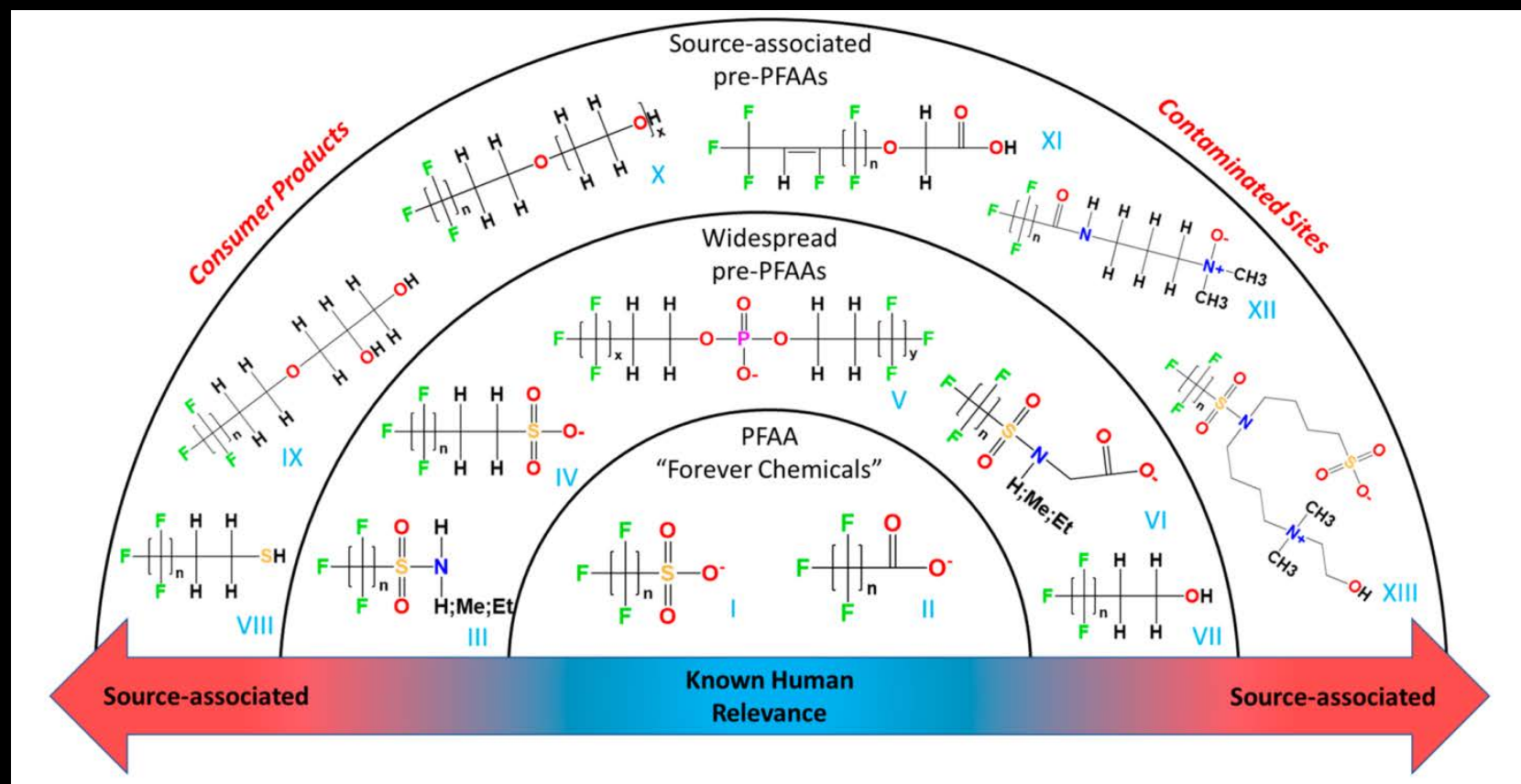


# "Forever Chemicals" in Your Body: Bioaccumulation of Per/Polyfluoroalkyl Substances (PFASs)

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Stony Brook University

Center for Clean Water  
Technology  
Clean Water Symposium  
June 17<sup>th</sup>, 2022



# PFASs in Our Bodies

The story of uncovering PFAS exposure in humans is a story about the advancement of analytical chemistry...

## Evidence that there are Two Forms of Fluoride in Human Serum

It has been assumed that there is only one form of fluoride in serum, the inorganic F ion. It would therefore seem that either the value for serum fluoride which I found (1  $\mu\text{M}$ ) (refs. 1 and 2) or that found by Singer and Armstrong (7.5  $\mu\text{M}$ ) (ref. 3) must be in error. While the diffusion method of Singer and Armstrong has been shown to produce erroneous values, the same cannot be

Taves 1968 analyzing his own blood –  
“ashing makes more fluoride available” ... “These findings are consistent with the presence of a fluorocarbon molecule”

With respect to the chemical nature of the non-exchangeable form of fluoride several lines of evidence suggested that it was some sort of organic fluorocompound of intermediate polarity, tightly bound to plasma albumin in the blood. It migrated with

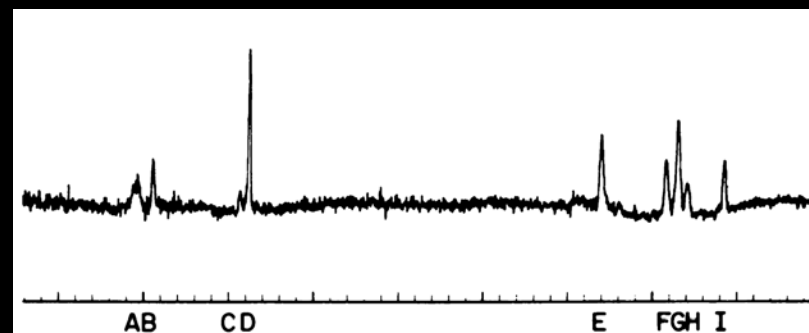


Figure 5. NMR spectrum of organic fluorocompound(s) isolated from human plasma

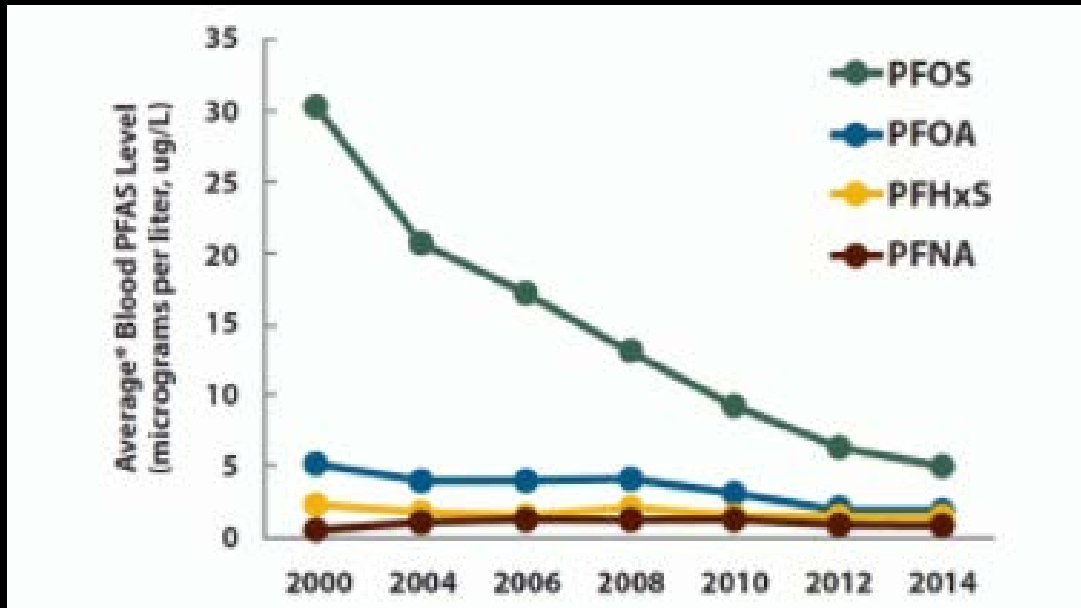
Guy et al. 1976: Characterization of C6-C8 perfluoro fatty acid derivatives “...findings suggest that there is widespread contamination of human tissues with trace amounts of organic fluorocompounds derived from commercial products.”

# PFASs in Our Bodies

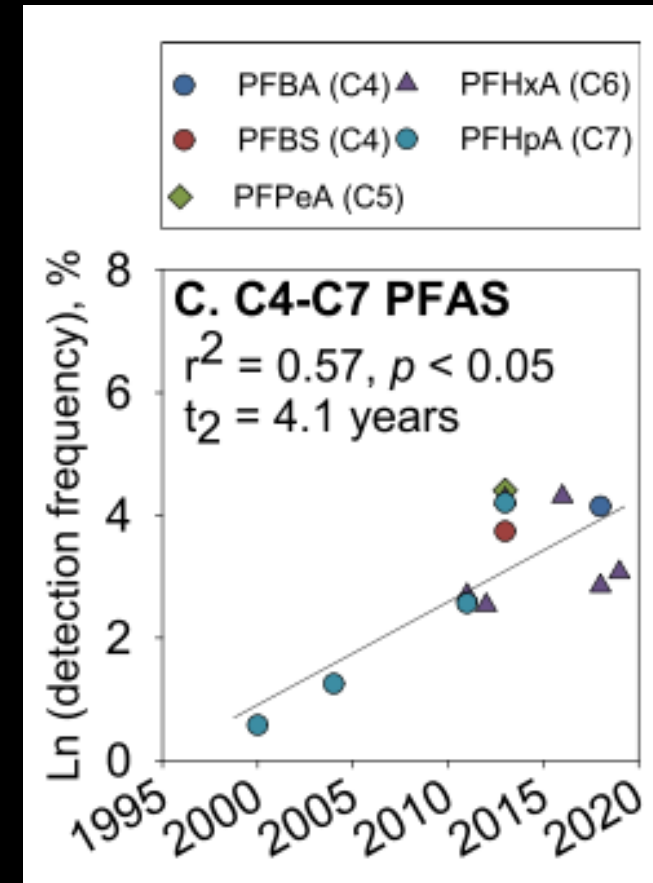
PFASs are present in the blood of **>99% of human beings...**

(CDC NHANES, January 2017)

Detection of shorter-chain PFASs is increasing in breast milk



<https://www.atsdr.cdc.gov/pfas/health-effects/us-population.html>

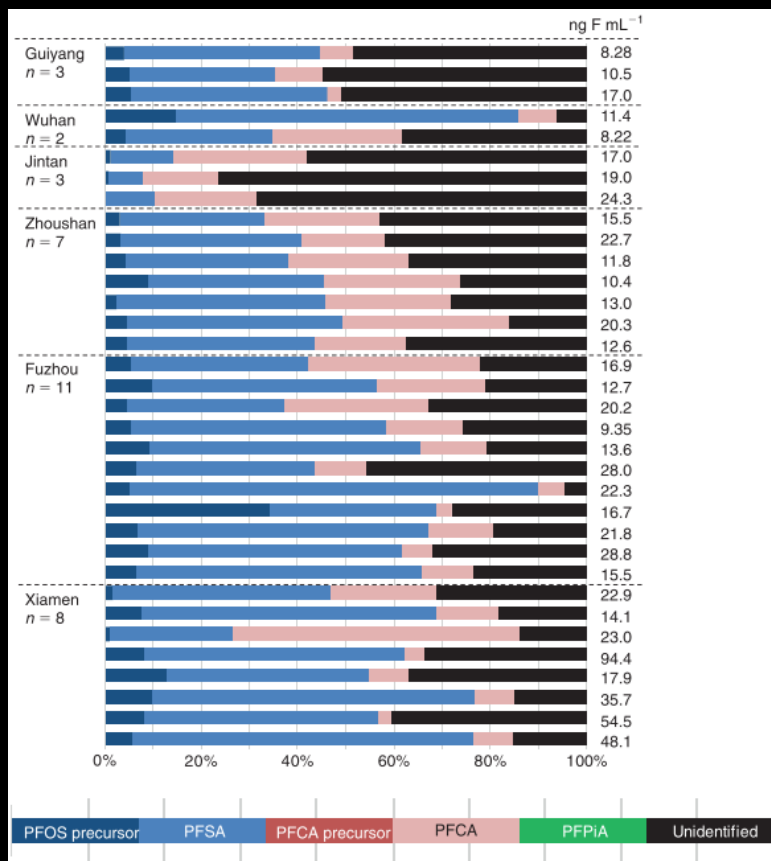


Zheng et al. *Environ. Sci. Technol.* 2021

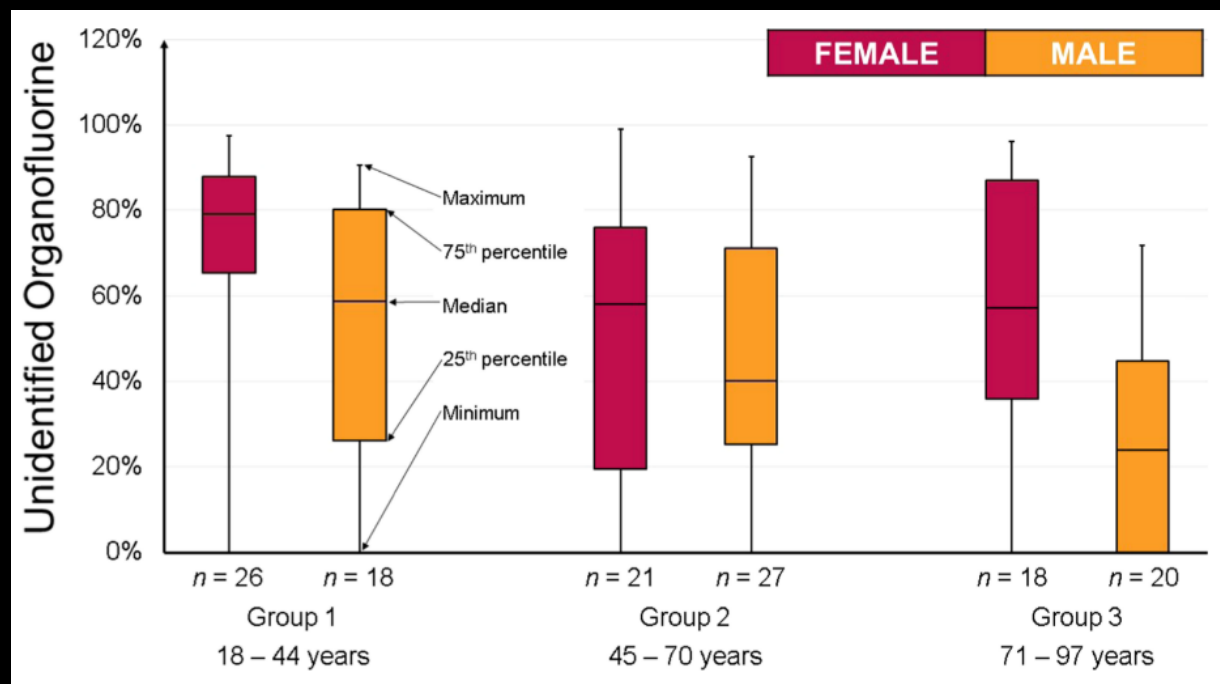


# PFAS “Dark Matter”

Studies measuring total organic fluorine (TOF) suggest significant contributions from unidentified PFASs



Extractable organic fluorine in blood from residents in Chinese cities (Yeung & Mabury 2016 *Environmental Chemistry*).

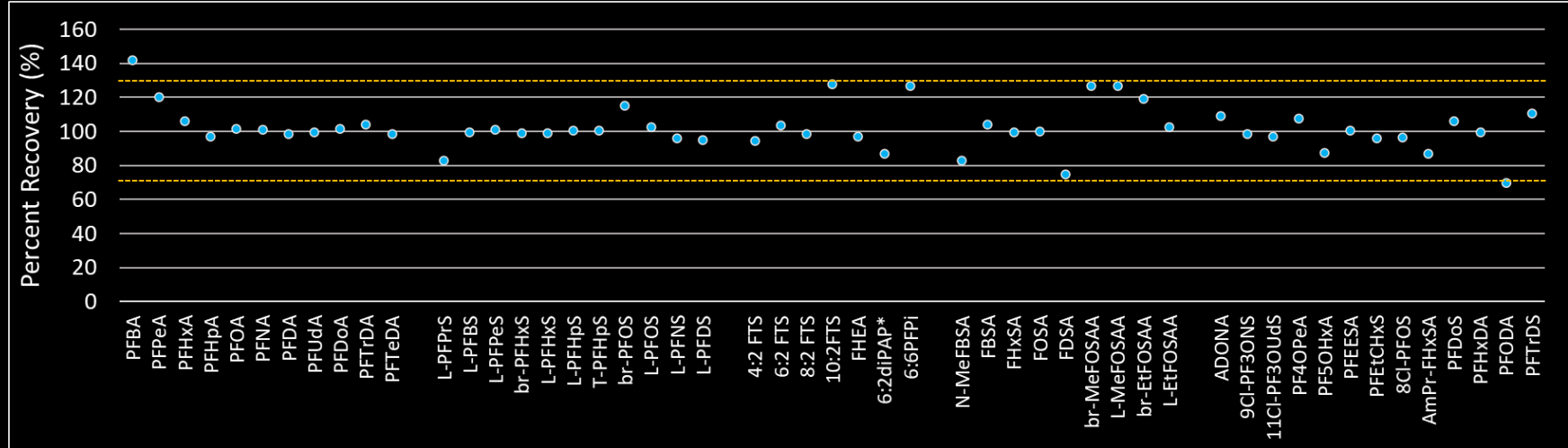


Fraction unidentified organofluorine in general Swedish population (Aro et al. 2021 *Environ. Sci. Technol.*)



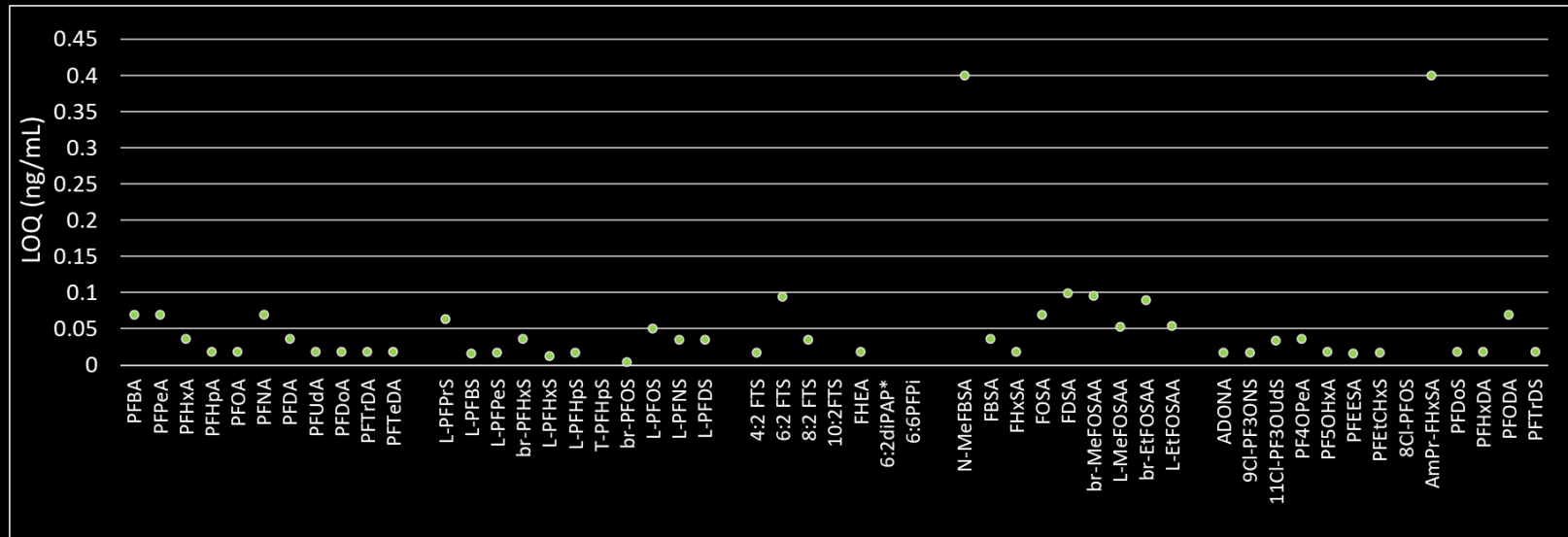
# PFAS in Serum: Targeted Analysis

## Serum Spike Recoveries (1.2 ng/mL) for 50 PFASs



\*data for 6:2 diPAP from 2 ng/mL serum spike

## Detection Limits (ng/mL serum)



# Suspect Screening: “Top-Down” Non-Target

## QToF-MS All Ions:

Scan and fragment all ions 100 – 1200 Da



Targeted quantitation: 50 PFASs with analytical standards



Screen for matches using **extracted ion chromatograph (XIC) list:** List of mass and formula for > **1000** characterized and theoretical PFASs



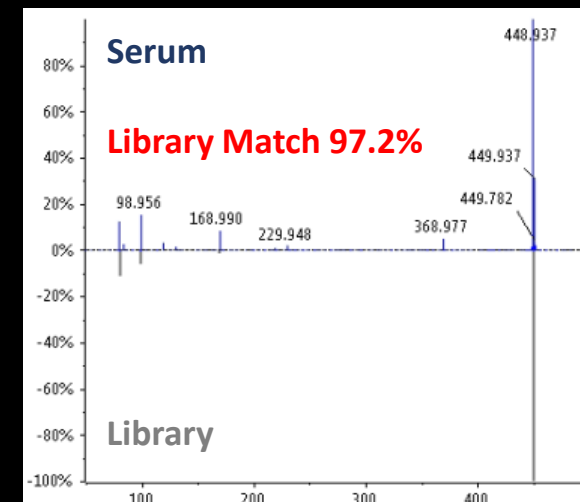
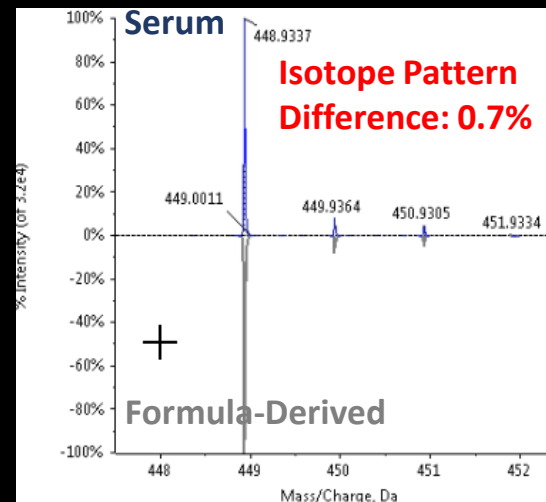
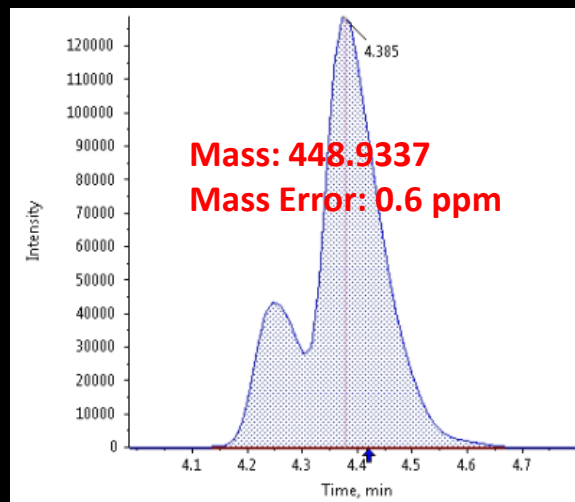
Precursor mass within **5 ppm** of known value



Isotope pattern match within **10%**



Screen for peak ID matches using **high-res MS/MS spectral libraries**



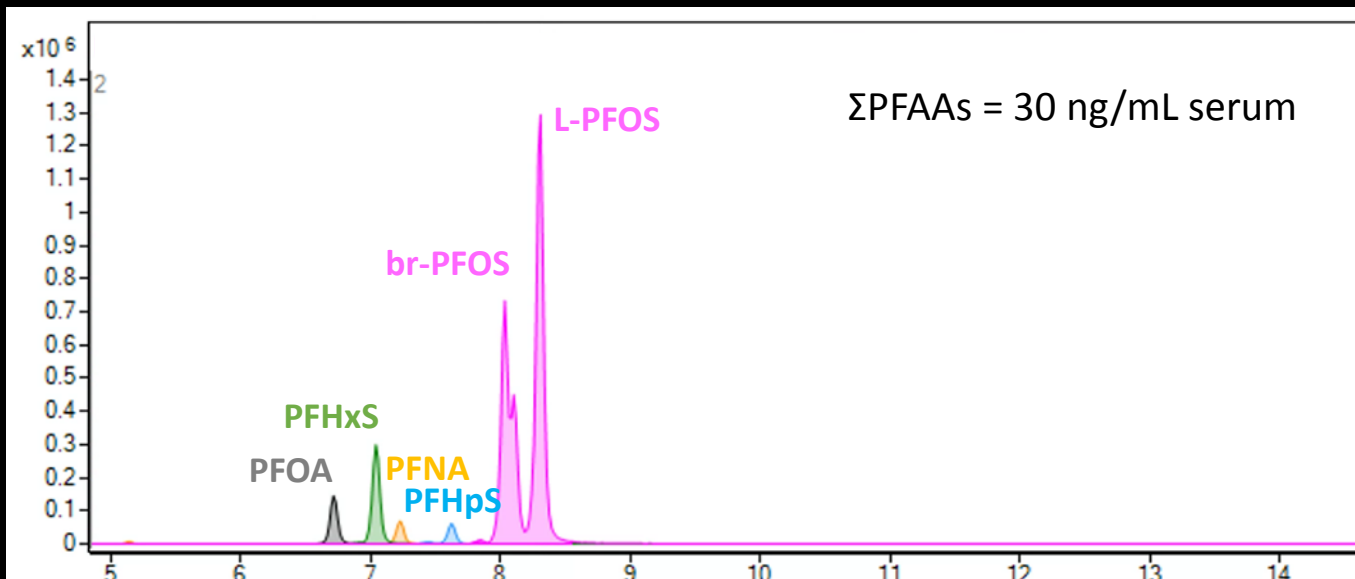
Fragments matching score  $\geq 70\%$

# PFAS Fingerprints Are Everywhere

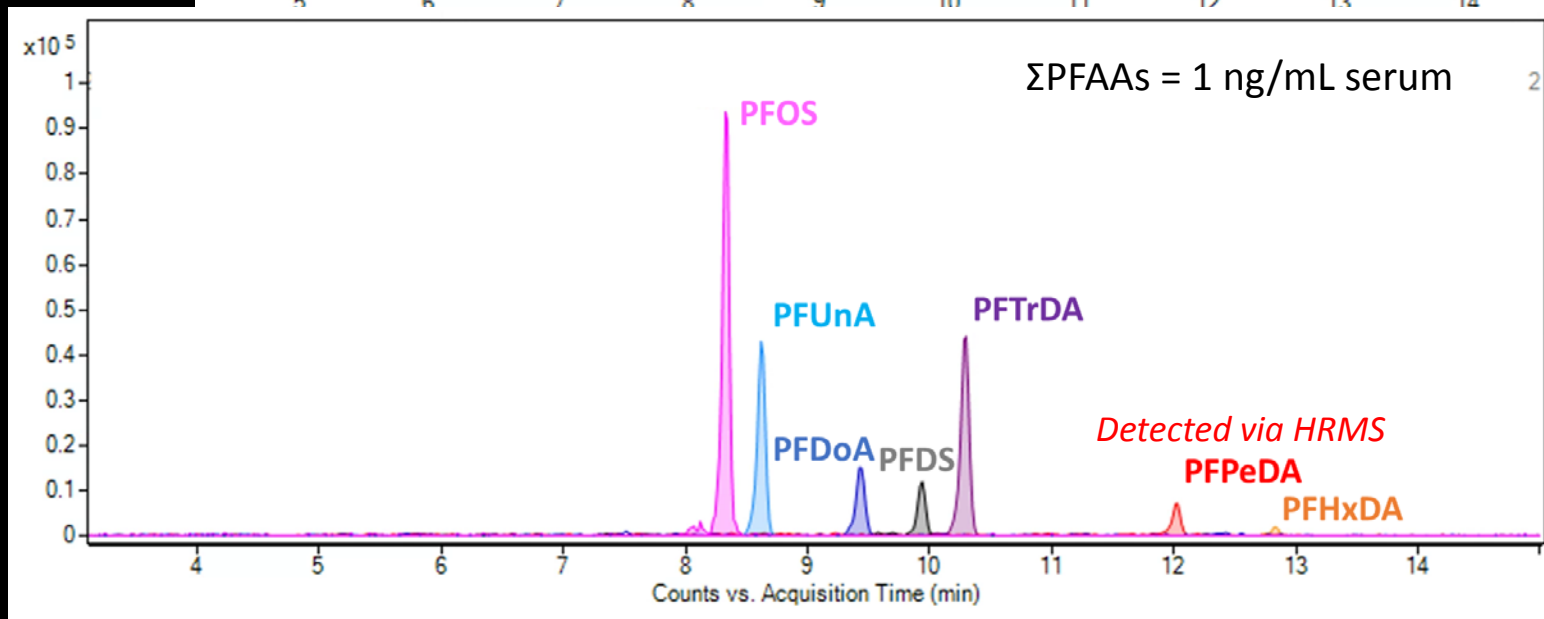
*from our first responders to marine organisms...*



U.S. Air Force photo  
by Senior Airman  
Christopher Quail



[www.readersdigest.ca/](http://www.readersdigest.ca/)





# PFAS Fingerprints Are Everywhere

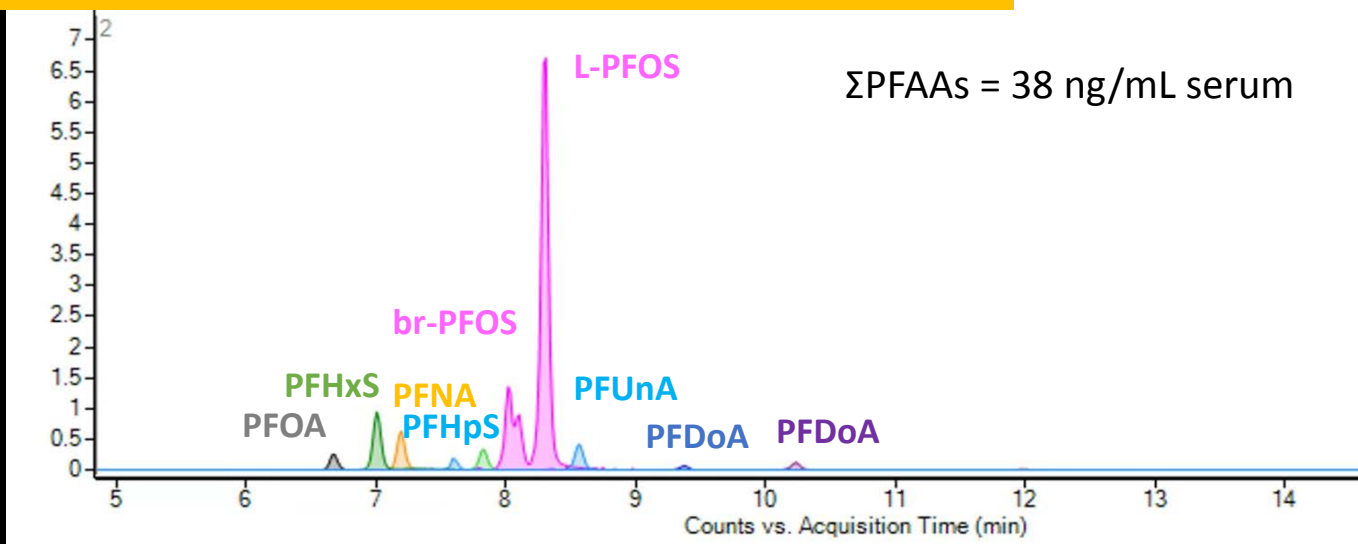
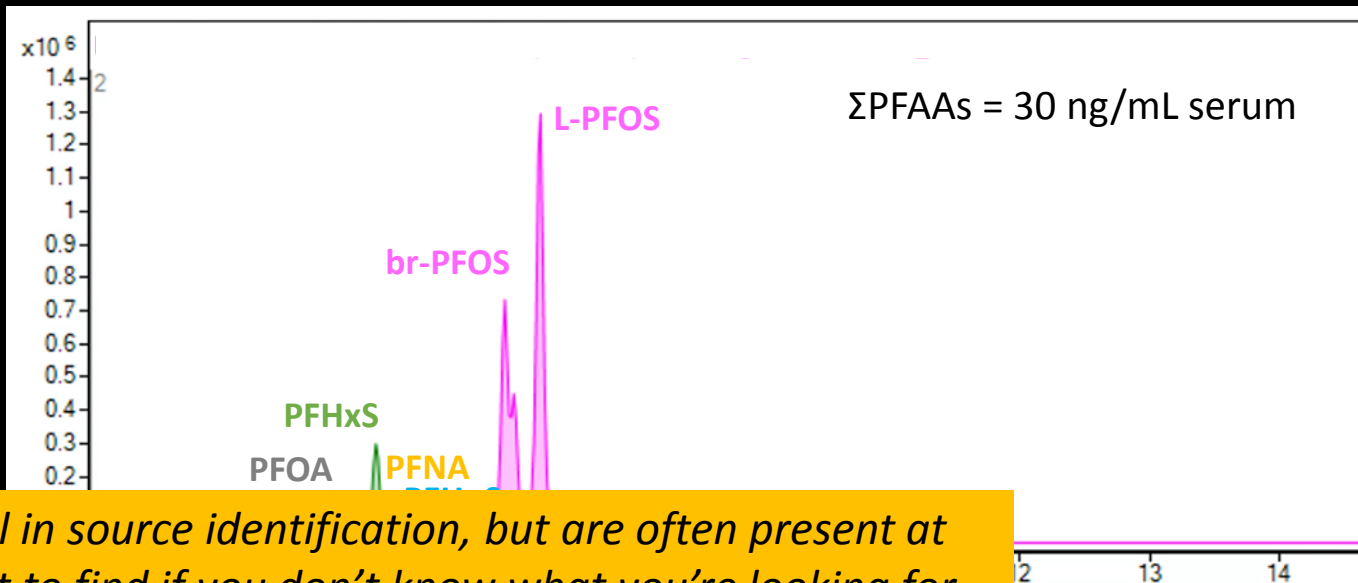
*from our first responders to marine organisms...*



*Novel PFASs can be very helpful in source identification, but are often present at low concentrations and difficult to find if you don't know what you're looking for...*

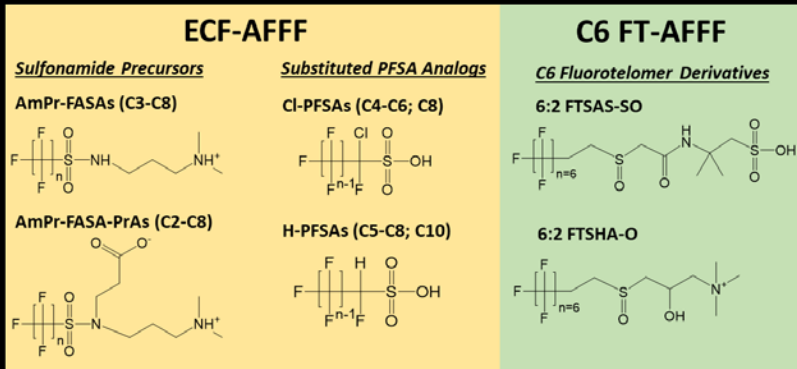


[www.longislandpress.com](http://www.longislandpress.com)



# Biological prioritization of novel and unknown PFASs

## Commercial PFAS mixtures

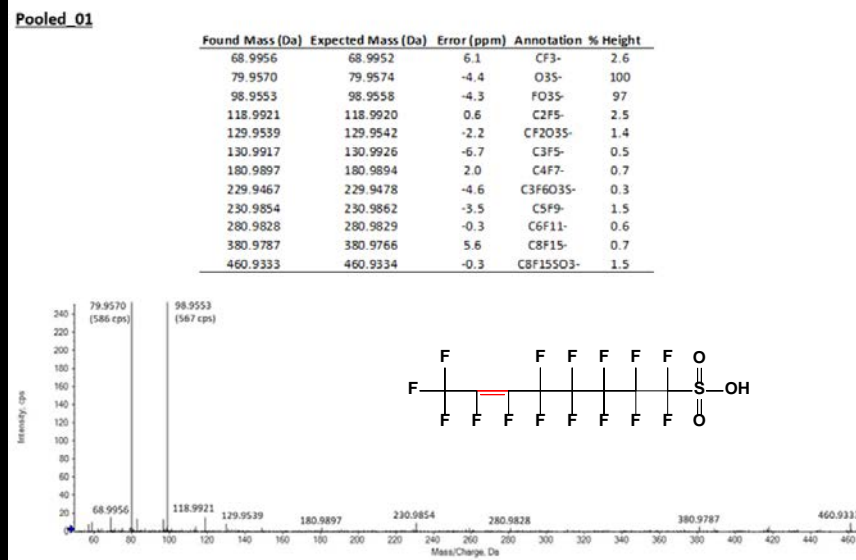
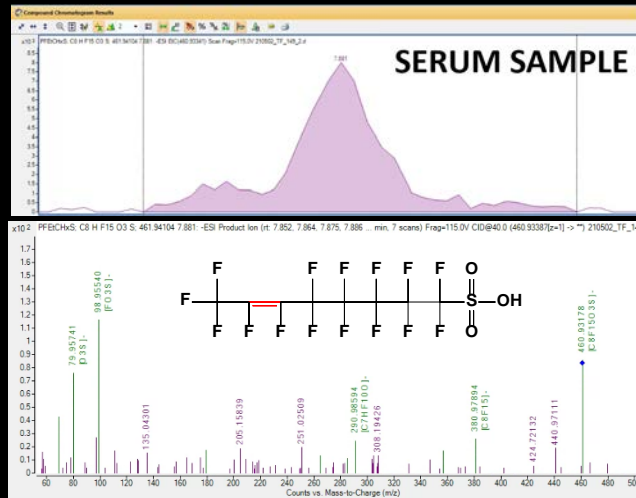


*In vitro* metabolism assays

*In vivo* dosing studies

Biological transformation products  
Novel bioaccumulative compounds

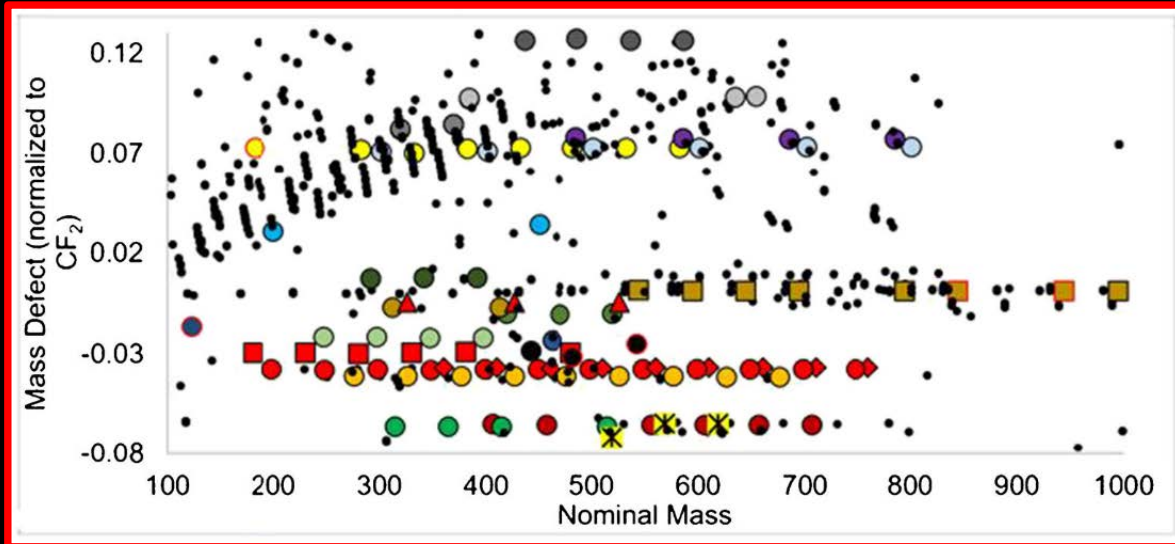
## Inform human biomonitoring



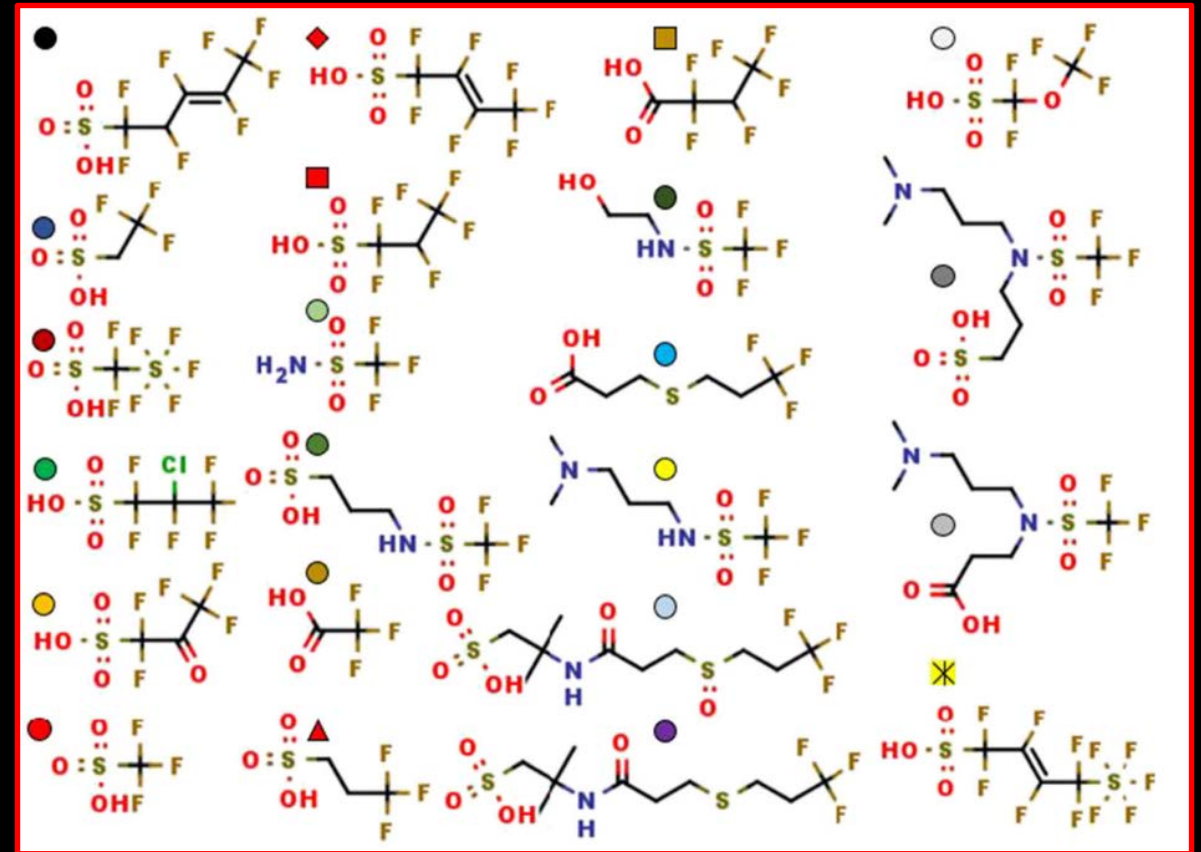
HRMS Libraries

# Aqueous Firefighting Foam (AFFF) Composition

Field-collected AFFF (primarily 3M Lightwater) characterized by iterative MS<sup>2</sup> and FluoroMatch screening software

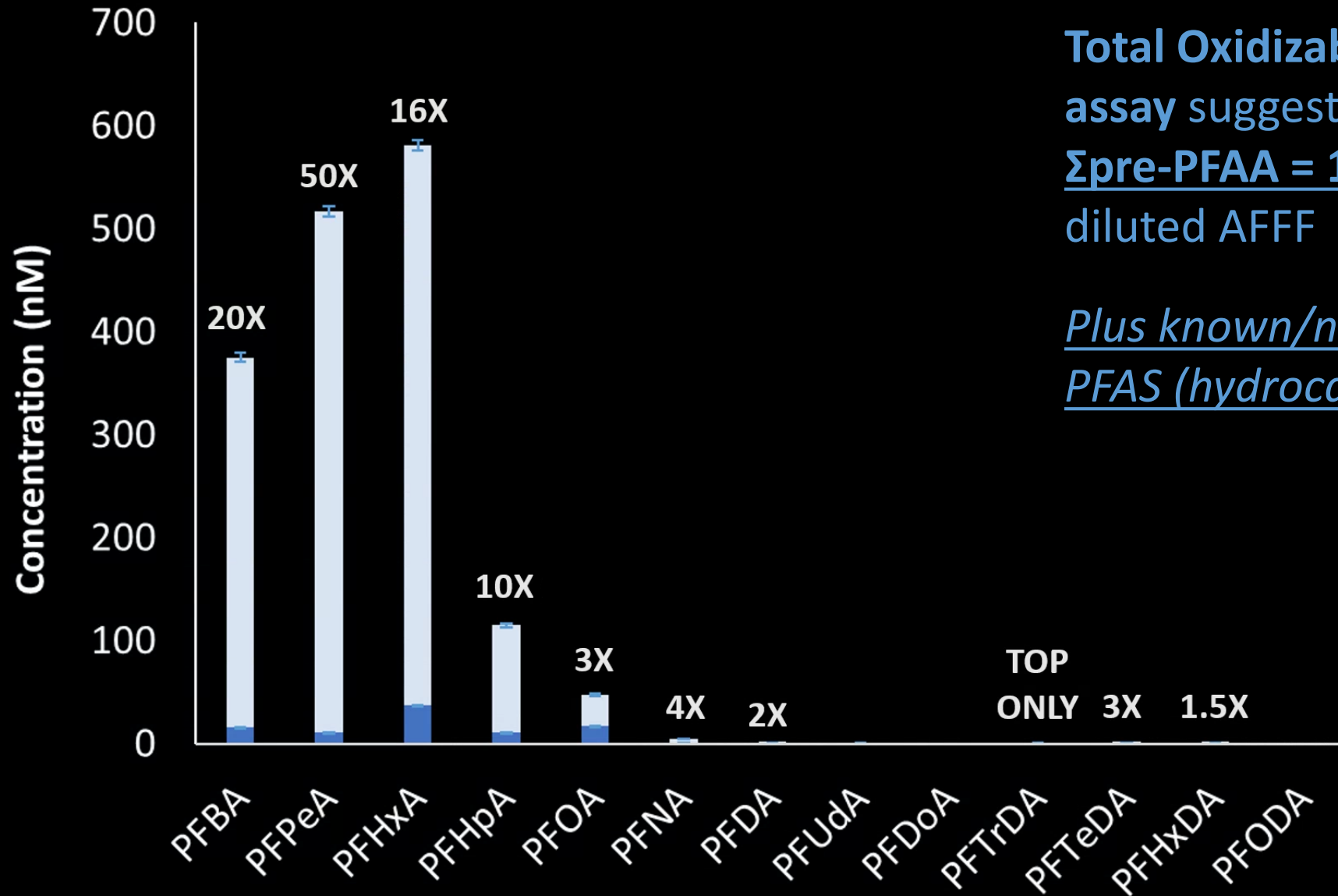


Koelmel et al., *Analytical and Bioanalytical Chemistry*, 2021





# 3M LW AFFF: What's Transformable (Oxidizable)?



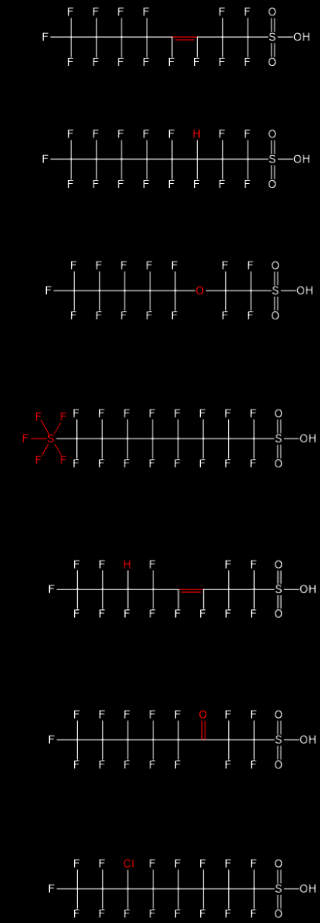
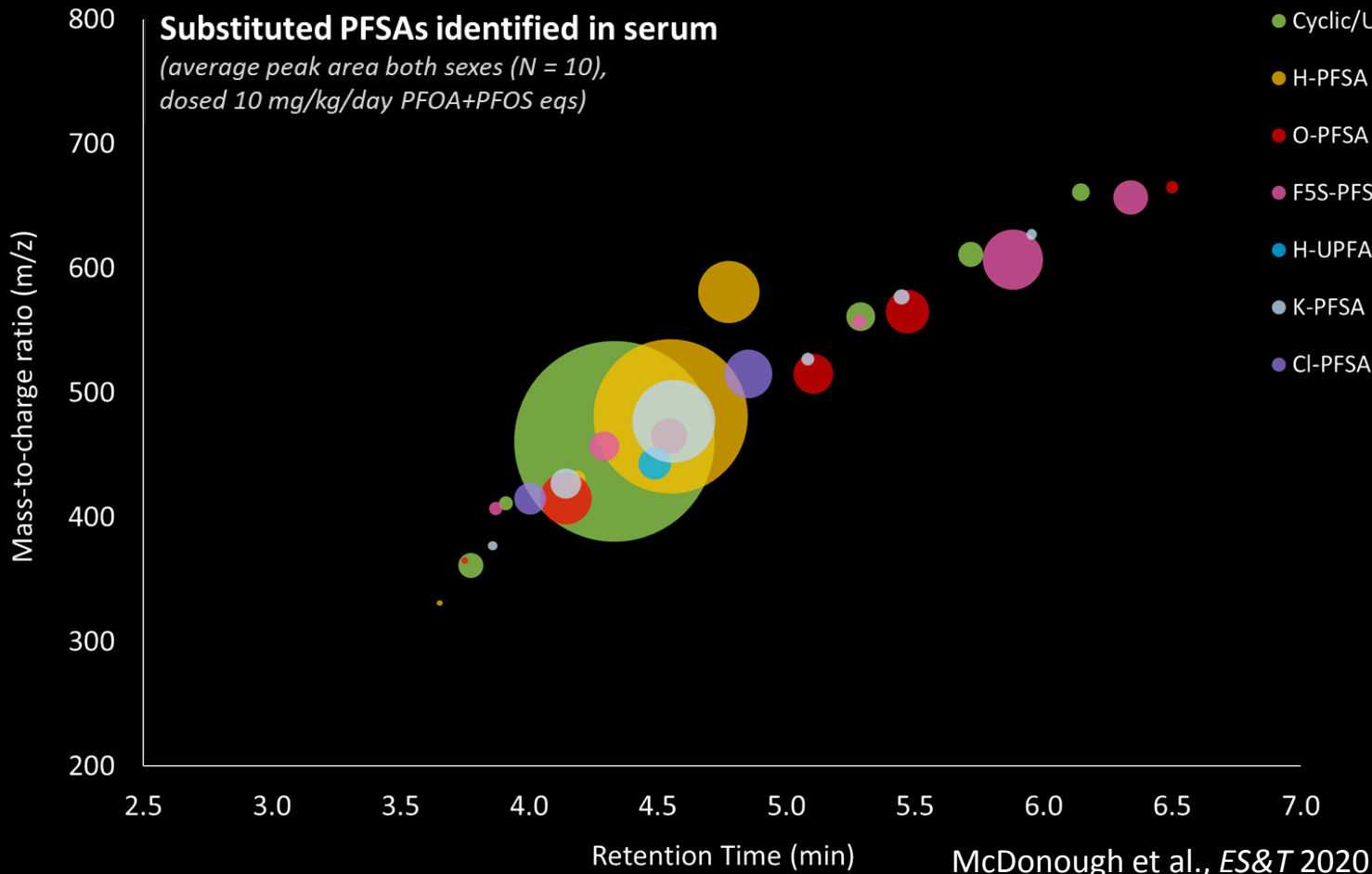
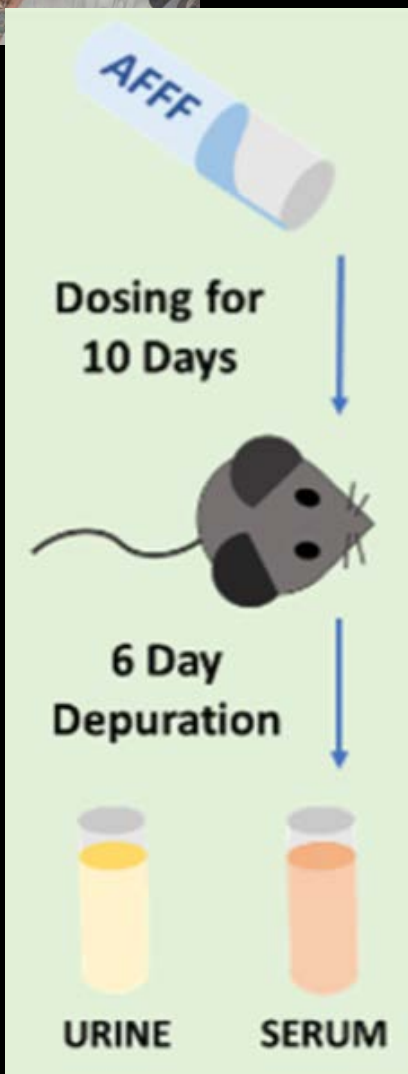
Total Oxidizable Precursor (TOP) assay suggests approximate  $\Sigma_{\text{pre-PFAA}} = 1500 \text{ nM}$  in 5000X diluted AFFF

Plus known/novel PFAAs AND non-PFAS (hydrocarbons, etc.)...

# AFFF PFAS Bioaccumulation *in vivo*



Jamie DeWitt, ECU





# What's Excreted?

Urine collected throughout dosing study; livers/kidneys at end

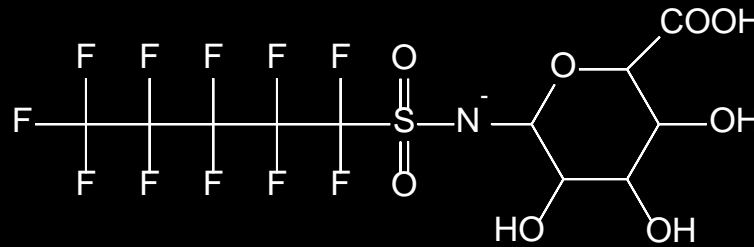


Iterative MS<sup>2</sup> on Agilent 6545 QToF-MS

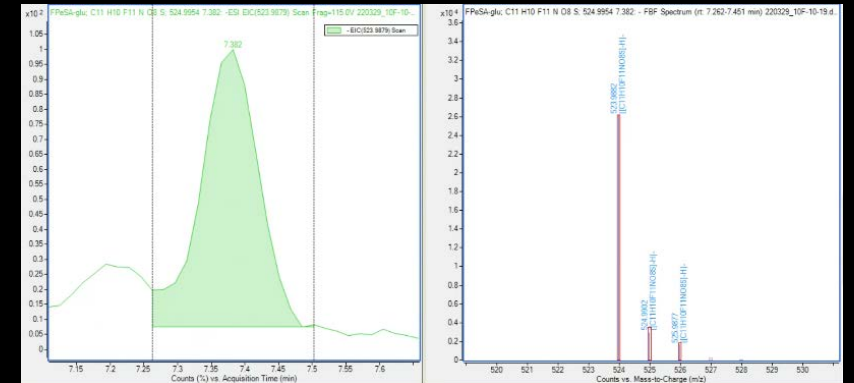


Suspect screening for previously observed and predicted phase II conjugates using Batch Targeted Feature Extraction in Agilent Profinder

## Glucuronidated perfluoroalkyl sulfonamides (C4-C6)

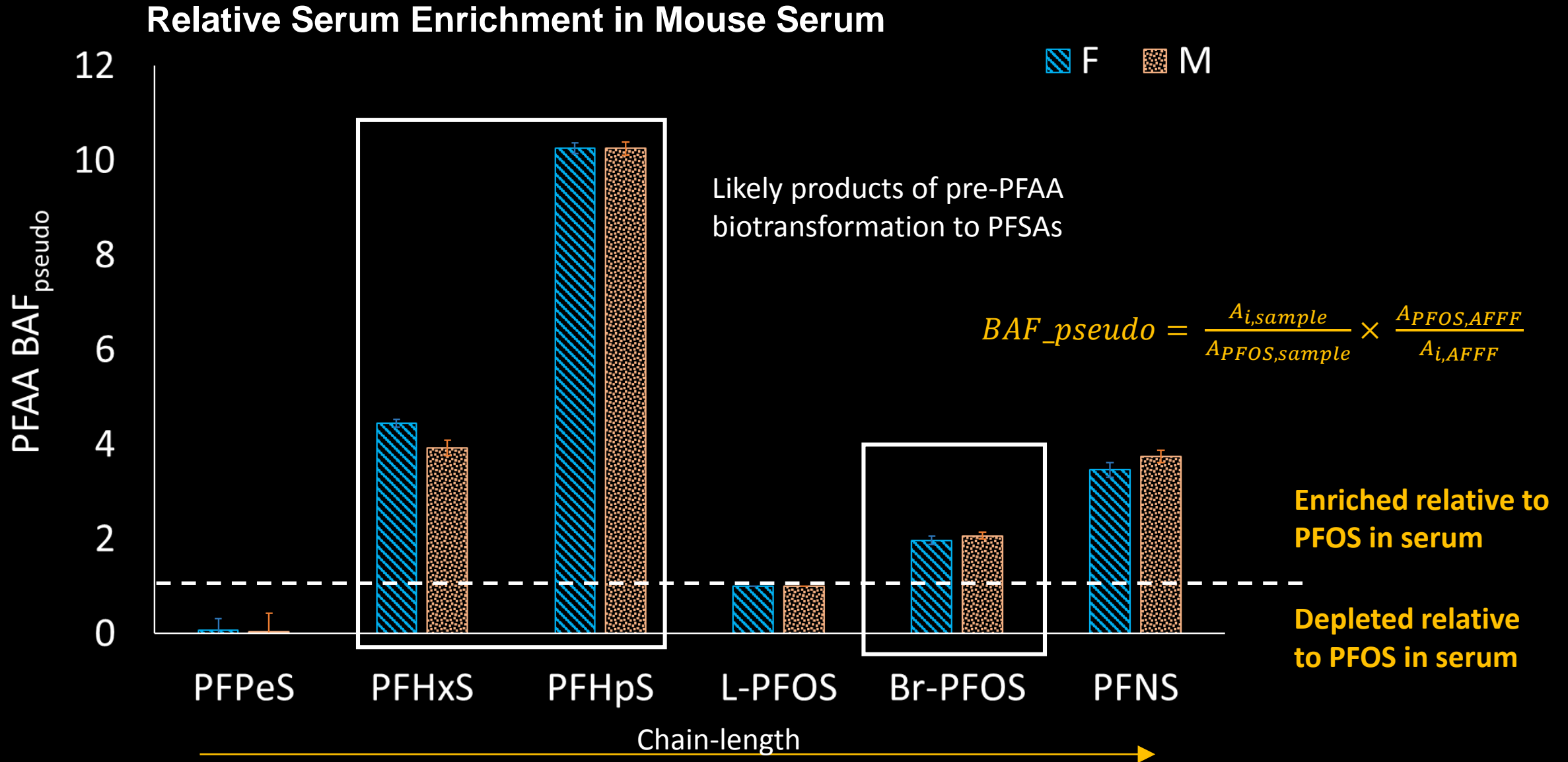


FPeSA-glu [C<sub>11</sub>H<sub>10</sub>F<sub>11</sub>NO<sub>8</sub>S]  
Δm/z 0.97 ppm  
Fragments: [C<sub>5</sub>F<sub>11</sub>];  
[C<sub>5</sub>F<sub>11</sub>SO<sub>2</sub>]; [C<sub>7</sub>F<sub>11</sub>SO<sub>3</sub>NH<sub>2</sub>]



Level 3 (Schymanski et al.)  
Level 3b (2022 PFAS Confidence Scale)

# What's Transformed?



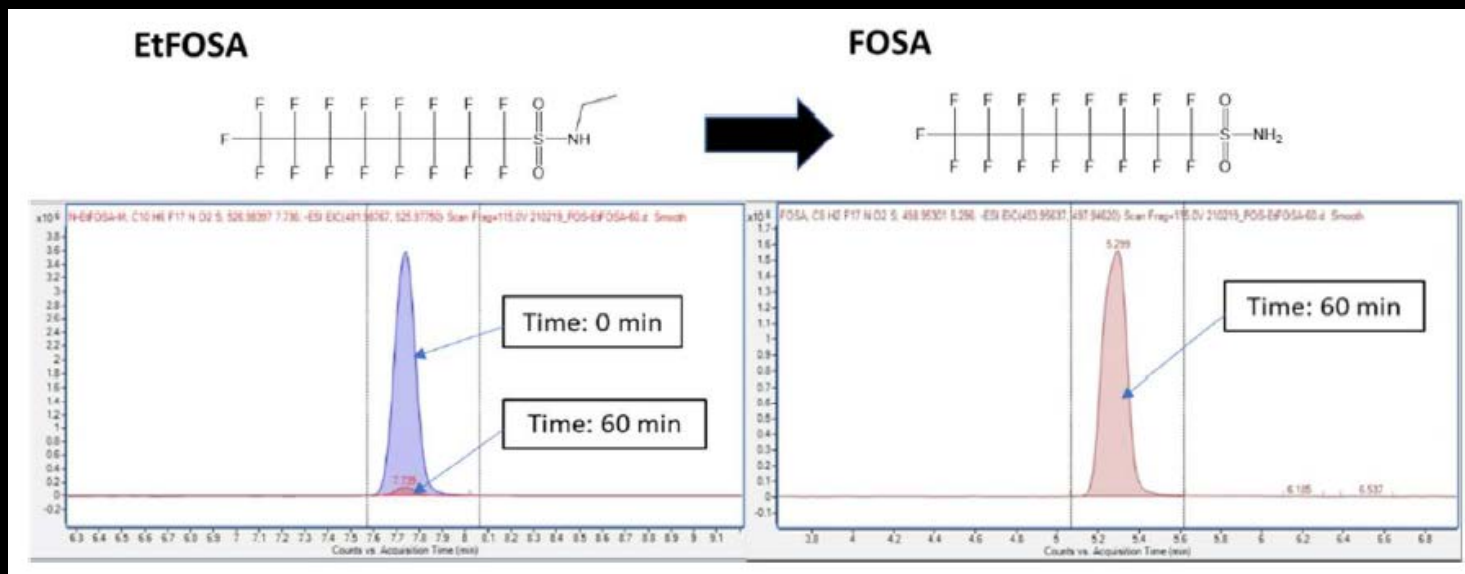
# Liver S9 Transformation of AFFF

Preliminary Data: C57BL/6 male mouse liver sub-cellular S9 fractions incubated with AFFF

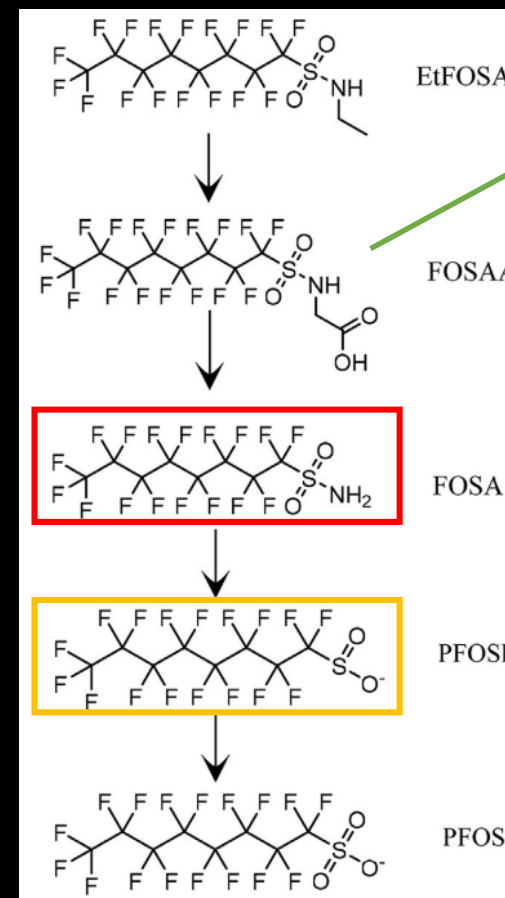
Positive Control: 40 nM EtFOSA + AFFF (7 nM  $\Sigma$ pre-PFAA)

## Dealkylation of EtFOSA

## Formation of FOSA

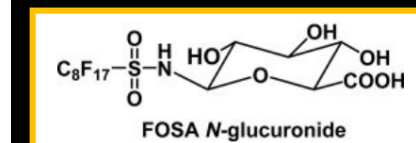


*FOSA, not PFOS, appears to be primary product*



AFFF-dosed with no EtFOSA (60 min):

FHxSAA (C6) [10<sup>5</sup>]  
FHpSAA (C7) [10<sup>4</sup>]



Joudan et al. TrAC 2020

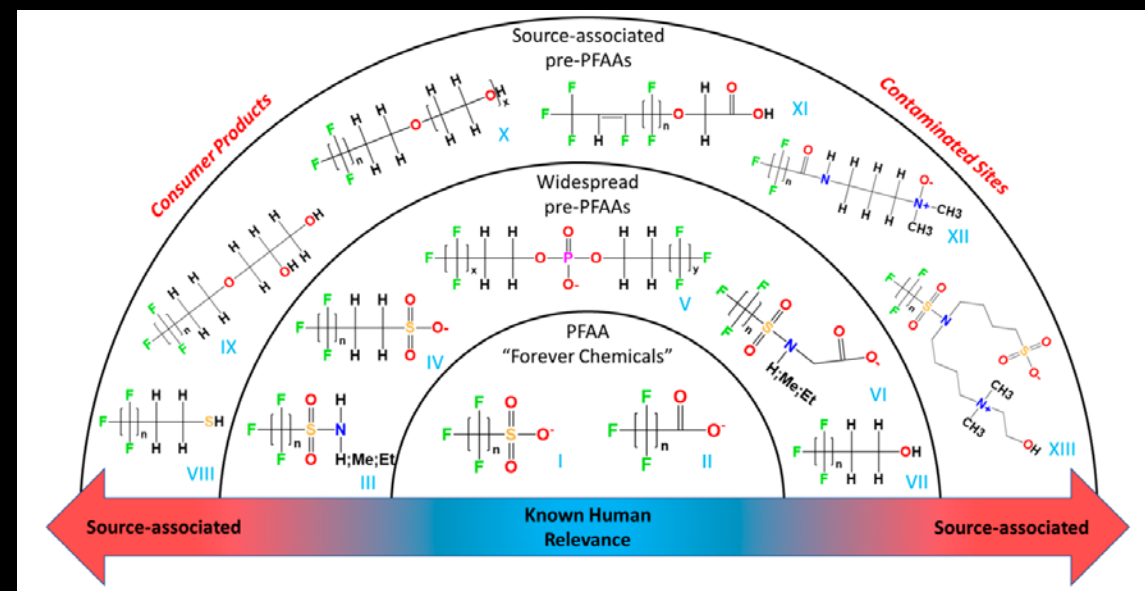
# Conclusions & Next Steps

## We Need to Test Exposure Relevant Mixtures

- Fractionation, purification, and bioaccumulation-directed prioritization of real mixtures containing knowns and unknowns

## We Need to Develop MS/MS Libraries for Biologically-Relevant PFASs and PFAS TPs

- *In vitro* formation through dosing studies → inform *in silico* fragmentation
- Moving towards **FluoroMatch + BioTransformer** for more comprehensive metabolite coverage
- More clues on sources and ADME from novel compounds



# Acknowledgments



## Collaborators:

**Agilent Technologies, Inc.:** Emily Parry, Tarun Anumol, Doug Postl

**University of Arizona:** Jefferey Burgess

**Colorado School of Mines:** Christopher Higgins; Sarah Choyke

**East Carolina University:** Jamie DeWitt

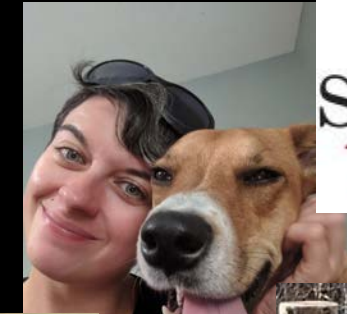
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**SBU School of Medicine:** Bruce Demple; Benjamin Luft; Sean

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# Questions?

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