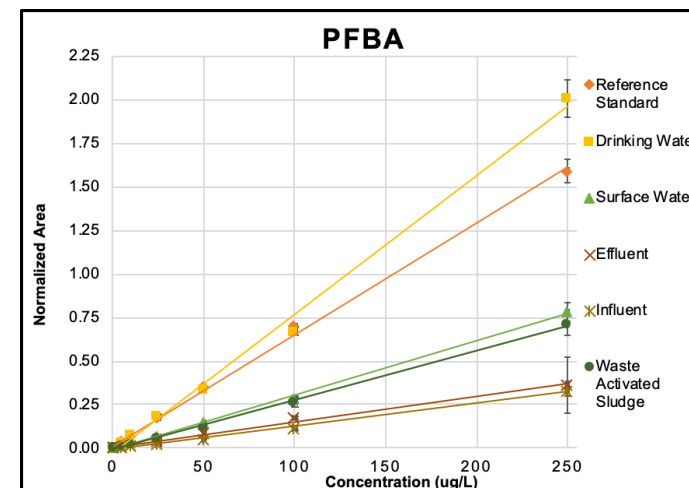
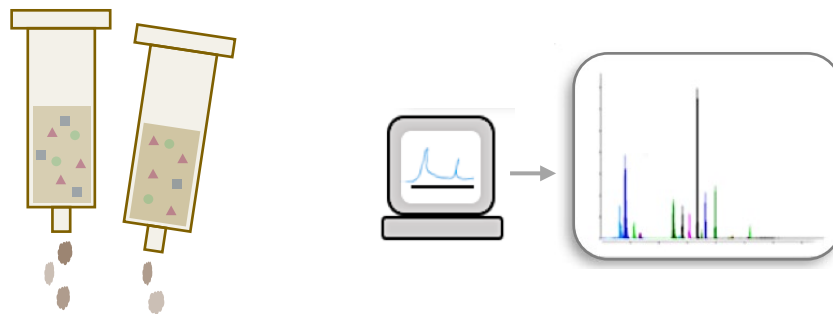
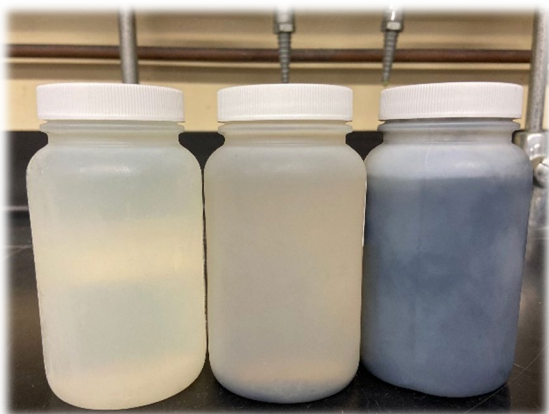


Towards Quantification Without Standards: Impacts of Environmental Matrices on the Solubilities and Ionization Efficiencies of Per- and Polyfluoroalkyl Substances (PFAS)

KC Donnelly Fellowship

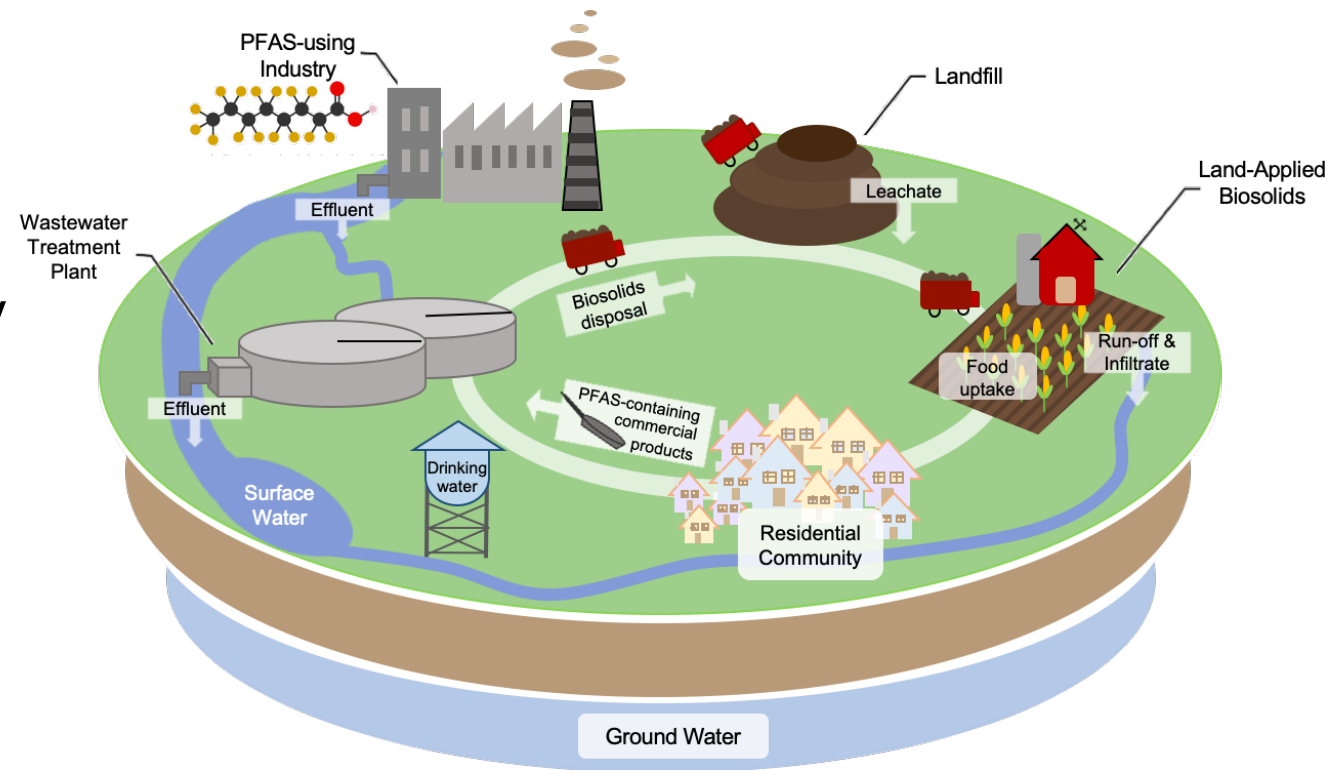
Rebecca A. Dickman¹, Shirley Pu², Nickolas Sayre-Smith², James McCord³, Jon Sobus³, Diana S. Aga¹

1. Department of Chemistry, University at Buffalo, SUNY, Buffalo, NY 14260
2. Oak Ridge Institute of Science and Education (ORISE) Participant
3. Office of Research and Development, United States Environmental Protection Agency



Background: Studying PFAS in the Environment

- **Per- and polyfluoroalkyl substances (PFAS)** have been detected on every continent in surface,^{1,2} drinking,^{1,2} wastewater and biosolids³
- Liquid chromatography mass spectrometry (LC-MS) is often used for PFAS targeted identification & quantification¹⁻³
- Non-targeted analysis has identified several new PFAS for which reference standards are not available and therefore can not be directly quantified⁴
- **How can we quantify PFAS without reference standards?**

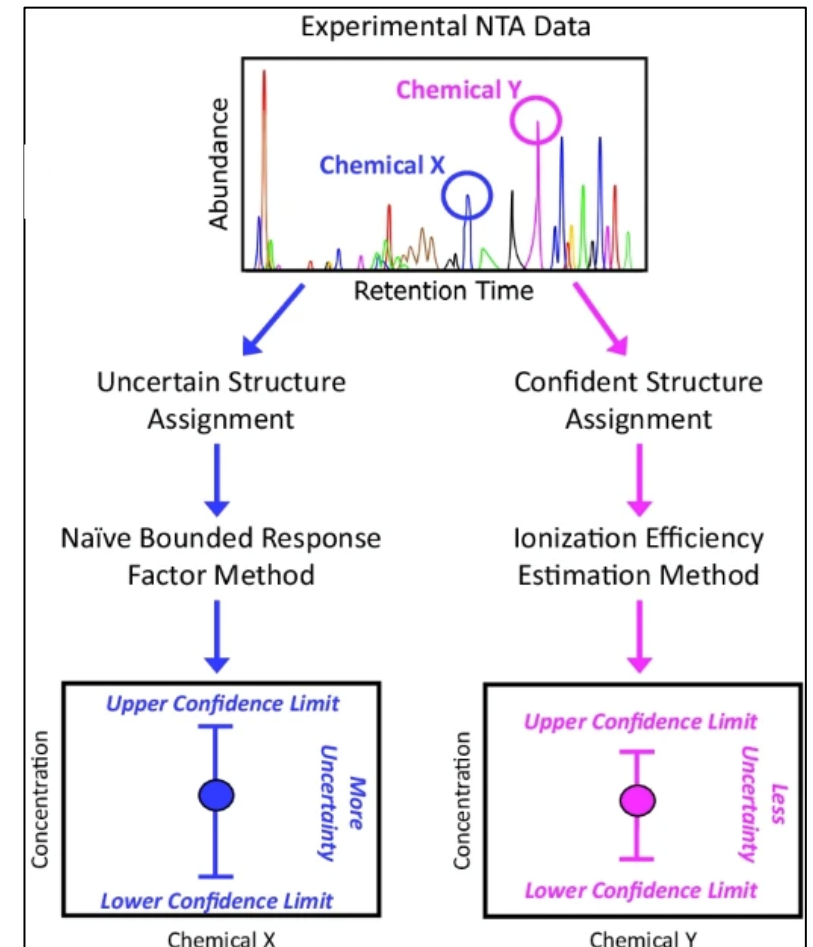


1. Kurwadkar, S., et al. Science of The Total Environment, 2022.
2. Vento, S.D., et al., Atmospheric Pollution Research, 2012.
3. Venkatesan, A.K., et al. Journal of Hazardous Materials. 2014.
4. Nakayama, S, F. et al. Trends in Analytical Chemistry

Background: Current inverse prediction models

- Inverse prediction models were used to quantify organic contaminants without reference standards in solvent
- **Naïve bounded response factor (RF) bootstrap method:**
 - All chemical RFs from training set used to bound the concentration estimate of an unknown
 - No assumptions about chemical structure
 - High uncertainty due to models generic nature
- **Ionization efficiency estimation method:**
 - Considers SMILES structures, analytical solvents, and analyte retention times
 - Lower overall uncertainty than bootstrap RF method but more error than ideal inverse predictions

→ But matrix effects & solubility were not considered



Background: Matrix effects on response factor

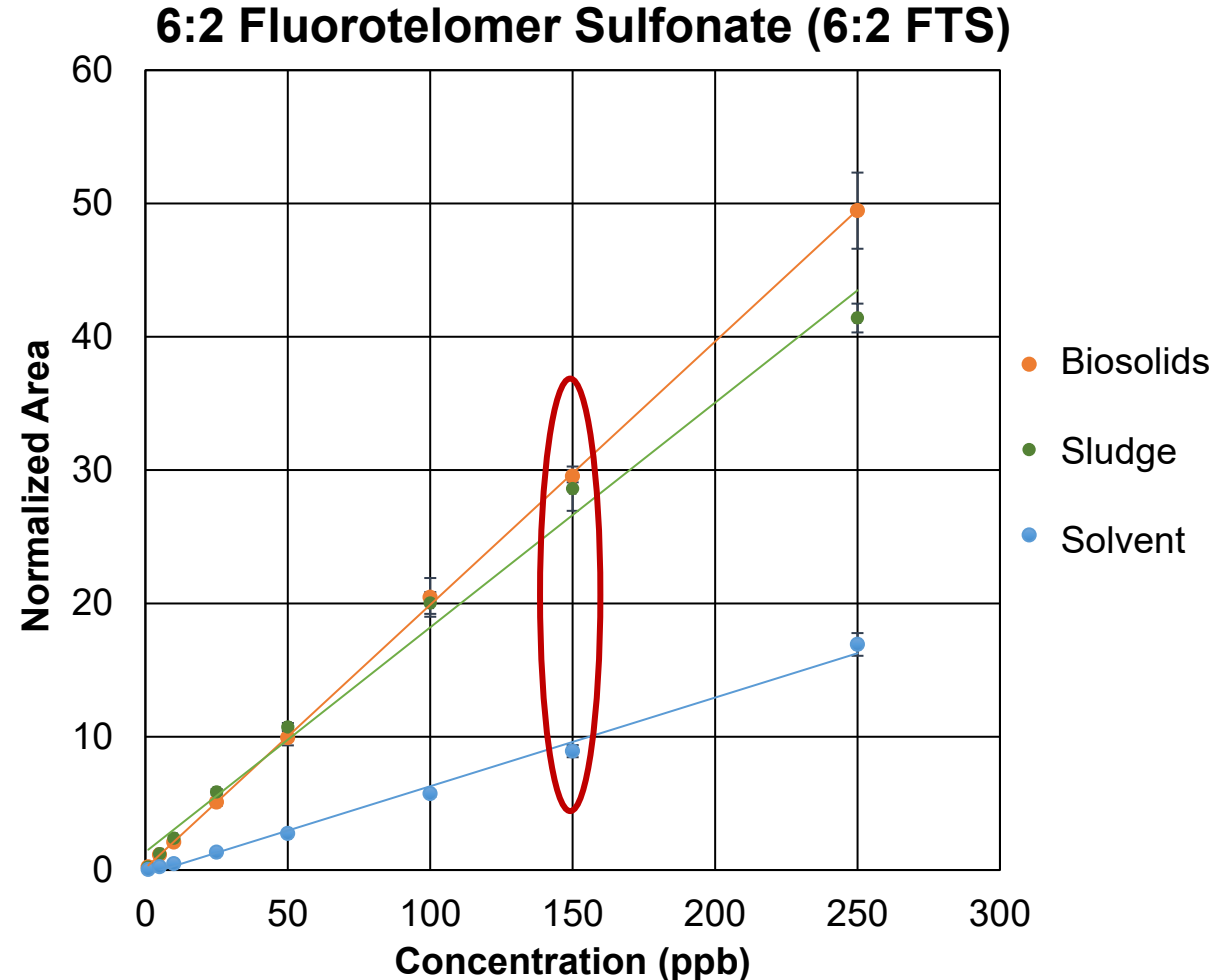
Targeted analyses use calibration curves to calculate analyte response factor (RF) and quantify detections

$$(RF) = \frac{\text{Analyte Area}}{\text{Concentration}}$$

- Assumed to be consistent across linear dynamic range
- Can be affected by matrix coextracted ions

Ex. RF is 3x higher in sludge & biosolids than solvent (150 ppb)

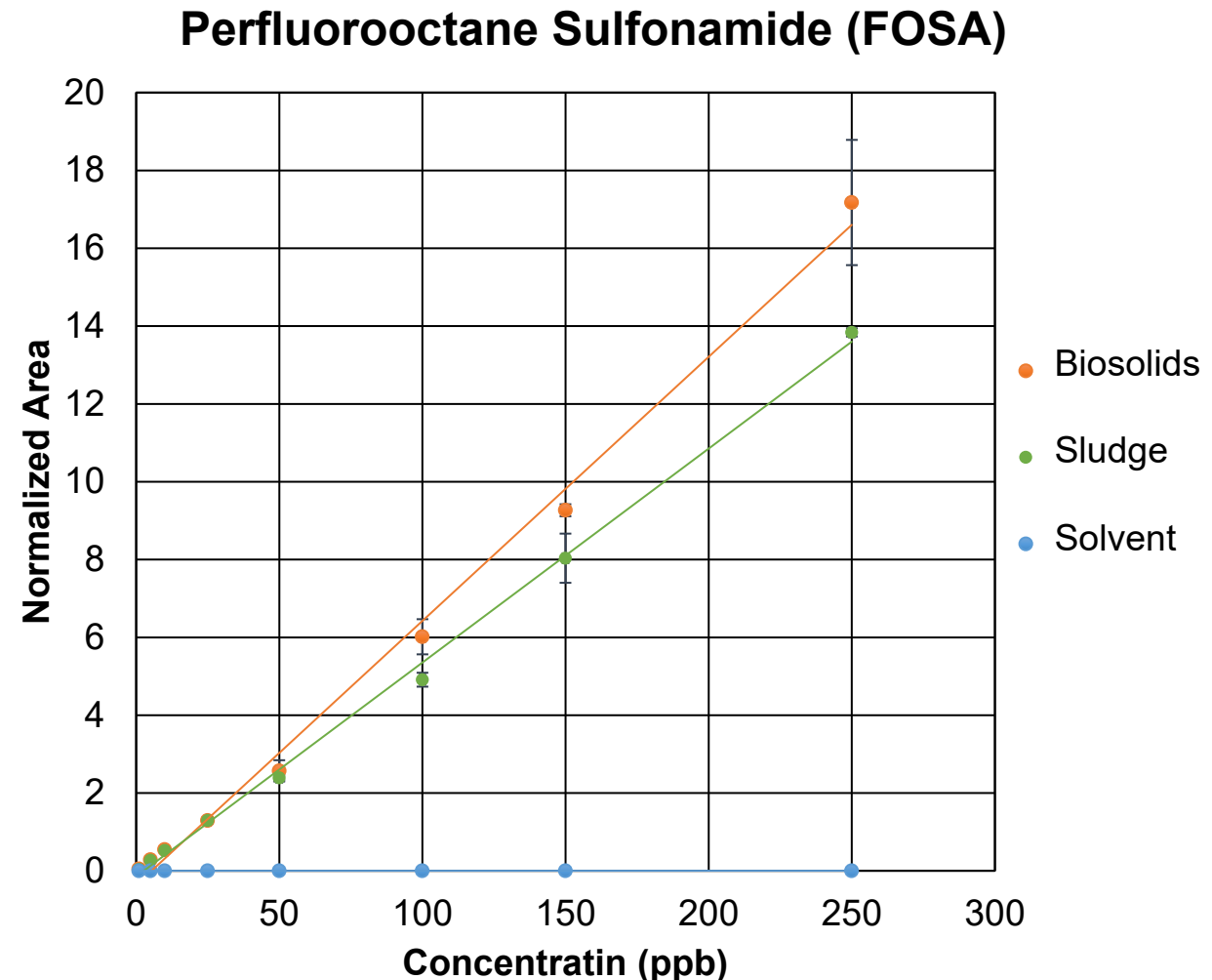
→ **Hypothesis 1:** Coextracted matrix influences the **RF** of PFAS in LC-MS analyses using electrospray ionization (ESI)



Background: Solubility effects on response factor

- No signal for FOSA in solvent (95% water, 5% acetonitrile)
- Aqueous solubility (OPERA¹ calculated) for FOSA = **$7.81 \times 10^{-7} \text{ mol / L}$**
- FOSA in sludge and biosolids samples had good signal and peak shape
 - Matrix can increase non-polar characteristics of water² and improve PFAS solubility in matrix extracts

→ **Hypothesis 2:** Variation in analyte solubility causes low observed RFs for some PFAS in aqueous solvent and can also vary between matrix coextracts



1. USEPA Comptox Dashboard, OPERA predicted properties. 2022

2. Renner, R. KOW controversy. ES&T 2002

Project goals

Project Goals:

→ Develop a technique for inverse quantification predictions for PFAS without reference standards that can correct for sample matrix & resuspension solvent

Tasks:

- (1) Evaluate the effects of matrix and solvents on analyte RF
- (2) Calculate inverse estimation error for each analyte with optimal techniques:
i.e. Matrix-matched calibration curves
- (3) Calculate inverse estimation error with naïve conditions with no assumptions about physico-chemical properties
i.e. naïve bounded RF method
- (4) Optimize an inverse error estimation method that considers matrix & solvent effects
- (5) Validate the optimized method through bootstrapping and cross-validation

Analytes & sample matrices

Training analytes: Isotopically labelled PFAS standards including -
carboxylates (10), sulfonates (3), fluorotelomer sulfonates (3), and sulfonamides (3)

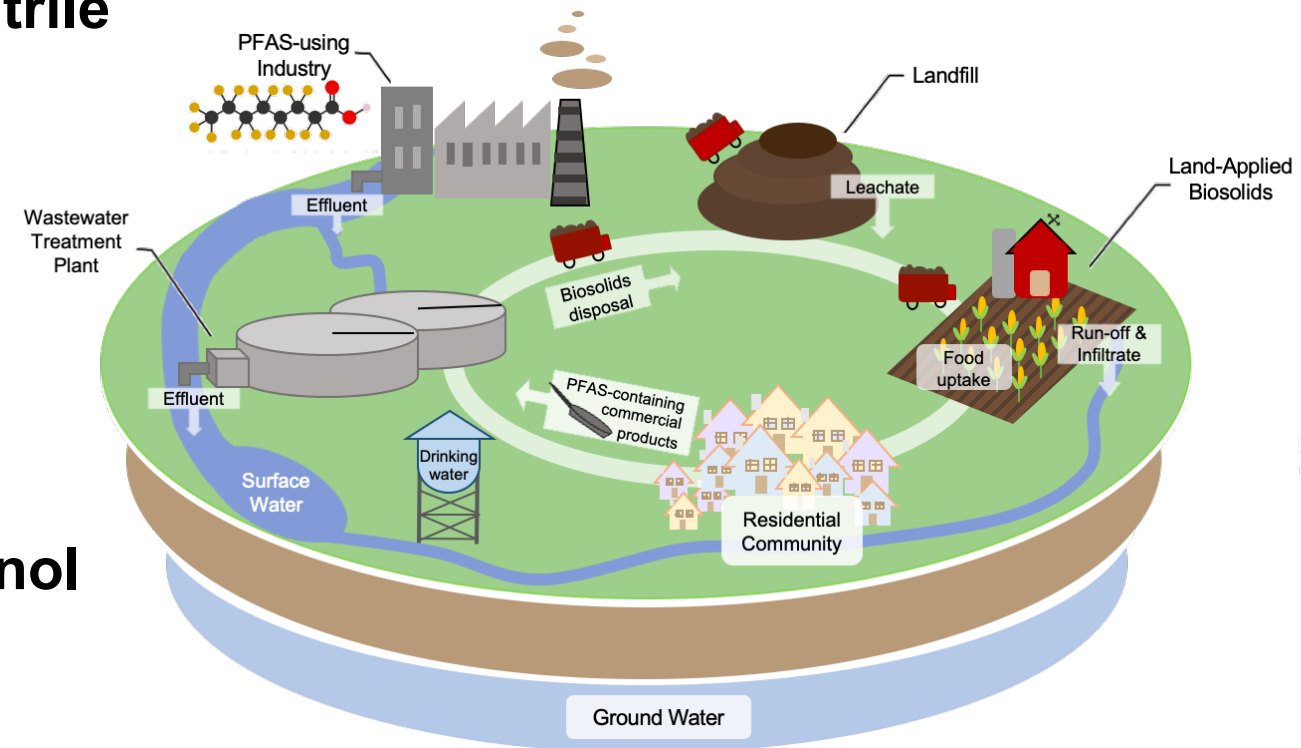
Linear range: 0.5 – 250 ppb

95% 5mM ammonium acetate, 5% Acetonitrile

- Reference standard
- Drinking water (500 mL)
- Surface water (500 mL)
- Secondary effluent (100 mL)
- Raw influent (100 mL)
- Waste activated sludge (250 mg)

25% 5mM ammonium acetate, 75% Methanol

- Reference standard
- Drinking water (500 mL)
- Waste activated sludge (250 mg)



Matrix & solubility effects on RF

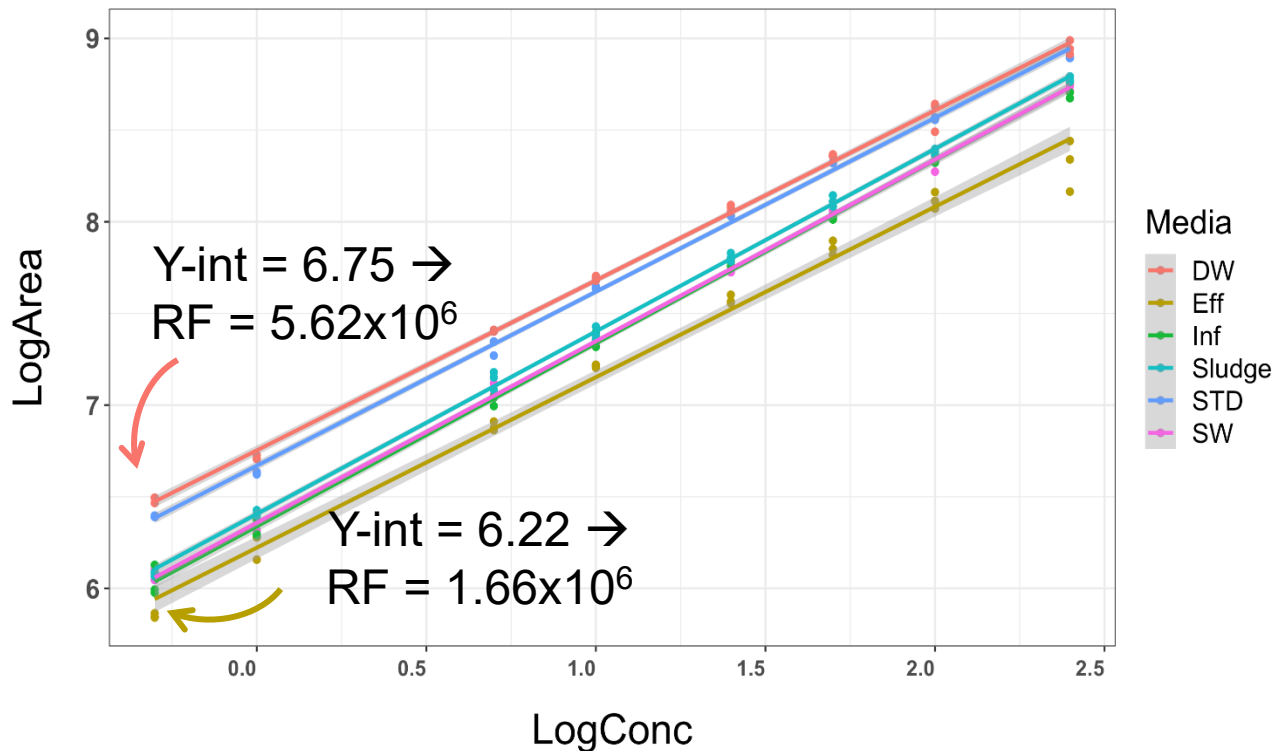
Log Concentration vs. Log Area plots:

Slope ~ 1

Log(RF) = Y-intercept

→ Matrix coextracts are shown to affect RF

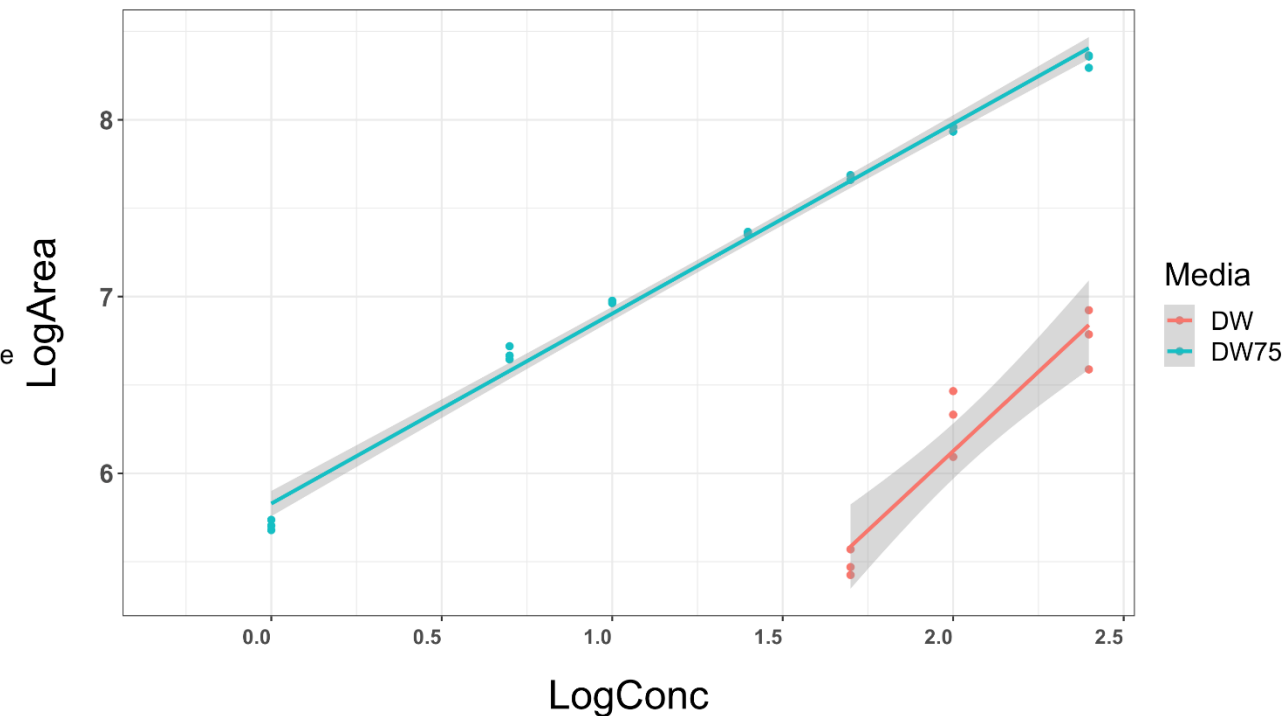
PFHxA



The linear range of some analytes & matrices have only 3 calibration points & slopes $\neq 1$

→ Analyte solubility in aqueous solvent is poor for certain analytes

N-Me-FOSAA



Project goals

Project Goals:

→ Develop a technique for inverse quantification predictions for PFAS without reference standards that can correct for sample matrix & resuspension solvent

Tasks:

- (1) Evaluate the effects of matrix and solvents on analyte RF
- (2) Calculate inverse estimation error for each analyte with optimal techniques:
i.e. Matrix-matched calibration curves
- (3) Calculate inverse estimation error with naïve conditions with no assumptions about physico-chemical properties
i.e. naïve bootstrap RF method
- (4) Optimize an inverse error estimation method that considers matrix & solvent effects
- (5) Validate the optimized method through bootstrapping and cross-validation

Inverse predictions: Ideal conditions

Matrix-matched calibration curves:

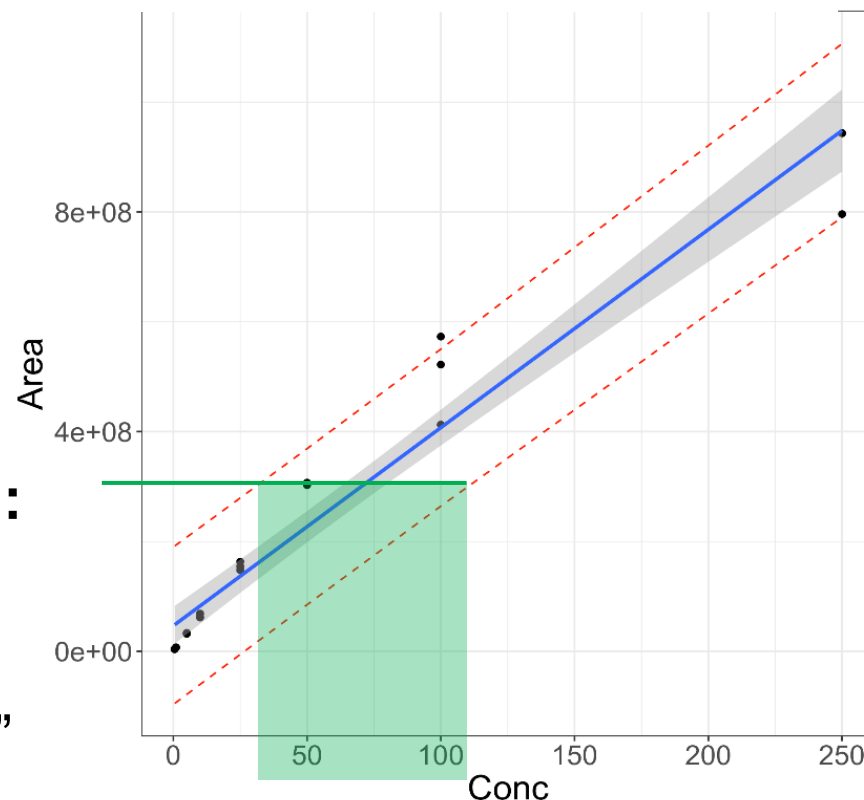
- Ideal quantification technique
- Benchmark for error to compare other techniques

“Leave-one-out” validation:

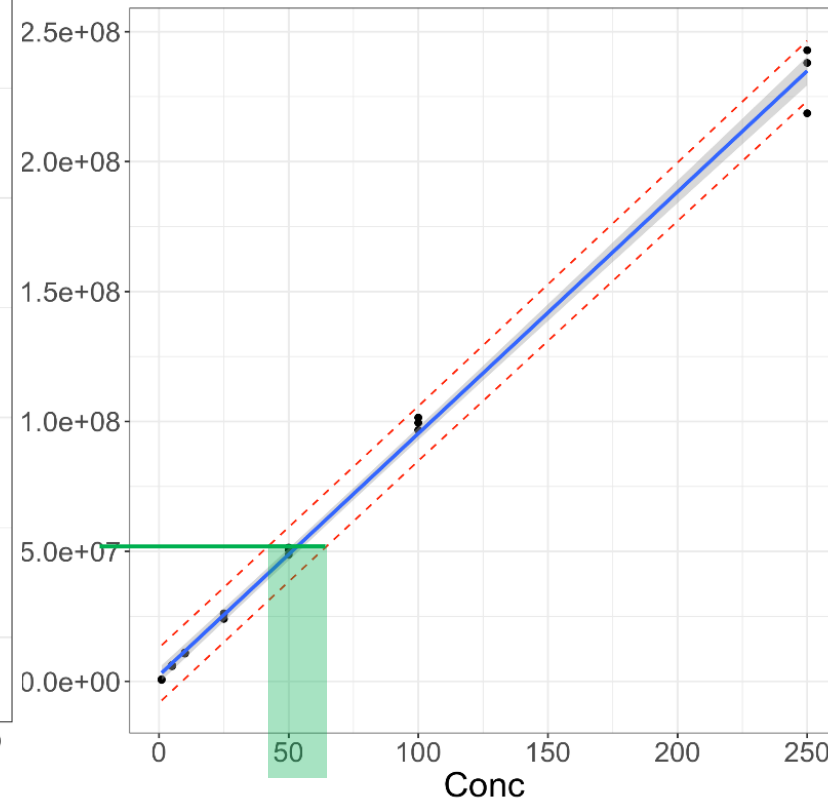
All but 1 injection included in calibration curve

Concentration of the “left out” injection is calculated to determine uncertainty

Drinking water (DW):
PFOA



Drinking water 75% MeOH
(DW75): PFOA

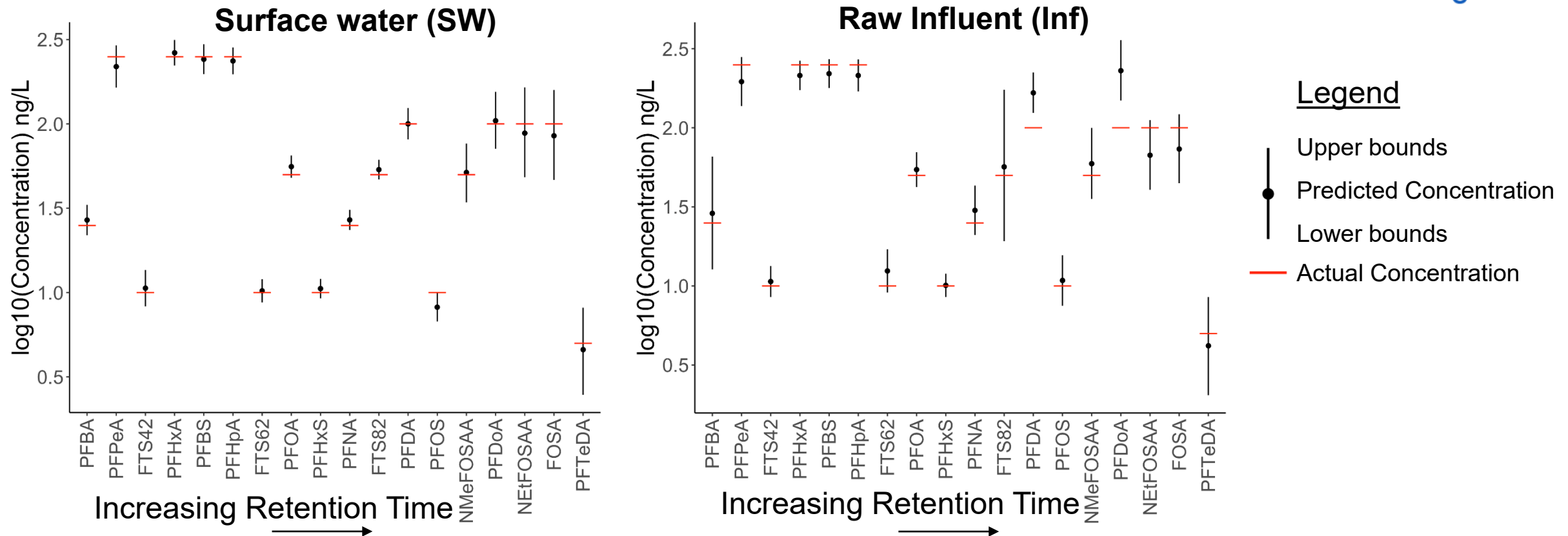


Ex. Quantify PFOA 50 ppb, replicate #2

1) DW area = 3.0×10^8
Concentration bounds : ~30 – 110 ppb

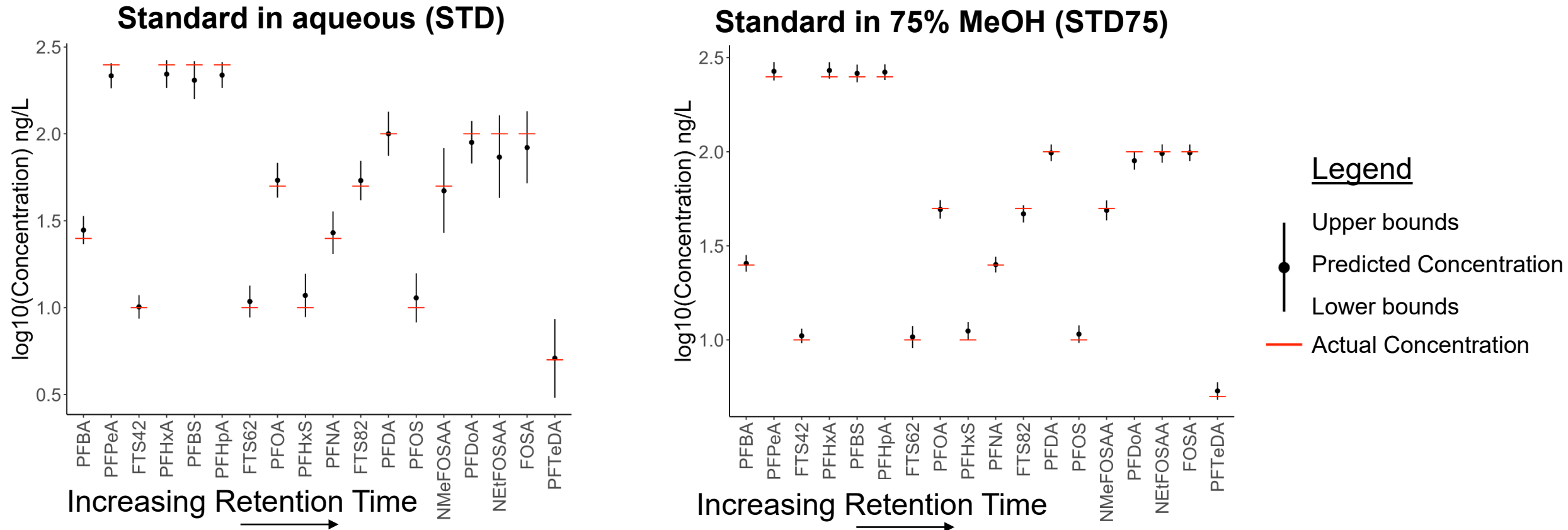
2) DW 75 = 5.1×10^7
Concentration bounds: ~45 – 65 ppb

Matrix effects on inverse estimation error



- Complex matrices (e.g., Inf) often had higher error intervals vs. cleaner matrices (e.g., SW)
- Long chain and sulfonamide PFAS often had larger intervals vs. shorter chain analytes

Solubility effects on inverse estimation error



- More error in aqueous standard than in 75% MeOH standard
- Uncertainty in the aqueous standard increases with retention time
- 75% MeOH standard had smaller and more stable error overall

Project goals

Project Goals:

→ Develop a technique for inverse quantification predictions for PFAS without reference standards that can correct for sample matrix & resuspension solvent

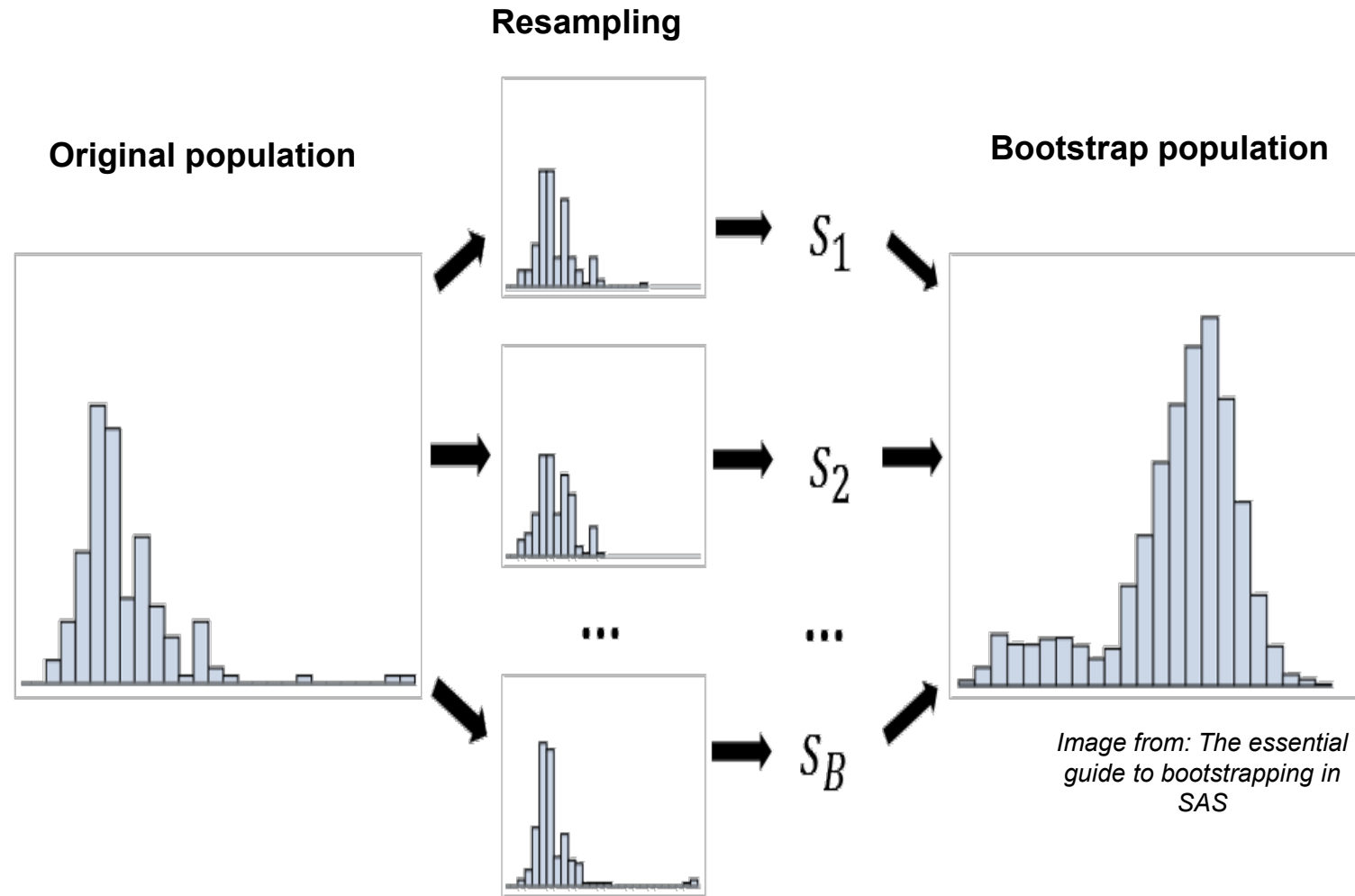
Tasks:

- (1) Evaluate the effects of matrix and solvents on analyte RF
- (2) Calculate inverse estimation error for each analyte with optimal techniques:
i.e. Matrix-matched calibration curves
- (3) Calculate inverse estimation error with naïve conditions with no assumptions about physico-chemical properties
i.e. naïve bootstrap RF method
- (4) Optimize an inverse error estimation method that considers matrix & solvent effects
- (5) Validate the optimized method through bootstrapping and cross-validation

Naïve bounded RF predictions

Naïve bootstrap predictions:

- Predict concentrations for unknown PFAS using other 18 PFAS as surrogates for RF
- RF bootstrap distribution quantiles can be used to bound uncertainty in the predictions
 - 97.5 \rightarrow upper uncertainty bound
 - 2.5 \rightarrow lower uncertainty bound
 - Median \rightarrow best estimate



Benchmark vs. naïve inverse estimations

- Naïve bootstrap RF predictions had a higher uncertainty fold-range than the targeted calibration inverse predictions
- Simpler/aqueous matrices including the standard had a higher uncertainty range than the 75% MeOH resuspension or complex matrices (i.e. Sludge)

	Percent Exceedance		Uncertainty Fold-Range	
	Calibration Curve	Naïve Bounded RF	Calibration Curve	Naïve Bounded RF
Aqueous resuspension				
Standard	3.1%	19.7%	1.9	68.2
Drinking Water	5.3%	21.5%	2.0	54.8
Surface Water	5.6%	20.0%	1.8	77.1
Secondary Effluent	5.7%	13.1%	2.6	27.9
Raw Influent	5.7%	12.1%	2.6	11.9
Sludge	5.6%	11.4%	1.9	12.2
75% Methanol resuspension				
Standard	4.7%	13.7%	1.2	5.2
Drinking Water	5.5%	16.9%	2.8	14.4
Sludge	4.2%	10.9%	1.5	9.1

Conclusions

- Matrix effects and solubility effects impacted the observed RFs for PFAS
- Analytes with a retention time > 21 minutes had poor solubility in aqueous solvents
 - Artificially low RF in aqueous solvent causing wide RF distributions
 - Naïve bootstrapping had highest error in simple, aqueous samples (drinking water, surface water, standard)
- Naïve bootstrap predictions had higher uncertainties compared to inverse calibration predictions

Future work

- Test expert surrogate inverse prediction to bootstrap RF distributions based on chemically specific surrogates (n=3)
- Develop an inverse prediction technique for PFAS which considers matrix and solubility effects
 - Apply a mixed model with predicted ionization efficiencies to a training set in sample matrix
 - Include variable to correct RF for solubility limitation for late eluting analytes
- Validate the optimized method through bootstrapping and cross-validation

Acknowledgements

EPA Collaborators: Shirley Pu, Dr. Nickolas Sayre-Smith, Dr. James McCord, Dr. Jon Sobus



UB group: Dr. Diana S. Aga & Aga lab members



Funding: NIEH SRP Award # R01ES032717

KC Donnelly Externship supplement

