Application of an antibody-based biosensor for rapid assessment of PAH fate and toxicity at contaminated sediment sites

SRP Progress in Research
Biogeochemical Interactions Affecting Bioavailability for in situ Remediation
May 13, 1-3 pm EDT

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Application of an antibody-based biosensor for rapid assessment of PAH fate and toxicity at contaminated sediment sites

• PAH and their importance as environmental contaminants
  • Sources & concerns

• PAH biosensor, what is it and how do we make it?

• Biosensor applications to PAH fate and transport
  • Elizabeth River, VA: Evaluating PAH transport
  • Oil spill detection: ExxonMobil and Ohmsett

• Biosensor applications to PAH bioavailability/toxicity
  • Factors affecting bioavailability in sediments
  • Baltimore Harbor, MD: Toxicity of contaminated sediments

• Current and future work
  • Kristen Prossner’s SRP Research-Bioaccumulation in oysters
  • Krisa Camargo SRP TAMU Research- Soil screening
  • Continued Technology Development-Sapidyne and VIMS
  • Fate and Toxicity Assessment
Polycyclic Aromatic Hydrocarbons (PAH)
Potentially toxic and carcinogenic
Common target of Superfund cleanup (historical/legacy contaminants)
Oysters are potential vector for human exposure
Sources include: combustion products, creosote, oil

Superfund driven by reducing Human Risk

Limited water solubility
“hydrophobic” very low concentrations in water

Under “equilibrium” conditions
High affinity for lipid material “Lipophilic”
organic carbon rich sediments and biota (bivalves) are a “sink” or reservoir

NIEHS-SRP Research Focus
Can we predict how PAH fate will affect bioaccumulation from contaminated sediments?
**Available Analytical Methods for Organics** can be **Slow and Expensive**

**How slow?**

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

Multiple steps to separate, isolate and concentrate the target molecules-
Instrument and time intensive
Days- Weeks up to $1000/sample (data point)

- **FTS Dura Dry Bulk Freeze Dryer**
  - hours or until dry, aliquots removed for % solids, grain size, and organic carbon
  - 2 days

- **Spike with surrogate standards**
  - PCB 30, PCB 65, PCB 204, 1,1’binaphthyl, BDE-77, perinaphthenone, d-10 acenaphthene, d-12 chrysene, d-8 naphthalene, d-12 perylene, d-10 phenanthrene, and 1,4-dichlorobenzene
  - 1-2 days

- **Dionex ASE 300 extracted**
  - 100% methylene chloride at 100°C and 1500psi
  - 1 days

- **Copper Column to remove sulfur**
  - 1 day

- **HPLC-SEC**
  - Waters HPLC with a Phenomenex Envirosep
  - ABC GPC column in methylene chloride
  - 1 day

- **Silica gel to remove polar compounds**
  - 1 day

- **Spike with Internal Standards**
  - pentachlorobenzene, p-terphenyl, decachlorodiphenyl ether(DCDE), & BDE-166
  - 1 day

- **Evaluate QA/QC**
  - Varian Saturn GCMS-SIMS
  - 1-2 days
Near real-time PAH analysis: VIMS Biosensor

Our Approach

Bio
Monoclonal Antibodies against Contaminants

Sensor
Electronic detection of mAb Binding

Boise, Idaho
How to make new antibodies to PAH and other small targets?

↑ not immunogenic

immunogenic →
How to make antibodies to pollutants?

1. Immunize
2. Monitor sera for titer

Pollutant → Hybridoma-antibody producing cells

Screening of Hybidomas an important step
Several month process from immunization to mAb
(Li et al 2016, Immunoassay and Immunochemistry)

Provides an endless supply of antibodies in cell culture
Goal: Quantification of mAb binding

Inline Sensor (Biosensor) features:

1. Automated sample handling
2. Precise fluidics for analyzing small quantities accurately
3. Fluorescence emission/detection for heightened sensitivity

Boise, Idaho
Sample with NO PAH
Sample with PAH

samples

Fluorescent source ➔
high signal

Flow cell ➔

reagents

← Beads
antigen

← AF647 labeled
mAb
Sample with NO PAH

Sample with PAH

Fluorescent source → low signal

Flow cell →

Sample with NO PAH = high signal

Sample with high PAH = low signal

Beads

Antigen

AF647 labeled mAb

NIH

VIMS

Virginia Institute of Marine Science
**VIMS Antibody Biosensor**: new technology for contaminant analysis allows quantification at low concentrations at new spatial and temporal scales

**Good correlation to GC-MS**

**SMALL** volume samples (1-5 ml)

**FAST** analysis (8 m) near real-time

**LOW** concentrations (0.1 ppb total PAH)

**Environmental Fate Studies**: spatial and temporal resolution to identify sources and transport mechanisms

**Toxicity Evaluation**: spatial and temporal resolution to understand what is driving bioavailability and toxicity

**2G8 Affinity for a wide range of PAH (3-5 ring)**

Study Site Money Point: Contaminated with PAH and DNAPL from Historical Creosote Facilities in the Southern Branch of the Elizabeth River, VA

Methods are needed to better understand and predict PAH transport at sediment remediation sites to assure long-term success.
Methods: Porewater sampling surface sediments

- Real-time analysis can be used to map [PAH] in water/sediment porewater in the field

- Dissolved phase (0.47 μm) porewater samples are collected and analyzed on board and up to 30 stations can be surveyed in 1 day

- Small volume samples analyzed on board by biosensor and larger volume samples can be brought back to the lab for GC-MS

- Good correlation between biosensor & GC-MS in complex environmental samples
Results: Money Point, Phase 2
Mapping water/porewater in a day

Surface water  <1μg/L-3μg/L
Porewater  50μg/L – 450 μg/L
Phase 2 remediation area

<table>
<thead>
<tr>
<th>Id</th>
<th>Conc(μg/L)</th>
<th>Station</th>
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<tbody>
<tr>
<td>1</td>
<td>0.08</td>
<td>MP-5 Bot</td>
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<tr>
<td>2</td>
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<td>11</td>
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<td>MP-5 PW</td>
</tr>
<tr>
<td>27</td>
<td>50</td>
<td>MP-4 PW</td>
</tr>
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</table>

Mapping of site porewater and surface water and bottom water in one day
27 samples

Surface water <1μg/L-3μg/L
Porewater  50μg/L – 450 μg/L
Phase 2 remediation area
PAH Transport within sediment: Methods

In-situ porewater measurements

Salinity by refractometer

Total PAH by biosensor
Saline surface water is mixing with more contaminated fresh pore water at depth in the sediment.
PAH Flux Transport to the water column: Seepage meter/Biosensor data

Porewater sampling stations-Money Point

MPF-6
MPF-5
MPF-4
MPF-3
MPF-2
MPF-1

Dredged and capped

Seepage Meters
Direct hourly flow measurements
PAH concentrations by biosensor
Short-term concentration/flux measurement

MPF-2 Porewater Flow

<table>
<thead>
<tr>
<th>Avg Water Height (m)</th>
<th>Ebb Flow Rate</th>
<th>Flood Flow Rate</th>
<th>PAH Flux (μg/m²/hour)</th>
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<tbody>
<tr>
<td>0</td>
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<tr>
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</tr>
<tr>
<td>0.6</td>
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<td>0.8</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.2</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

Highest flux at remediated sites with coarse sediment cap and low total PAH

CTD data logger provides evidence of tidal driven advection

Data from the Biosensor is now helping to guide future remediation plans to limit flux to the water column. Revisit problem sites and engineered caps in new areas.
Can the Biosensor help to better understand the fate and effects of oil?

Dissolution is important for the exposure and bioavailability to aquatic organisms.

While PAH are a minor component in the total hydrocarbons in oil they represent a major fraction of the dissolved potentially toxic compounds.
Collaboration to evaluate PAH plume identification during an oil spill

**Lab Study:** Water soluble fractions from three different oils at two oil loadings - Model prediction vs. Biosensor measurements

**Field Trial:** October 2017 Ohmsett Leonardo, NJ. Simulated spills PAH fate and transport by Biosensor real time
Biosensor analysis of PAH has helped elucidate the mechanisms controlling the fate and transport of PAH in water and sediments.
**Paracelsus, Father of Toxicology** (1493-1541)

"All substances are poisons; there is none which is not a poison. The right dose differentiates a poison...."

- The dose makes the poison!!

Simple concept but what is the **DOSE** in contaminated sediment???

2015 paper, 2017 SETAC Europe: New methods are being proposed to consider more accurate measurements addressing bioavailability in management decisions

Ortega-Calvo et al, ES&T 2015, 49, 10255-10264
What is the Bioavailable fraction in sediments?

Even Chemists don’t get it all!

Biological response: true measure of bioavailability

Typical organic analysis

Mild extraction

PSD, etc.

Biosensor

Ortega-Calvo et al, ES&T 2015, 49, 10255-10264

Decreasing Concentrations

Can we use new antibody based measurement methods to directly analyze the bioavailable/toxic component in porewater?
Porewater Toxicity Evaluation via Biosensor
VIMS/University of Maryland Research Collaboration: Sharon Hartzell, Lance Yonkos

Test species – Estuarine *Leptocheirus plumulosus*

Acute 10-d test - Whole sediment collected from field

PAH concentrations in porewater measured by VIMS Biosensor

PAHs in porewater and sediment were strongly correlated with toxicity.

So were: Nickel, Chromium, TPH
PAH spiked control sediments: 18 compounds from site adjusted for relative composition and total PAH.
Results-Spiked Control sediment from Baltimore Harbor

PAH concentrations in whole sediments aren’t very good predictors of toxicity

Biosensor measurement of PAH porewater concentrations predicts toxicity

Porewater analysis by Biosensor can be used to rapidly identify toxicity in field sediments

PAH & Metals

New Research: Kristen Prossner SRP Trainee at VIMS

WHY?—Current state of the science for seafood PAH contamination
Public distrust from inaccurate or slow response during spills or floods

After Deepwater Horizon:

Rapid Sniff Testing

AND

Slow GC-MS Tissue Analysis

From policy standpoint: Fast, quantitative analysis allows quicker turnaround time to get data on seafood status back to stakeholders, build trust

From science standpoint: Allows analysis of PAH dynamics within individual oysters on temporal scales not possible with GC-MS
Shift the scales of equilibrium partitioning

\[ K_p \text{ predicts distribution of PAHs in the environment} \]

\[ K_p = \frac{[\text{PSD}]}{[\text{Aqueous phase}]} \]

Does it predict distribution of PAHs in a bivalve?

\[ K_{\text{PAH}_{\text{oyster}}} = \frac{[\text{lipid tissue}]}{[\text{oyster aq. phase}]} \]

\[ K_p = \frac{[\text{Sediment}]}{[\text{Aqueous phase}]} \]
Methods

Collect mantle fluid-
Aqueous phase

Field oysters from contaminated sites in Elizabeth River
-28-day lab exposure oysters

0.45µm PTFE syringe filter

Biosensor (Li et al. 2016)

Freeze-dry homogenate

ASE extraction

Gel permeation chromatography

Silica gel column chromatography

~1g-7g dry wt.

GC-MS

6 individuals per homogenate
Results—Biosensor vs. GC-MS

\[
y = 955 \\
R^2 = 0.87
\]

\[
[\text{whole tissue}] = [\text{oyster mantle fluid}] \times K_{\text{tiss-mf}}
\]
RESULTS—Variability among individual oysters

n=6 individual oysters per site/exposure treatment

Sensitivity of biosensor for small volume samples allows for total 3-5 ring PAH concentration measurements at an INDIVIDUAL level—GC-MS analysis usually requires composite samples

Better understanding of individual variability

Elizabeth River field oysters

Lab Exposure oysters
New Research: Collaboration with TAMU SRP Center
Tony Knap and Krisa Camargo (SRP trainee and KC Donnelly Fellow)

Working on a Biosensor based method for rapid screening of PAH in soil and sediments

• Use Biosensor data to guide future sampling in the field for compound specific analysis by GC-MS to delineate sources

• Map potential PAH gradients during flood events

• Scheduling for summer/fall 2019 to map PAH in near real time in Houston to guide future areas of focus

• Lessons learned in Houston area have potential to advise flood prone areas like Chesapeake Bay

Source: City of Houston GIS Open Data, Texas Natural Resources Information System Study Area 25)
Summary Biosensor Technology

- Total PAH concentrations (3-5 ring) in minutes from small volume samples allows spatial and temporal measurements not possible by conventional methods: good correlation to GC-MS analysis in split samples.

- Mapping of concentration gradients in the water column and within sediments is possible to identify contaminant sources, transport and flux. It can provide a measure of the toxic or bioavailable fraction.

- Similar initial instrumentation costs but a few dollars/analysis vs. 100s dollars for GC-MS, data in minutes, green technology: no solvents.

- Prioritize samples for compound specific GC-MS based on total PAH measurements by biosensor (don’t pay for non-detects!)
Summary Sediment Remediation Needs

- Bioavailability is governed by contaminant partitioning and transport-whole sediment measurements alone are not good for assessing remediation effectiveness for reducing exposure to biota/humans.
  - Reducing contaminant bioavailability and flux to the water column should be the metrics for success- We are now advising environmental managers on the need for redefining regulatory goals to reflect bioavailability
  - Future remediation strategies should consider ways to mitigate porewater transport. (i.e. barriers, sorptive amendments, etc.)
  - Can we convince regulators that remediation may involve leaving contaminated sediments in place? Change the partitioning and you change the bioavailability/toxicity. Funds will potentially go farther to improve greater areas of the watershed
Current and Future Biosensor Work

- Biosensor hardware development, smaller, more portable - Sapidyne Instruments & commercialization of current mAbs
  - Portable, battery powered easy to operate

- Detection of oil spills and sediment toxicity
  - ExxonMobil-water soluble PAH, porewater, SPME & toxicity

- New antibodies for other new hydrocarbons, PFAS, HAB toxins or ???

[Graph showing VIMS Biosensor vs EMBSI SPME]
Acknowledgements

NIEHS-SRP Grant #R01ES024245
Impact of groundwater-surface water dynamics on in situ remediation
efficacy and bioavailability of NAPL contaminants
PIs: Michael Unger, Aaron Beck, Collaborator/RTC: Josef Rieger, The Elizabeth River Project, Portsmouth, VA

Steve Kaattari, Mary Ann Vogelbein, George Vadas, Kristen Prossner, Aaron Beck, Michele Cochran, Xin Li, Ellen Harvey, Matt Mainor

Joe Rieger, Dave Koubsky

Paracelsus

Sharon Hartzel, Lance Yonkos, Yonkos lab members: Wenqi Hou, Amy Wherry and Shannon Edmonds

Terrance Lackey

Dave Marsell

Chris Prosser, Tom Parkerton
Questions?

Relevant PAH Biosensor Publications


Hartzell, S. E., M. A. Unger, B. L. McGee and L. T. Yonkos. 2017. Effects-based spatial assessment of contaminated estuarine sediments from Bear Creek, Baltimore Harbor, MD, USA. *Environmental Science and Pollution Research*. http://dx.doi.org/10.1007/s11356-017-9667-0
