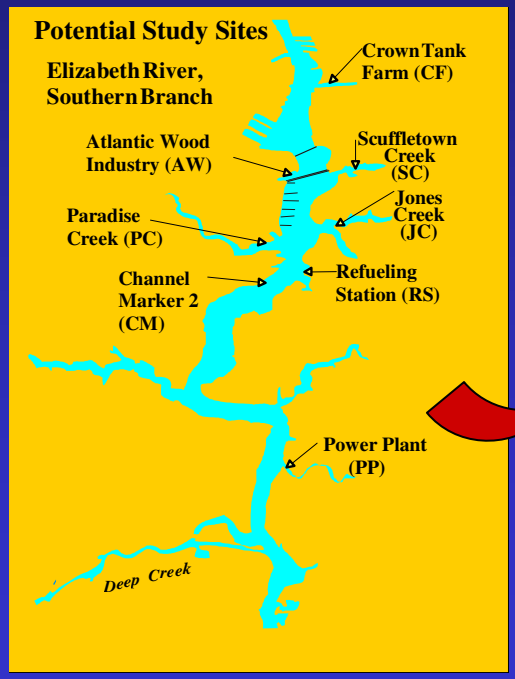
Objectives

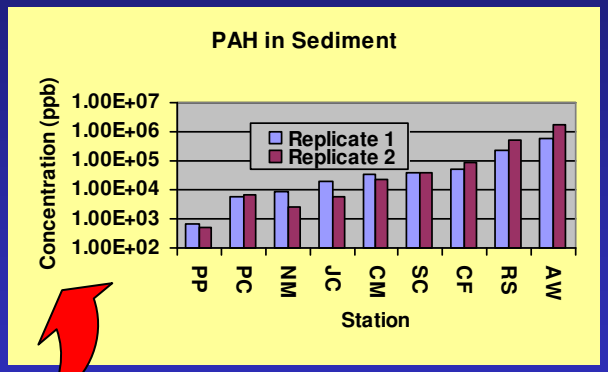
The over all goal of this proposal is to validate a new field deployable sensor technology for differentiating and quantifying specific petrogenic PAH's from pyrogenic PAHs in aquatic systems. This will provide water quality managers, as well as environmental monitoring and compliance officer's access to real-time data, allowing knowledge-based decisions.

Sensor Development & Validation: Basic and Applied Research

Develop New Antibodies

Using real time chemical analyses
to answer difficult questions for science
and environmental management





Contaminant Sources
Oil, Creosote
Groundwater
Stormwater Runoff