Development of Methodologies, Instrumentation, and Assay Kits for Screening of Toxic Organic Pollutants in Environmental Sources

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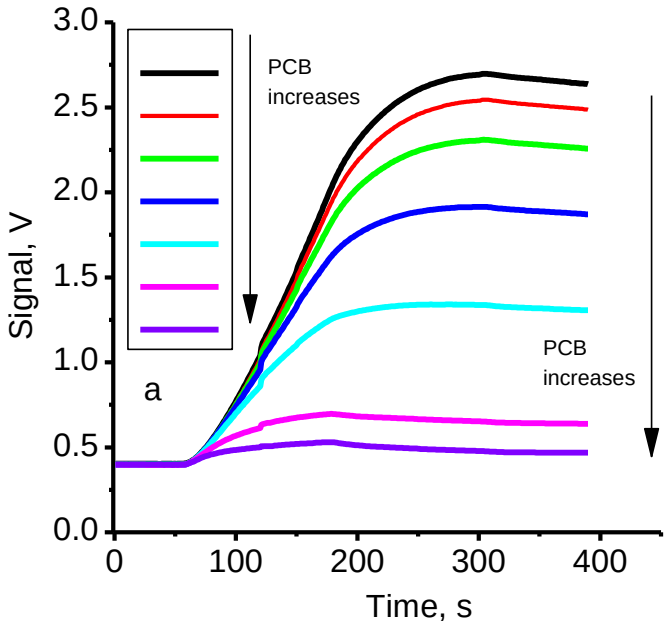
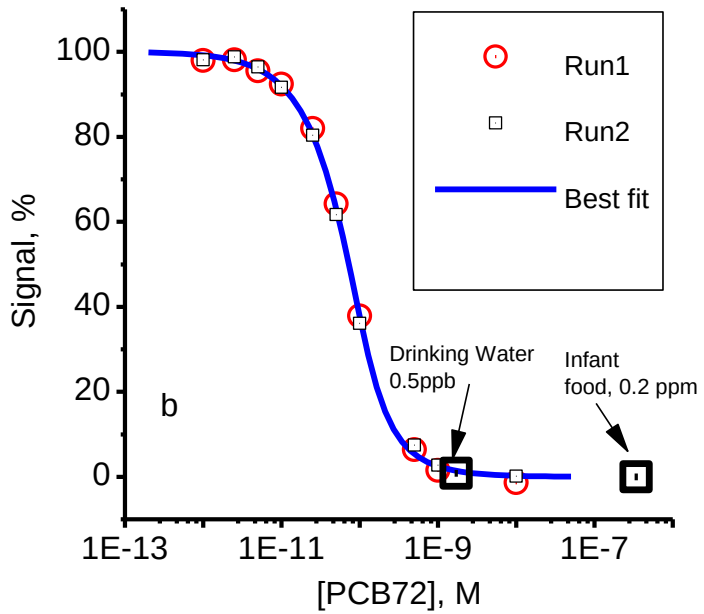
- Grant #: IGEM25-001
- Reporting Period: 7/1/24 –12/31/24

Commercial partner: Sapidyne Instruments, Boise, ID

Summary of progress towards proposed milestones

Milestone 1: **Develop aptamer-based methodologies and assay kits for detection and measurements of Polychlorinated Biphenyls (PCBs) in water-based solutions**	• A performance aptamer for PCB 72 was **selected and produced** in the PI's lab by employing a novel method of selection •The aptamer **was tested for binding to the target in spiked solutions of PCB72 by using the KinExA technology** ; the measured affinity of the aptamer (~23 pM) **surpasses by up to two orders other PCB72 aptamers reported in literature**; this high sensitivity **enables quantitation of PCB72 in environmental samples at levels 100-1000 times less than MCLs**; • Minor Roadblocks: The EHS at Boise State notified EPA (form 7710-53) in November to inform on testing the specificity of aptamers by employing mixtures (Aroclors, and Congeners). This is only a notification that PCB waste will be generated on campus, but the acknowledgment is still pending. **The Standard Operational Procedure was approved by the EHS at BSU.**





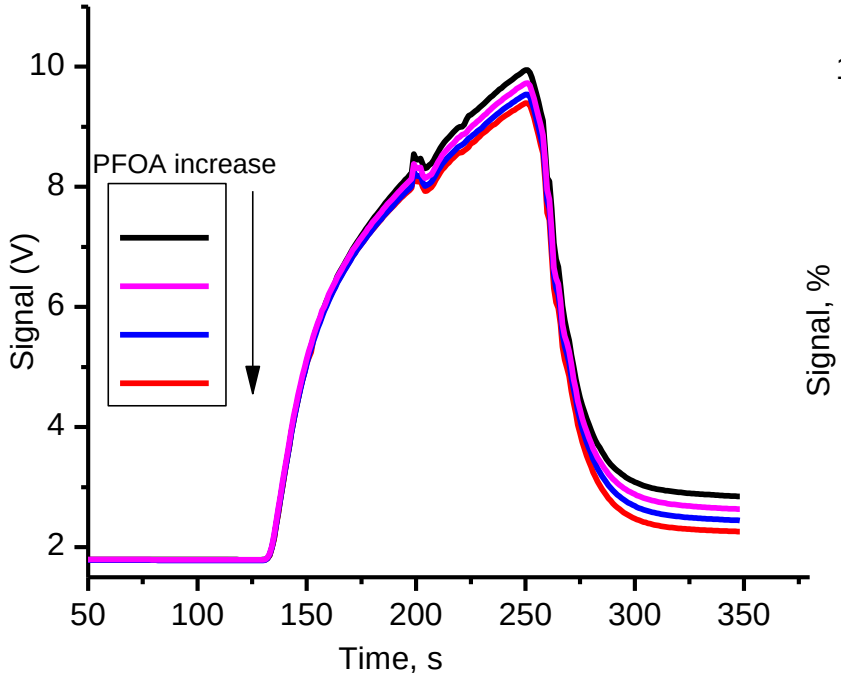
Regulations Maximum Contaminant Levels (MCLs)

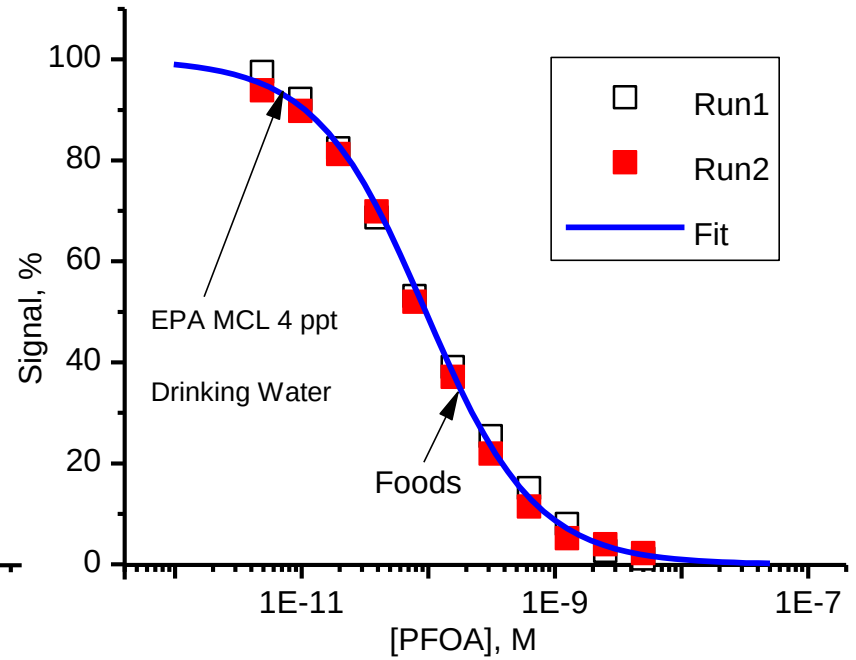
EPA
-drinking water, 0.5 ppb
FDA
-infant/junior food, 0.2 ppm
-milk/dairy, 1.5 ppm
-fish, 2 ppm
-poultry/read meat, 3 ppm

Our estimated sensitivity: < 1 ppt!

Summary of progress towards proposed milestones

Milestone 2: **Develop antibody-based methodologies and assay kits for detection and measurement of PFAS in water-based solutions.**	•We **produced PFOA (perfluorooctanoic acid) antibodies** (IgY) by chicken immunization with custom-made PFOA bioconjugates •The PFOA antibodies were separated from chicken eggs by specific procedures and **tested for PFOA binding with the KinExA technology** •Successful binding of the polyclonal antibodies was observed •We determined on equilibrium dissociation constant K_D = 58.9 pM; this may **enable detection of PFOA in water at concentrations down to ~6 pM; the MCL for PFOA in drinking water is 4 ppt (~10 pM)** •The antibodies are polyclonal! Separation of monoclonal antibodies, sequencing, and production of recombinant antibodies is needed. •Roadblocks. Minor: low egg production during the cold months; **Major: a key member of the team was on sick leave for 4 months…**





EPA MCL PFOA
-drinking water, 4 ppt
-the MCL goal is zero!

Other potential sources of contaminants: country/state dependent

-for foods, > 0.2 ppb (~200 pM)

Summary of progress towards proposed milestones

Milestone 3: Potential economic impact	•Boise State and Sapidyne Instruments fulfilled and exceeded the objectives pertaining to the potential economic impact through: **1. Workforce development** -new hires/internships -Boise State University: **seven** undergraduate students, **one** graduate student, **four** high school students; four volunteers received training -Sapidyne Instruments: **two** undergraduate students, **one** graduate student, **two** high school students; four research scientists from Sapidyne dedicated their efforts to this project and were fully supported by the commercial partner 2. A **grant pre-proposal** has been sent to Department of Defense - Strategic Environmental Research and Development Program for the topic "**Real-Time Sensors for Detection and Quantitation of PFAS in Soil and Groundwater at AFFF-Impacted Sites"**
Milestone 4: Dissemination	•The dissemination of the PCB results is initiated through two conference presentations: 1. Conference for Undergraduate Women Physics, America Physical Society, Jan. 24th-26th, Washington State University, Pullman, WA. 2. American Physical Society, Global Physics Summit, March 15th-21st, Anaheim, CA. Selected for press release.

Summary of expenditures and budget performance

- ***Key Insights***
- Spending is ON TRACK with proposal for the first six months.
- Major expenditures included workforce development, and materials and supplies.
- The expenses exclude ~1month of OE due to the billing cycles and quoted/not yet delivered supplies

- ***Challenges/Changes***
- Antibody selection may be a challenge due to the health issues encountered by a key team member; we may consider re-budgeting and sub-contracting for portions of that work (OE, and Recharge Center categories)
- Validation may be expedited by using an external, EPA-accredited provider of analytical services

	Budgeted	Spent	+/-
Category 1	50	25	25
Salaries	$82k	$39k	$43k
Materials/Supplies	$39k	$12k	$27k
OE …	$40.9k	$1.9k	$30k
Recharge Center	$16k	0	$16k

Other Expenses		
Materials and Supplies		39,200
PCB aptamers and complementary DNA molecules (7 pairs x $2,700/pair)		18,900
PCB kits, spectrometry; includes columns, EPA-approved reagents, tubing, consumables, injectors		7,200
PFAS kits, spectrometry; includes columns, EPA-approved reagents, tubing, consumables, injectors		7,800
SPR chips		3,500
Recharge Center Services		15,600
Total Other Expenses		92,200

Quoted, lead time

Projection of work in next reporting period

- **Test the specificity of the PCB aptamers with Congeners and Aroclors; EPA notification pending**
- Perform and validate measurements with spiked samples and water from environmental sources
- Field-test the portable SCOUT instrument from Sapidyne
- **Accelerate the selection and characterization of monoclonal antibodies for PFOA; this may be seriously impeded by the precarious health of a key member of the team; we may seek re-budgeting and subcontracting some of the work to expedite the process**
- Initiate antibody production for other PFAS (included in the EPA first national drinking water regulation, released on April 17, 2024)
- Improve the hardware and software platform of the portable SCOUT instrument
- Continue workforce development
- Apply for funding from other sources
- Disseminate the results
- Work with the OTT at BSU for IP

Thank you for your time!