Analyzing the Accumulation of PFAS in Mussels from the Puget Sound

Evan Hoang, Gabriel Mykland, Henry Maldonado, and Joyce Dinglasan-Panlilio, PhD

Introduction

- Per- and poly-fluoroalkyl substances (PFAS) are colloquially known as **"forever chemicals"** due to their long-lasting chemical characteristics that allow them to persist in nature
- Their **carbon-fluorine bonds are extremely durable** which makes them valuable in coating non-stick cookware and waterproof materials
- Attached to the carbon chain are functional groups, such as carboxylic acids in perfluorocarboxylic acids (PFCAs) or in perfluorosulfonic acids (PFSAs), that allow them to **bind to blood** and biological substances that can cause many adverse health-related effects
- A major source of PFAS contamination in waterways is through the excessive and repeated deployment of **aqueous firefighting foams** used in training exercises on airbases and fire-stations
- PFAS are considered to be indestructible; they accumulate in waterways and **bioaccumulate** in filter feeders like mussels
- **97% of Americans have accumulated PFAS in their blood** via consumption of contaminated food or water
- Such exposure has been associated with an array of health concerns including but not limited to **impaired immune response, metabolic dysfunction, and cancer**
- **Indigenous populations** in the PNW are **more at risk** for the accumulation of PFAS and other bio-accumulative toxins, due a diet consisting of a greater proportion of shellfish than other populations

- Recent settlements for PFAS contamination by 3M in public water supply settled for **10 billion dollars**
- In April 2024, the Environmental Protection Agency (EPA) released the **first ever national drinking water standard**, with maximum contaminant levels of 4 parts per trillion perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS)

Figure 1. *Inside of a blue mussel (Mytilus edulis) used for extraction.*

Table 1: Listed classification, name, acronym, molecular weight and general structure for the analytes of interest.

Classification	Compounds	Acronyms	Molecular Weights (transition measured)	General Structure
Perfluorinated Sulfonic Acids (PFSAs)	Perfluorobutane Sulfonic Acid	PFBS (n=3)	299>99	$CF_3(CF_2)_n$—S(=O)(=O)—OH
	Perfluorohexane Sulfonic Acid	PFHXS (n=5)	399>99	
	Perfluorooctane Sulfonic Acid	PFOS (n=7)	499>99	
Perfluorinated Carboxylic Acids (PFCAs)	Perfluorohexanoic Acid	PFHxA (n=4)	313>269	$CF_3(CF_2)_n$—C(=O)—OH
	Perfluoroheptanoic Acid	PFHpA (n=5)	363>319	
	Perfluorooctanoic Acid	PFOA (n=6)	413>369	
	Perfluorononanoic Acid	PFNA (n=7)	463>419	
	Perfluorodecanoic Acid	PFDA (n=8)	513>469	
	Perfluoroundecanoic Acid	PFUnDA (n=9)	563>519	
	Perfluorododecanoic Acid	PFDoA (n=10)	613>569	
	Perfluorotridecanoic Acid	PFTrDA (n=11)	663>619	
	Perfluorotetradecanoic Acid	PFTeDA (n=12)	713>669	

Objectives

- To **validate and optimize extraction and clean-up methods** for mussel tissue samples collected from urban bays to measure perfluorinated acid contamination on these organisms
- To obtain **> 70% recoveries** for most of the analytes of interest (Table 1) in spike and recovery experiments

Methods

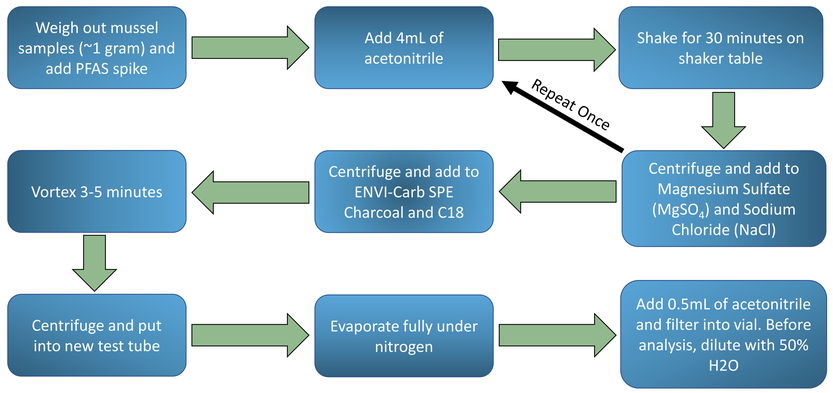


Figure 2. *Schematic of QuEChERS mussel extraction and clean-up method*

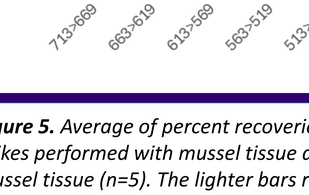


Figure 3. *Image of an Agilent 6495 LC/MS*

- Analyzed samples with 6495D Triple Quadrupole LC/MS
- Column: Agilent EclipsePlusC18
- Data analyzed with Agilent MassHunter Qualitative Analysis

Results

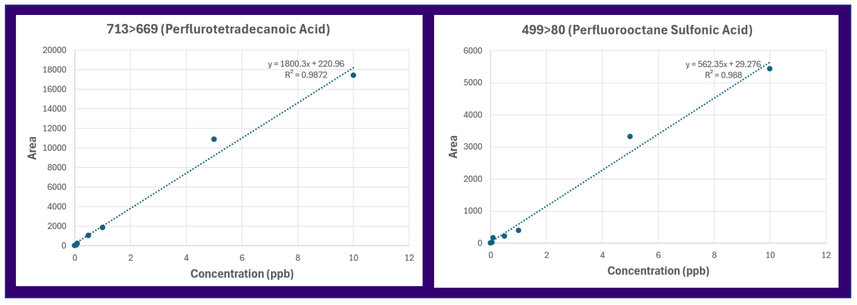


Figure 4. *Calibration curve PFTeDA derived from of 10, 5, 1, 0.5, 0.1, 0.05, and 0 ppb PFAS standards to relate the area counts to concentration of PFAS in samples. The R-squared value was used to ensure reliability of concentrations from standards.*

Figure 5. *Average of percent recoveries for PFAS analytes of interest with blue indicating the spikes performed with mussel tissue and orange indicating the spikes performed without mussel tissue (n=5). The lighter bars represent the PFCAs, the darker bars represent PFSAs.*

Results (continued)

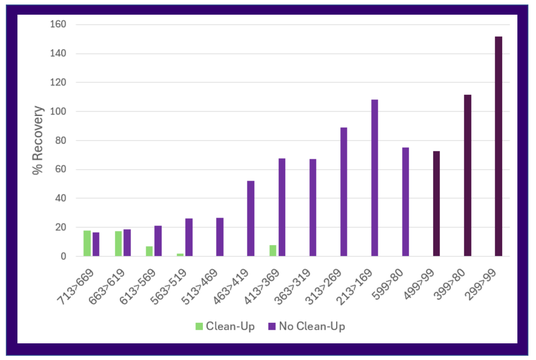
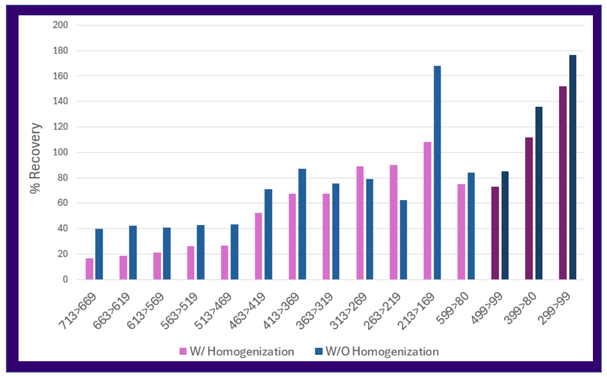


Figure 6. *Percent recoveries of PFAS analytes of interest without mussel tissue, with spike and recoveries performed with (green) and without (purple) the original clean-up procedure. The lighter bars represent the PFCAs, the darker bars represent PFSAs.*

Figure 7. *Percent recoveries of PFAS analytes of interest without mussel tissue, with spike and recoveries performed with (pink) and without (blue) the original homogenization procedure. The lighter bars represent the PFCAs, the darker bars represent PFSAs.*



Discussion/Conclusion

- PFCAs had an average of **46.7% less recovery without the mussel tissue** while PFSAs only had an average of 9.75% less recovery without mussel tissue
- This suggests that the **mussel tissue provides additional compounds that prevent the PFAS from being adsorbed** by the charcoal and C18, and that PFSA's are more resistant to binding
- This was further tested by performing a spike and recovery experiment without mussel tissue, testing the effect of the presence and absence of the clean-up chemicals (charcoal and C18)
- **With the clean-up**, the mean % recovery for all PFAS was a **3.76% recovery**, while **without the clean-up**, the mean % recovery was **64.6% recovery**, confirming these compound's importance in % recovery of PFAS
- Another source of loss of % recovery was the **homogenization** step. By cutting out the homogenization step, **% recoveries increased by 15.8% on average**.

Acknowledgements

I would like to extend gratitude to **Dr. Joyce Dinglasan-Panlilio** for being an amazing mentor and her unparalleled depth of knowledge on PFAS and organic chemistry, **the lab staff at UWT** for being accommodating to my N-Evap and centrifuge use, **Dr. Allison Gardell** for providing us mussels for the tissue extractions, **Gabe Mykland** and **Henry Maldonado** for setting a high standard for me, and **Dr. Marc Nahmani** for additional guidance on poster creation.

References