Rapid assessment bioaccumulation screening (RABS) for per-and polyfluoroalkyl substances in mice exposed to industrially impacted surface water

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SRP E-Learning Webinar Series

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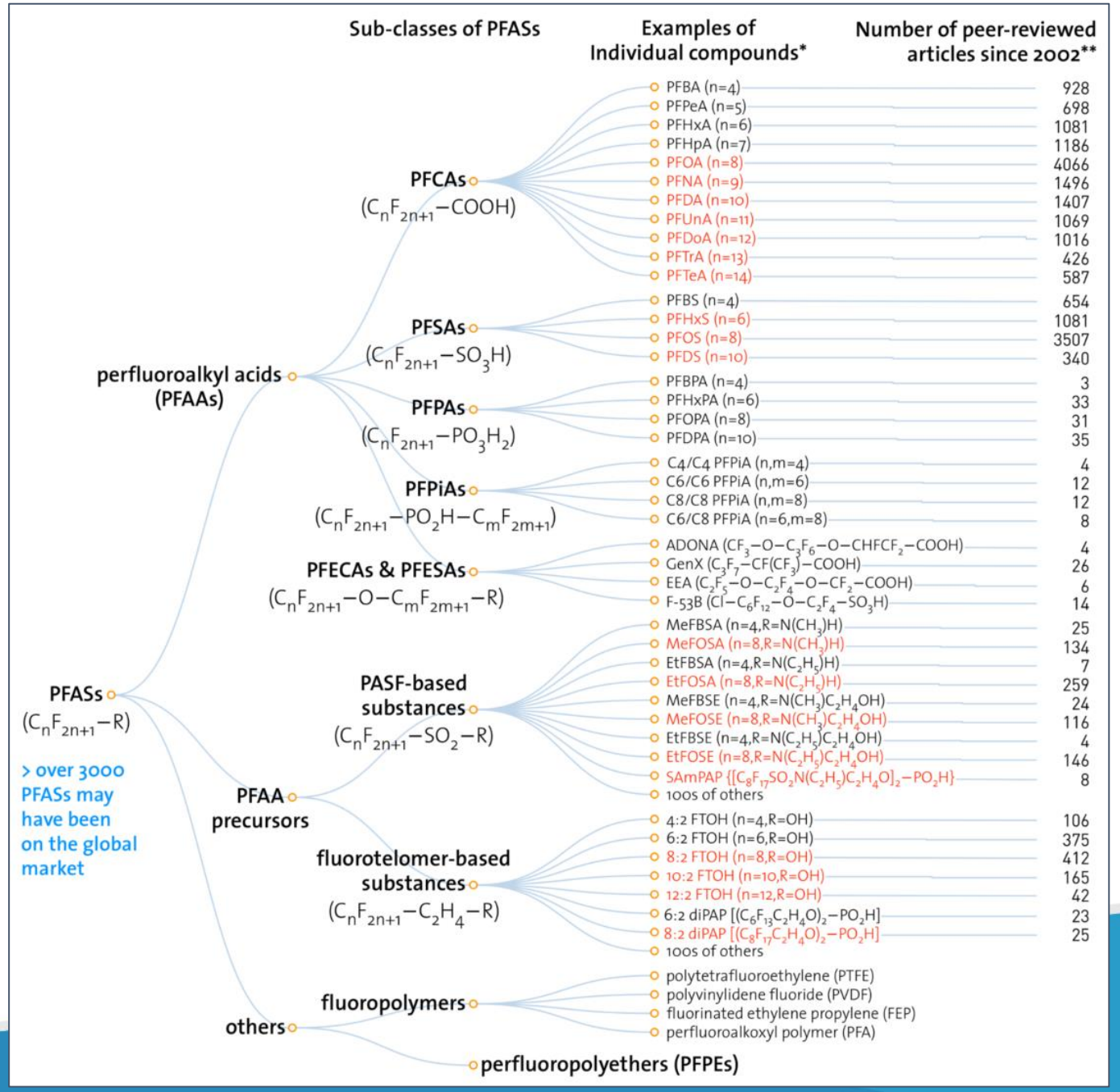
Office of Research and Development
Center for Environmental Measurement and Modeling

PFAS introduction

- Per- and polyfluoroalkyl substances (PFAS)
- Identified by a Carbon Fluorine moiety ($-C_nF_{2n+1}$) within their structure
- Estimated over 3,ooo individual PFAS chemicals on the market (Wang et al. 2017)



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What is Mass Spectrometry?

1 mg

=

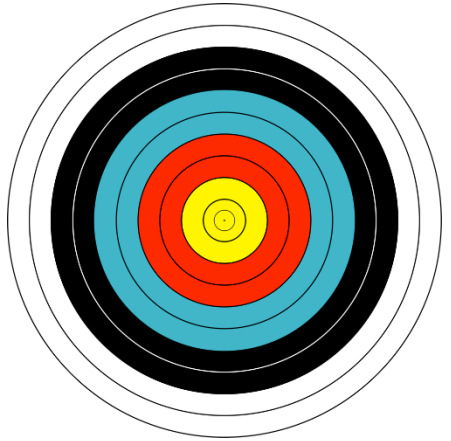
1.66e-24 mg

Various mass spectrometry methods for investigating PFAS

Targeted - analyzing for a suite of analytes with a validated method

e.g. How much PFOA is in my water?

- Reference standard needed



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Low Resolution Mass Spectrometry (LRMS)

High Resolution Mass Spectrometry (HRMS)

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Non-Targeted - no preconceived notion of chemical present

e.g. What chemicals are in my water?

- Informatics needed

↙ ↘

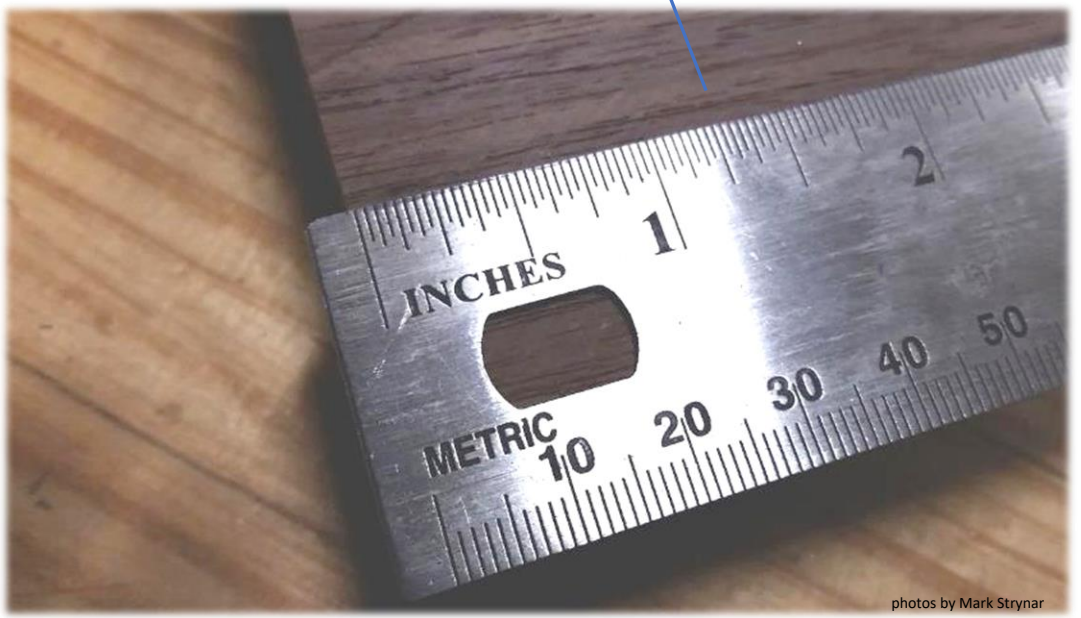
Suspect Screening – screening against a database of chemicals

NTA Discovery – novel compound identification

Low Resolution VS High Resolution
Mass Accuracy

PFOA: MW 413.0 +/- 0.1 to 0.5 Da

MW 412.9964 +/- 0.0004 Da (1ppm)



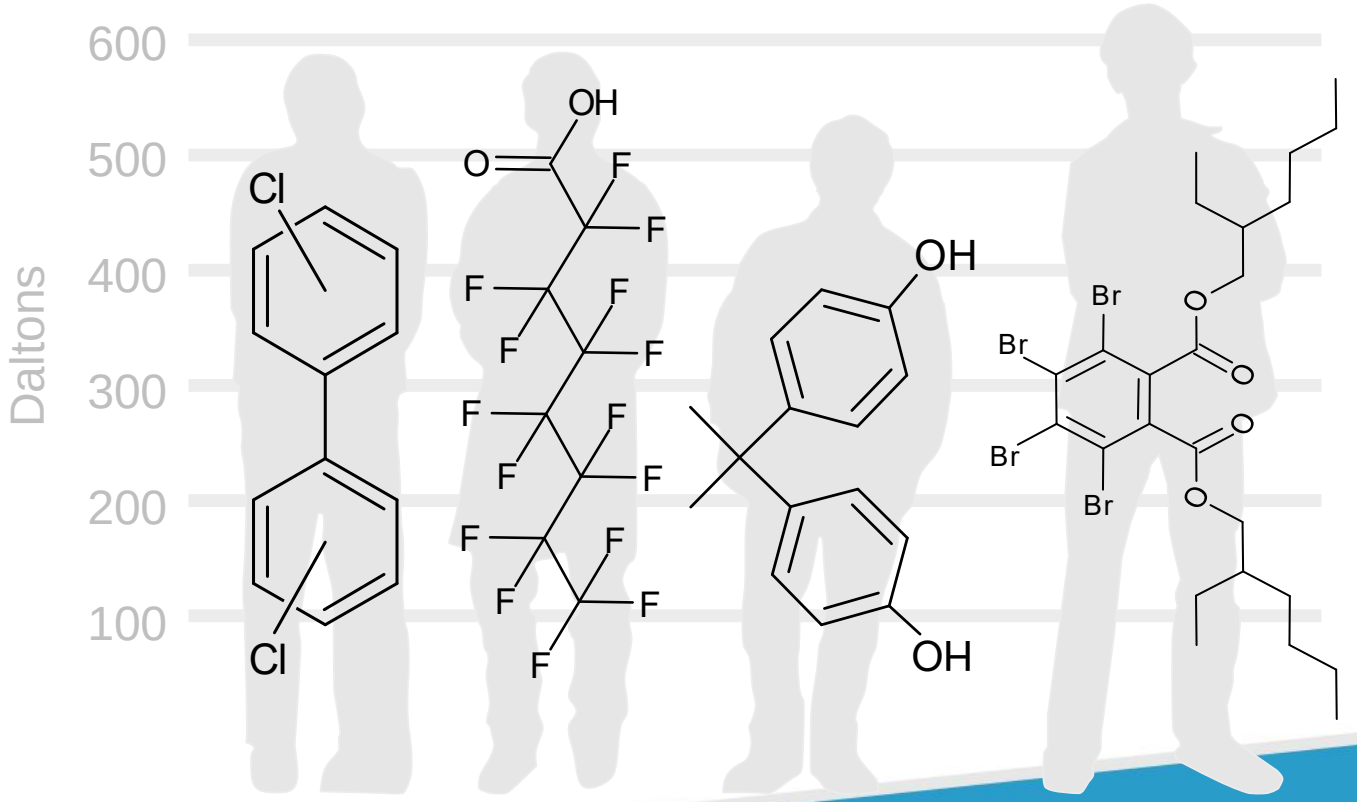
Mass accuracy is the ability to measure or calibrate the instrument response against a known entity. Usually expressed in parts per million (ppm), the measurement indicates the deviation of the instrument response from a known.

Balogh 2004 Debating Resolution and Mass Accuracy
LCGC NORTH AMERICA VOLUME 22 NUMBER 2

SRM 2585 dust example
Matched >90 (n=157) (4.2%)
Matched 80-90 (n=161)
Matched 70-80 (n=139)
Matched <70 or Unmatched (n=3,304, n= 3,060 unmatched) (81.4%)
m/z
RT (min)
0
200
400
600
800
1000
1200
1400
1600
0
5
10
15
20
25
30
35
40
45

Building a Case for Identification

- Some key tools:
  - Abundant peaks
  - Contain halogens (F, Cl, Br)
    - Isotopic signature



Key tools: Isotopic signature

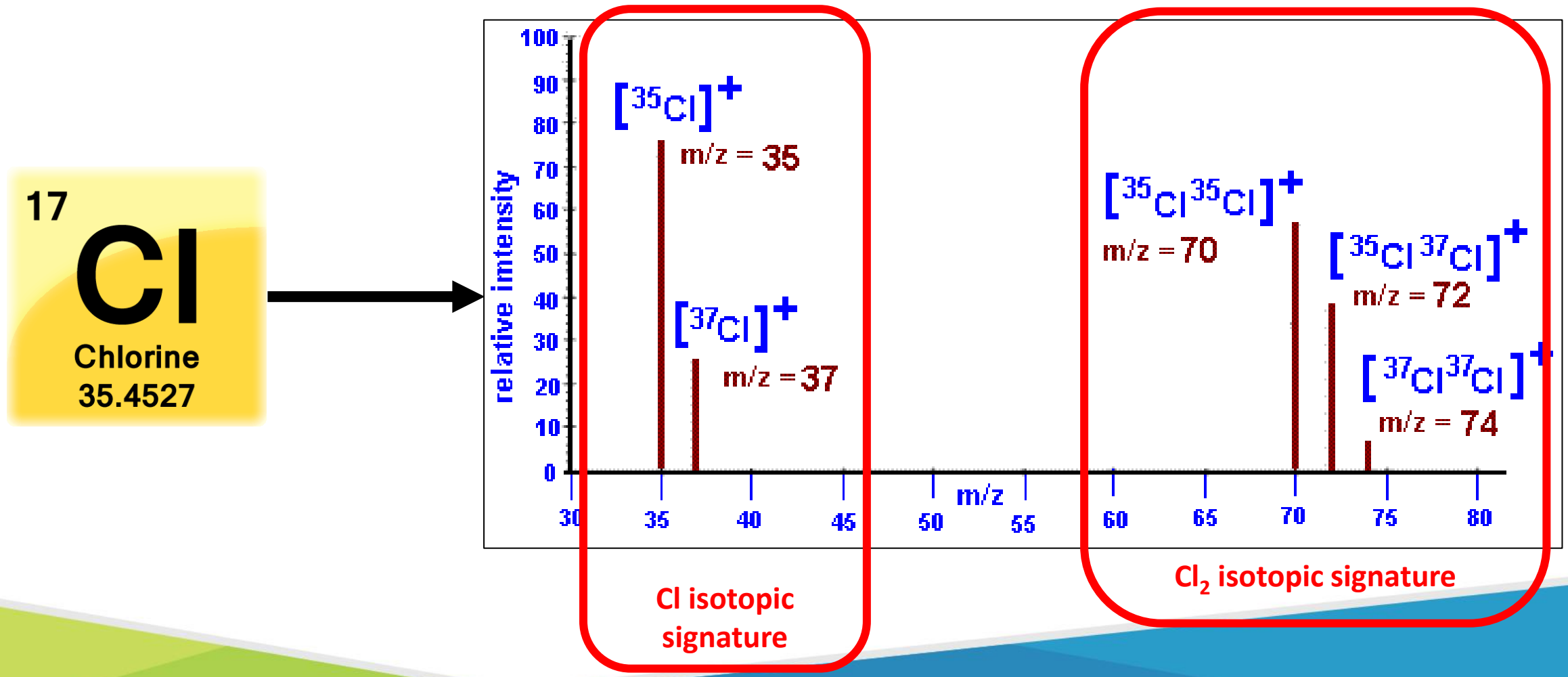
ISOTOPES

ISOTOPES OF CHLORINE

	$^{35}_{17}Cl$	$^{37}_{17}Cl$
ATOMIC NUMBER	17	17
MASS NUMBER	35	37
PROTONS	17	17
NEUTRONS	18	20
ABUNDANCE	75%	25%
RATIO	3 :	1

Varying number of neutrons

Key tools: Isotopic signature



Building a Case for Identification

- Some key tools:
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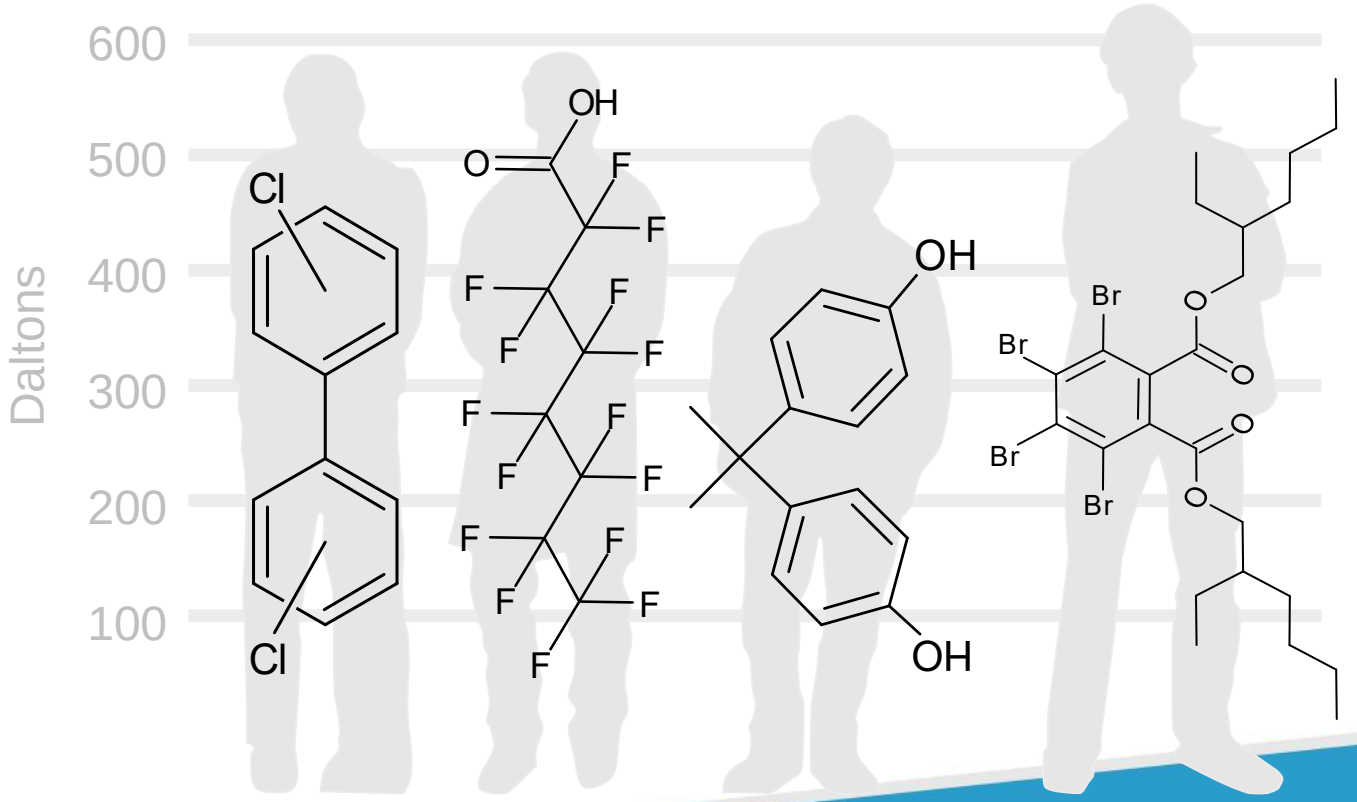
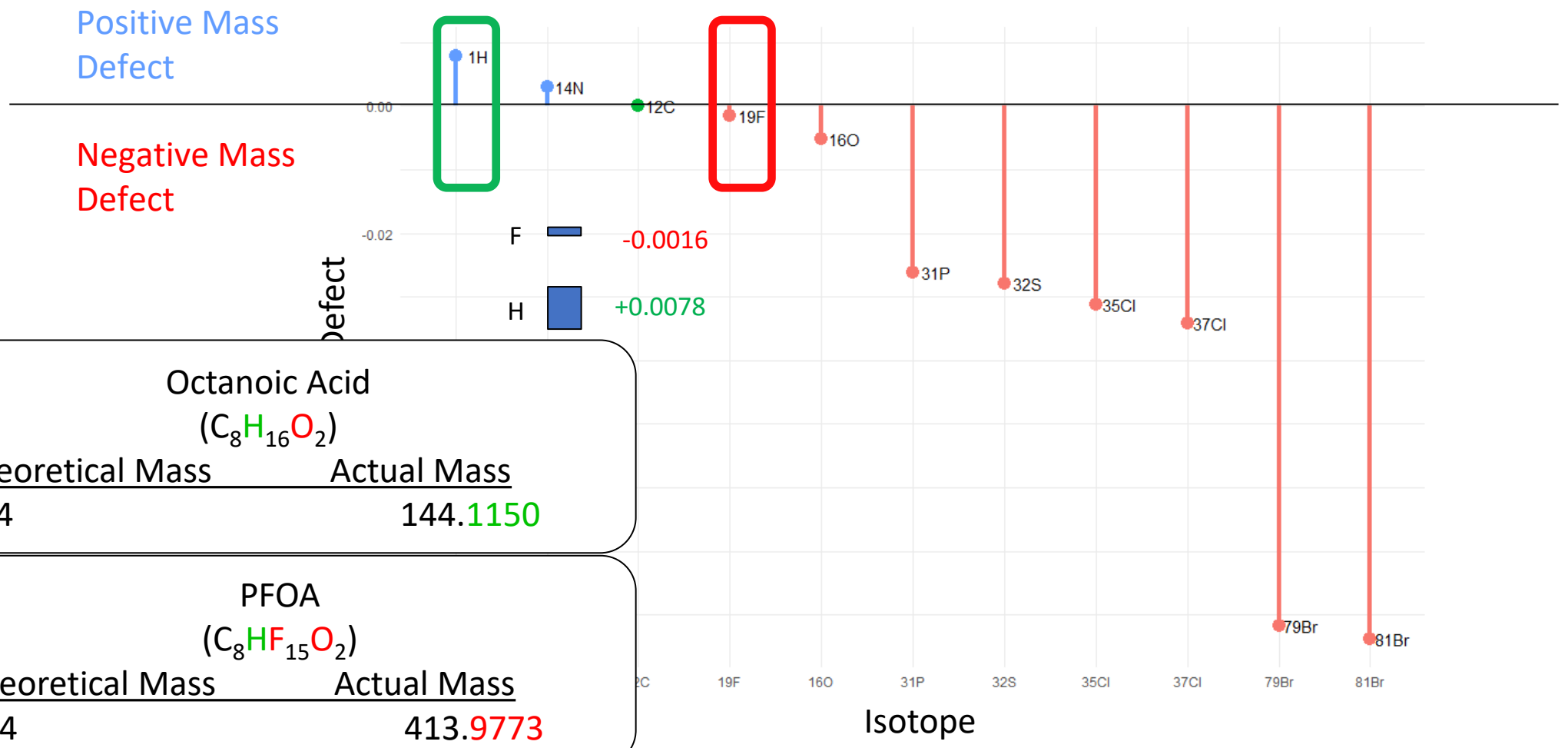
    - Negative mass defect



Key tools: Negative Mass Defect



Octanoic Acid

$(C_8H_{16}O_2)$

Theoretical Mass	Actual Mass
144	144.1150

PFOA

$(C_8HF_{15}O_2)$

Theoretical Mass	Actual Mass
414	413.9773

Building a Case for Identification

- Some key tools:
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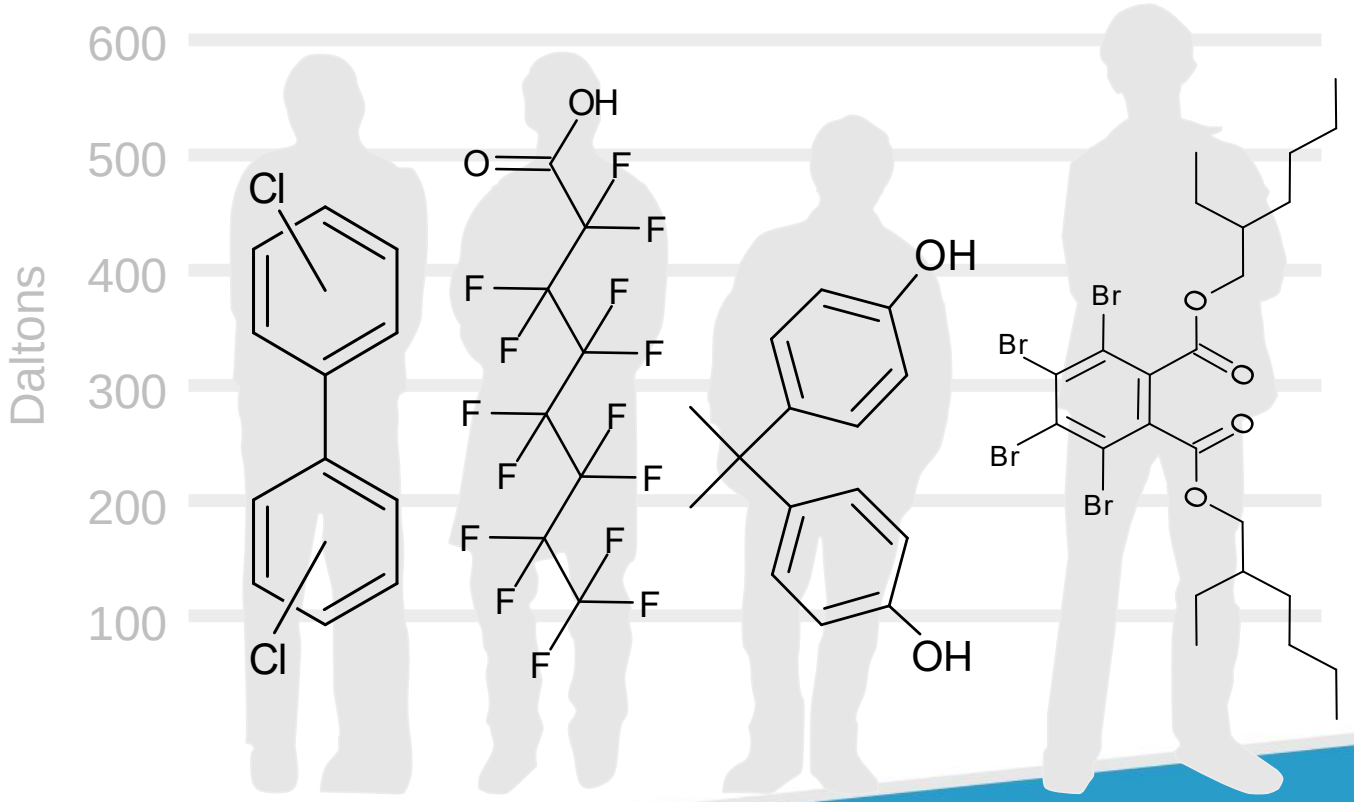
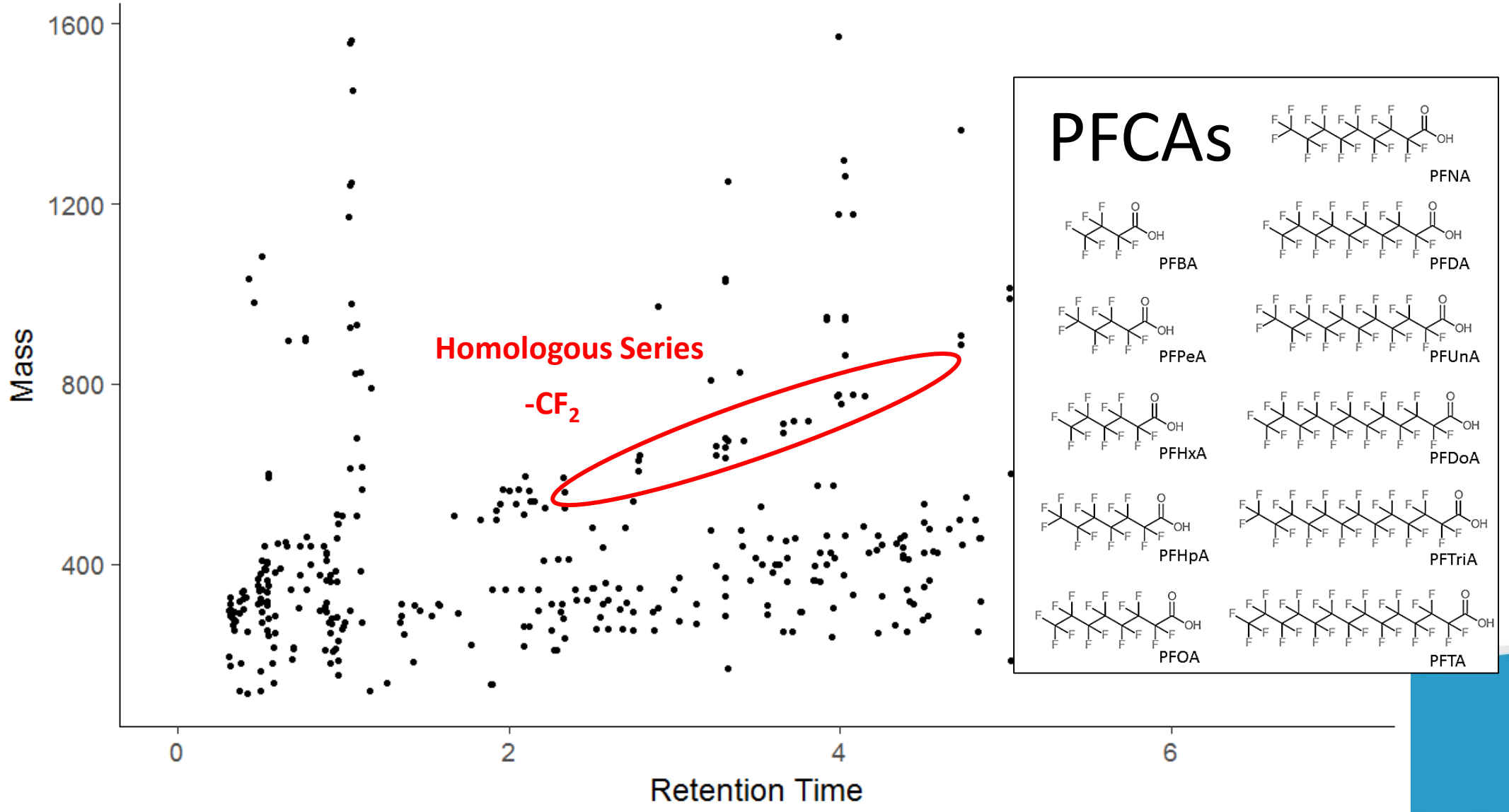
  - Contain halogens (F, Cl, Br)
    - Isotopic signature
    - Negative mass defect
  - Homologous series

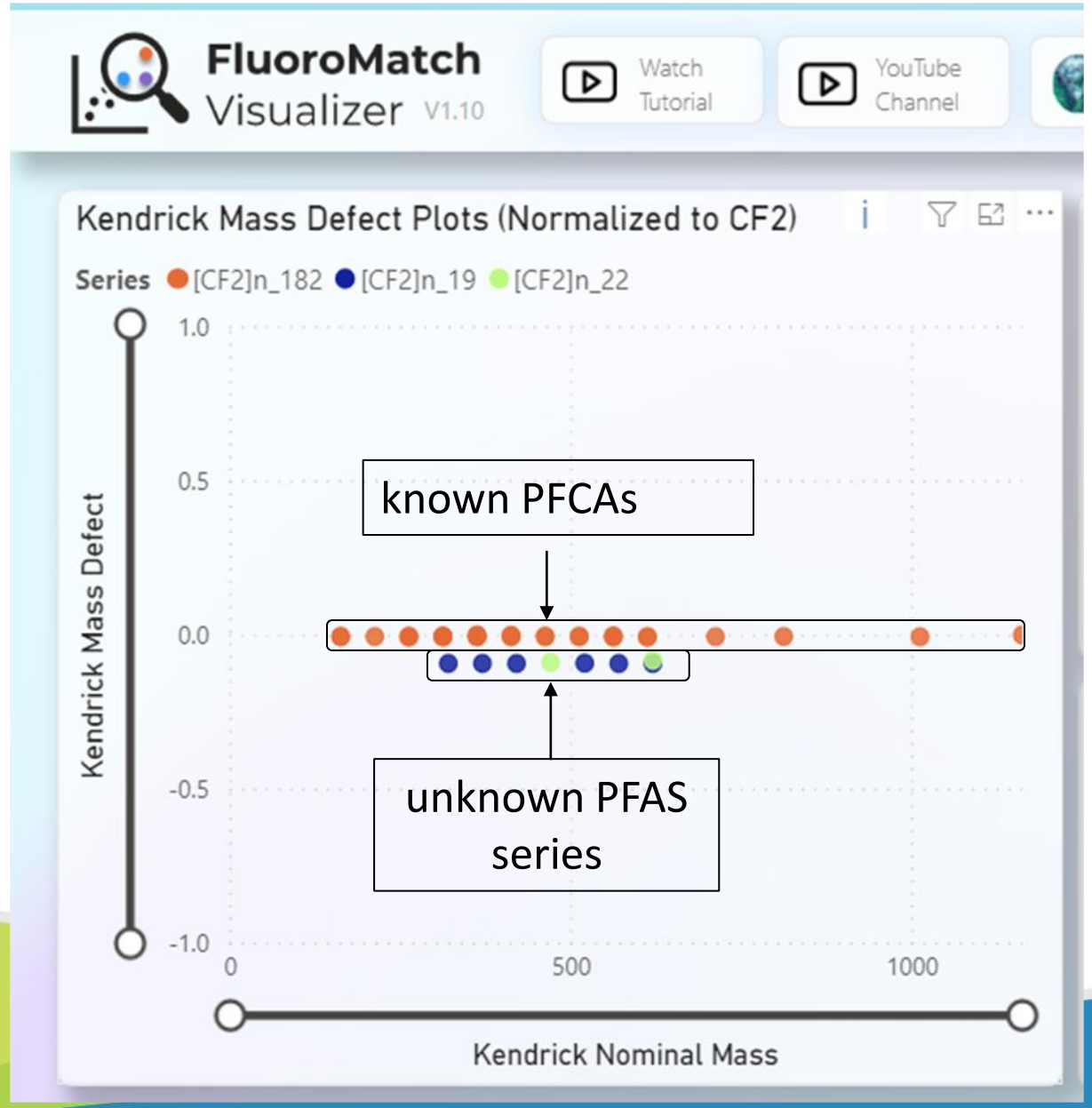


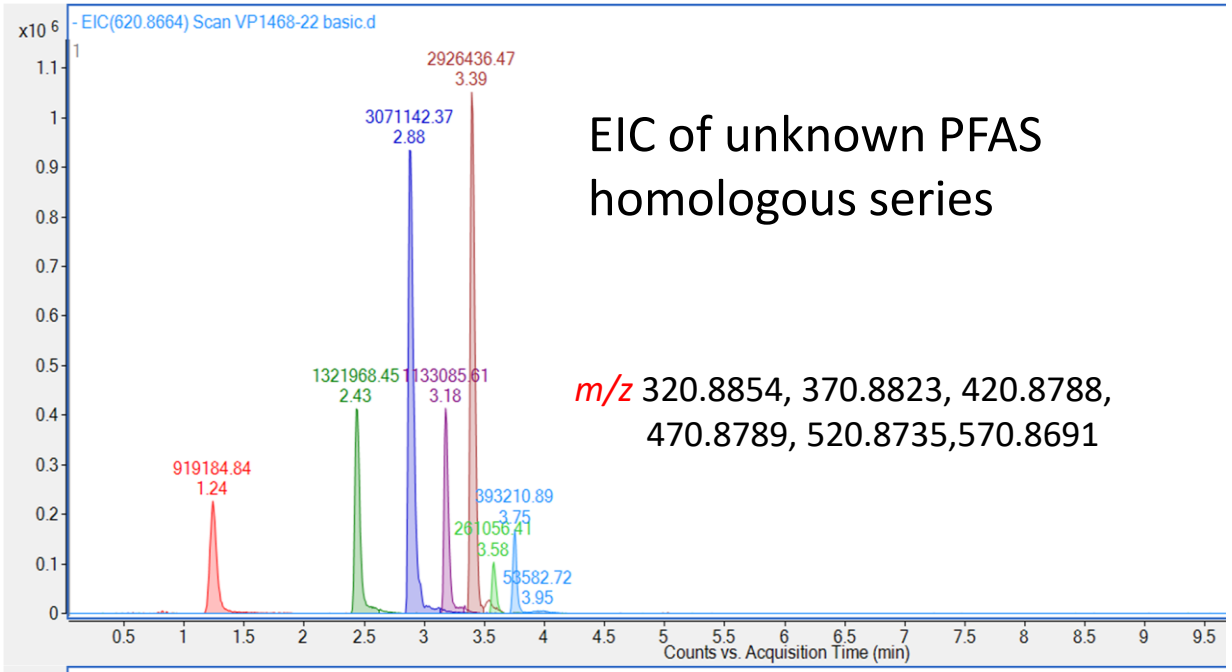
Key tools: Homologous series

A series of organic compounds of the same family which differ by a common structural unit, For PFAS, that structural unit is often a CF2 unit.



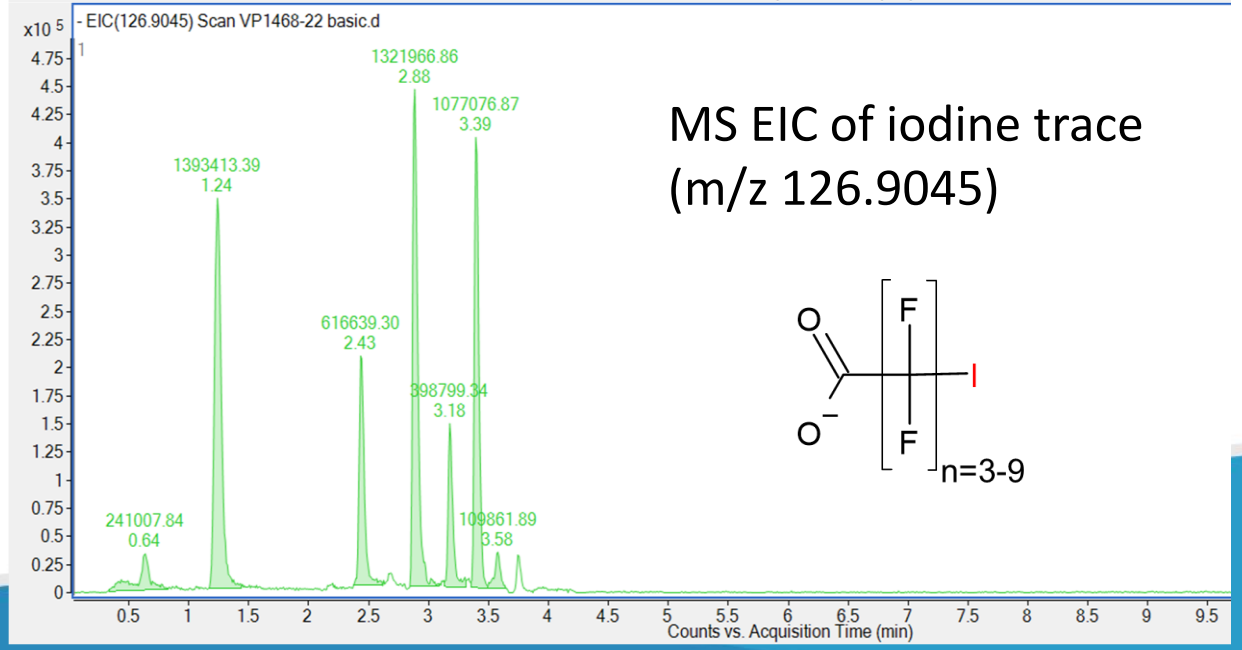
Key tools: Homologous series





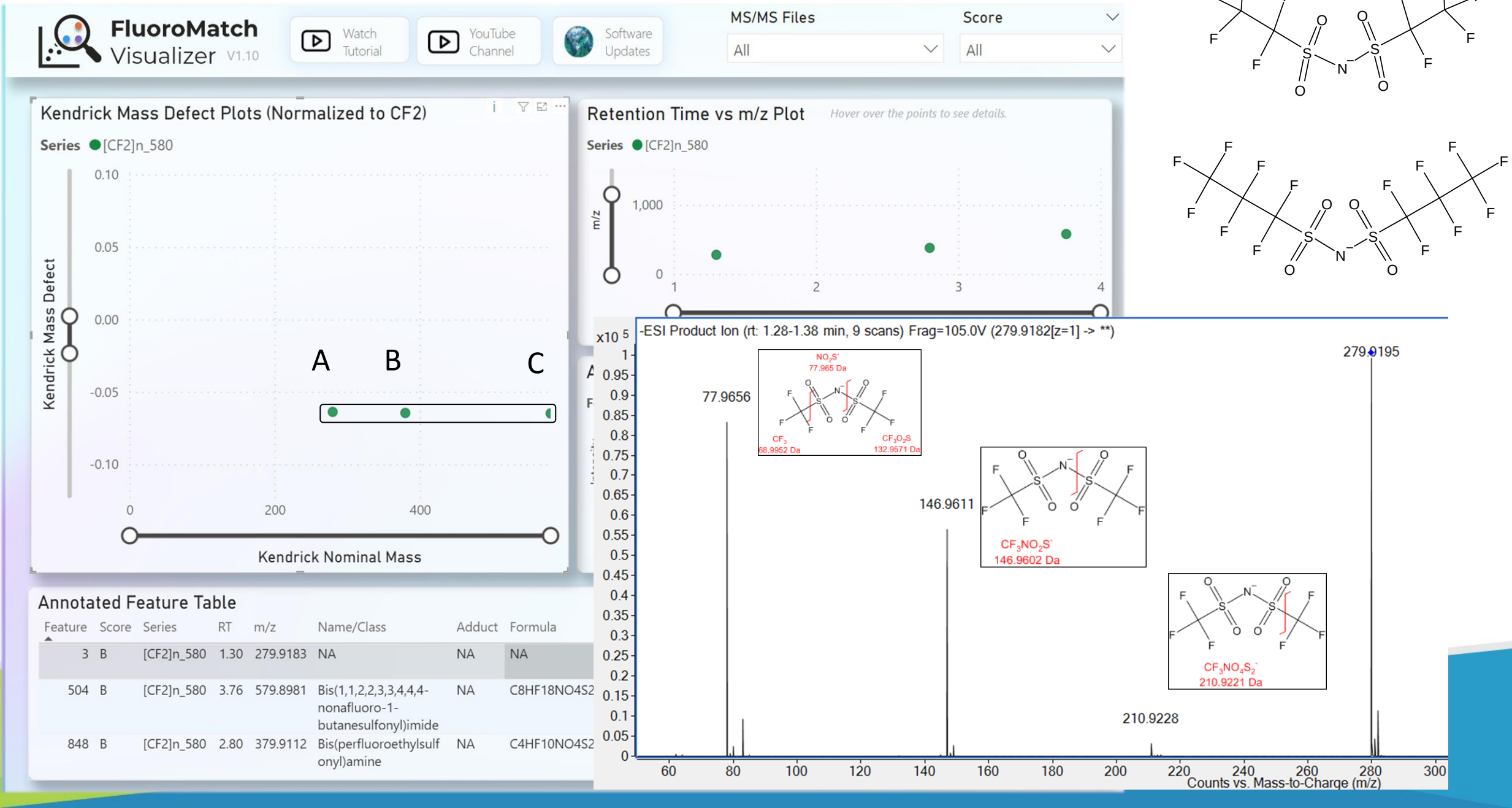
EIC of unknown PFAS homologous series

m/z 320.8854, 370.8823, 420.8788, 470.8789, 520.8735,570.8691

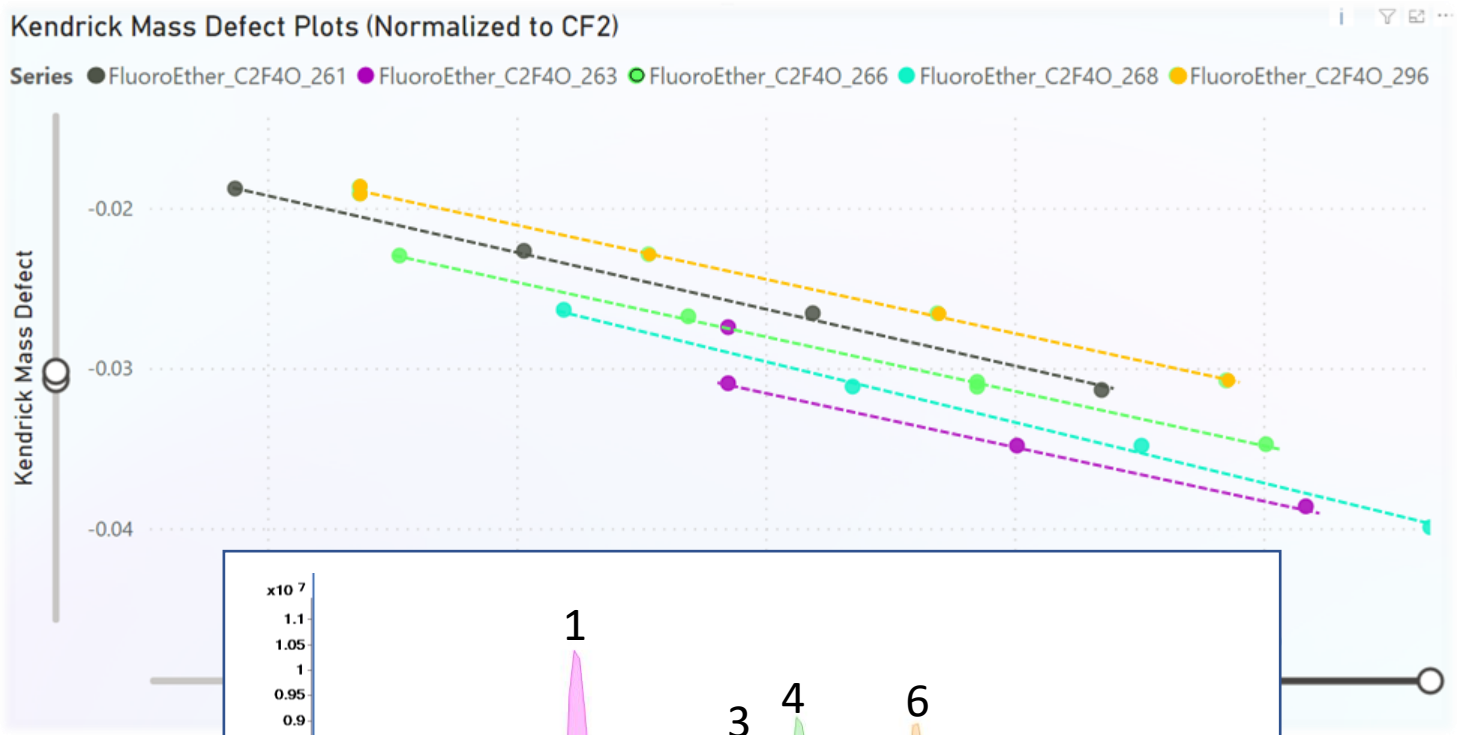


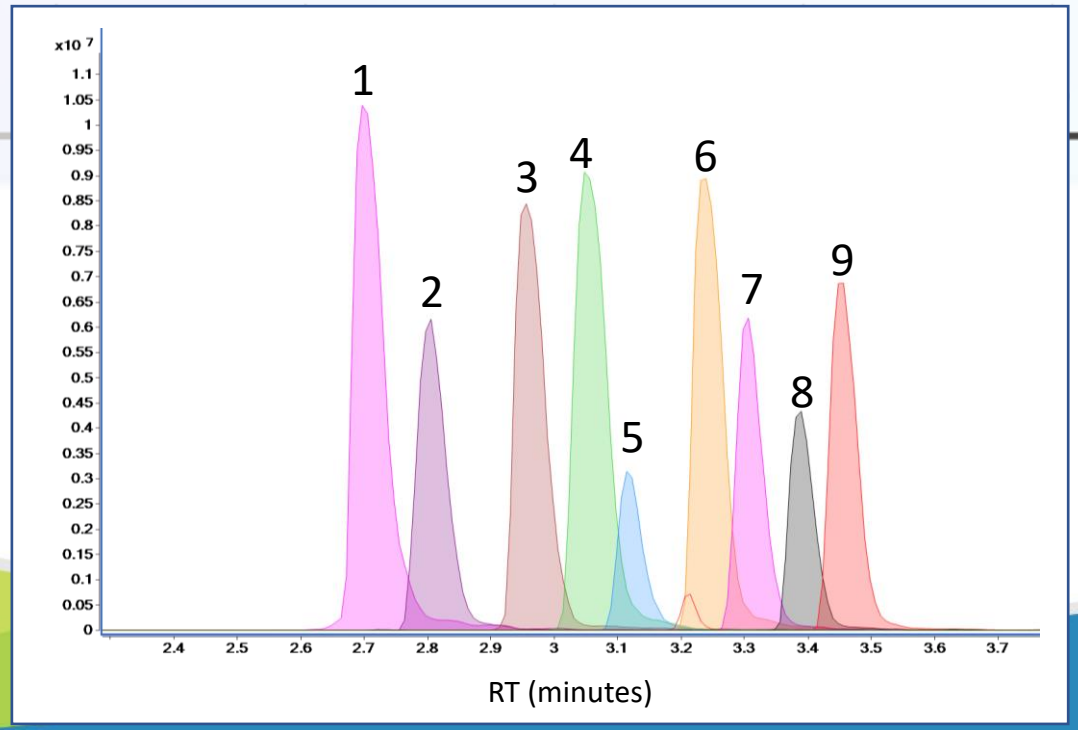
MS EIC of iodine trace (m/z 126.9045)

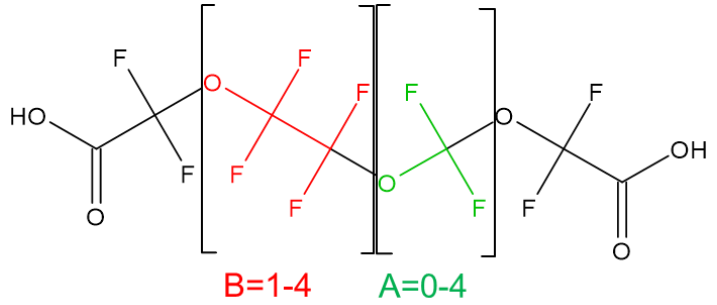
Key tools: Homologous series



Key tools: Homologous series







Series	Color	RT	m/z	Peak	Isomer
FluoroEther_C2F4O_261		0.97	386.9565		
FluoroEther_C2F4O_296		1.82	436.953		
FluoroEther_C2F4O_296		2.15	436.9534		
FluoroEther_C2F4O_266		2.36	452.9481		
FluoroEther_C2F4O_261		2.84	502.9452	1	AB2
FluoroEther_C2F4O_268		2.87	518.9405	2	A3B
FluoroEther_C2F4O_296		2.98	552.9418	3	B3
FluoroEther_C2F4O_266		3.09	568.9369	4	A2B2
FluoroEther_C2F4O_263		3.15	584.9317	5	A4B
FluoroEther_C2F4O_263		2.69	584.9352		
FluoroEther_C2F4O_261		3.27	618.9339	6	AB3
FluoroEther_C2F4O_268		3.33	634.9283	7	A3B2
FluoroEther_C2F4O_296		3.37	668.9307	8	B4
FluoroEther_C2F4O_266		3.46	684.9251	9	A2B3
FluoroEther_C2F4O_266		3.22	684.9254		
FluoroEther_C2F4O_263		3.52	700.9204		
FluoroEther_C2F4O_261		3.58	734.9217		
FluoroEther_C2F4O_268		3.62	750.9172		
FluoroEther_C2F4O_296		3.70	784.9191		
FluoroEther_C2F4O_266		3.74	800.9141		
FluoroEther_C2F4O_263		3.82	816.9092		
FluoroEther_C2F4O_268		3.95	866.9047		

Building a Case for Identification

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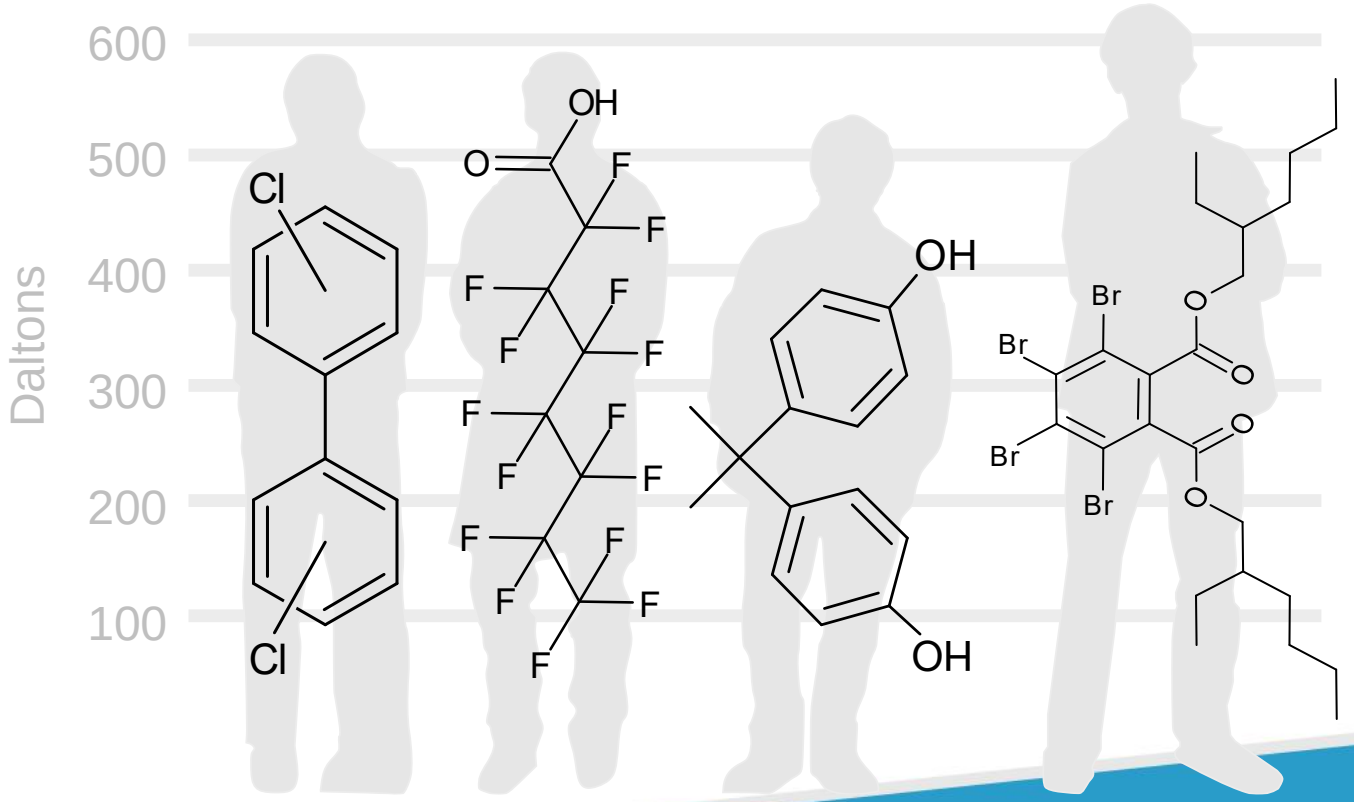
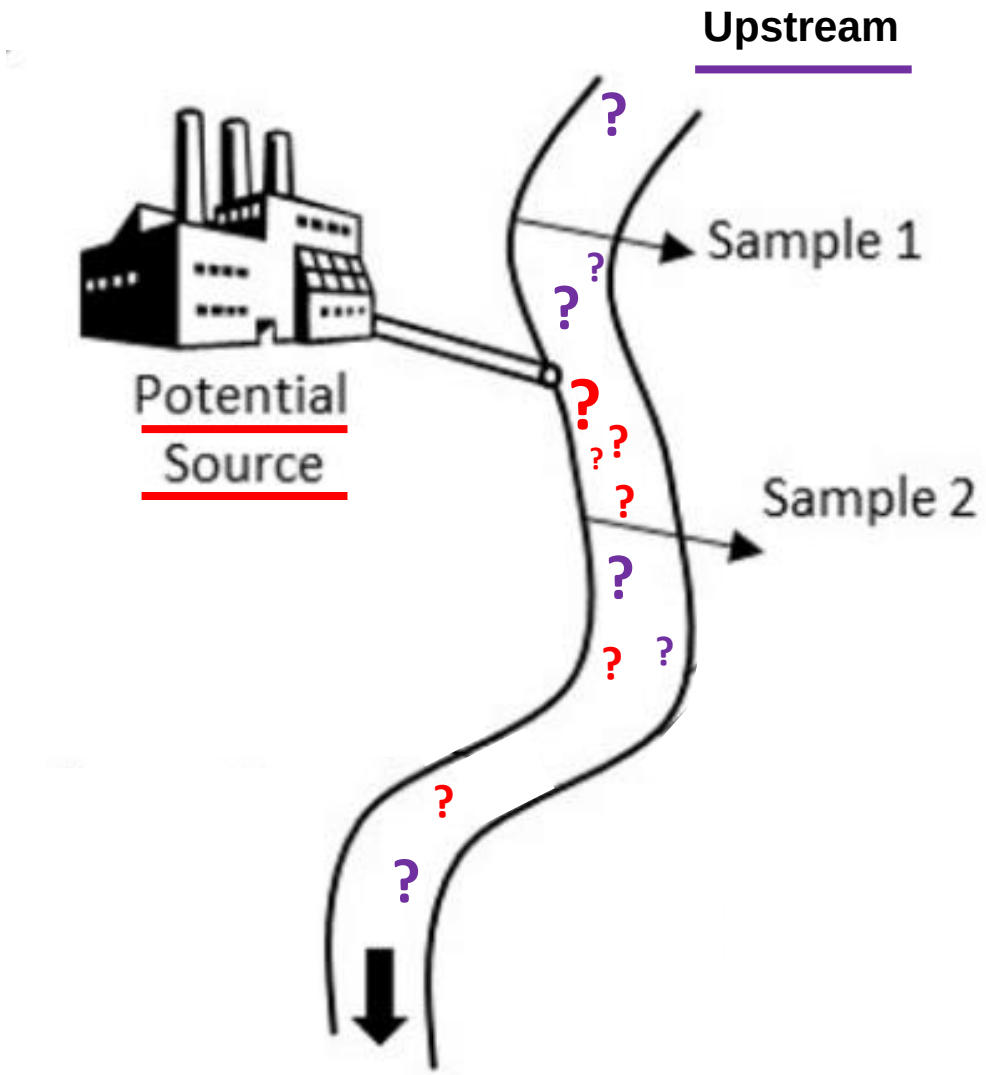
    - Isotopic signature
    - Negative mass defect
  - Homologous series
  - Background filtering

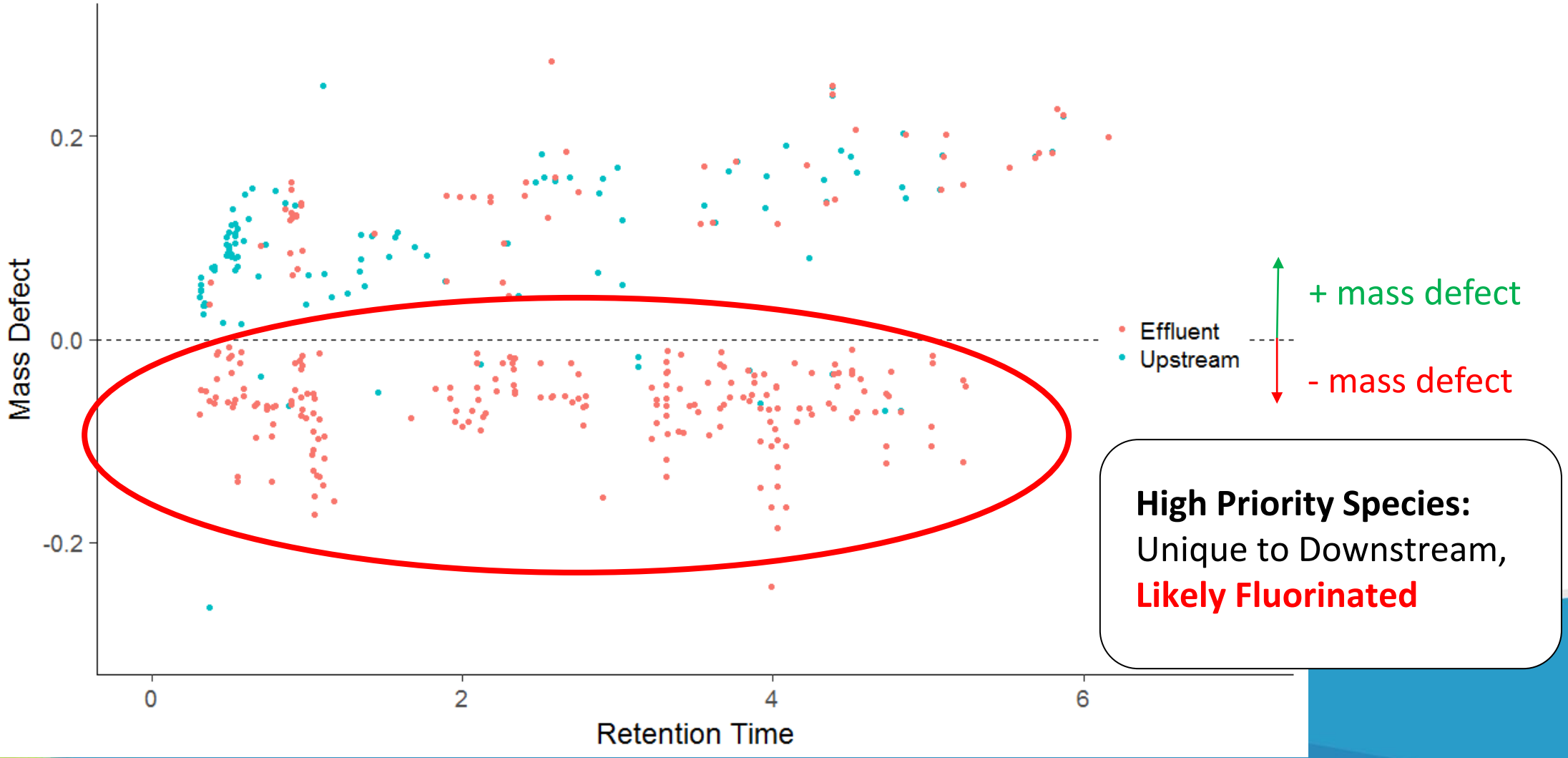


Identifying emerging PFAS point sources

HDPE Bottles



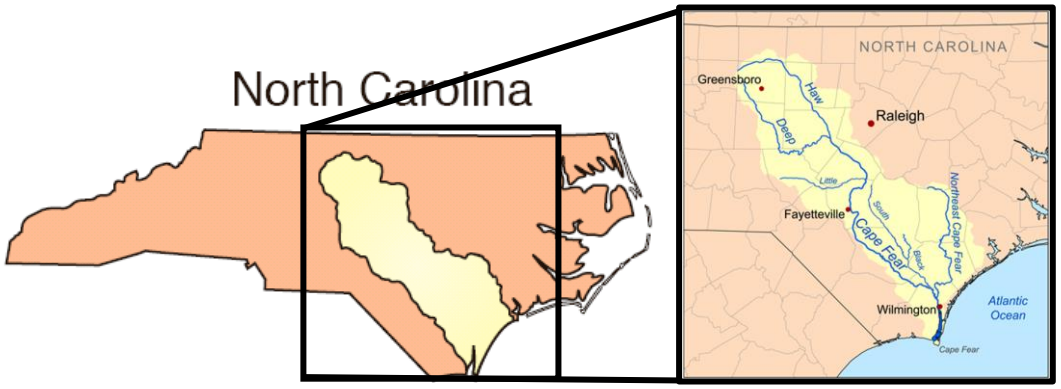
Identifying emerging PFAS point sources

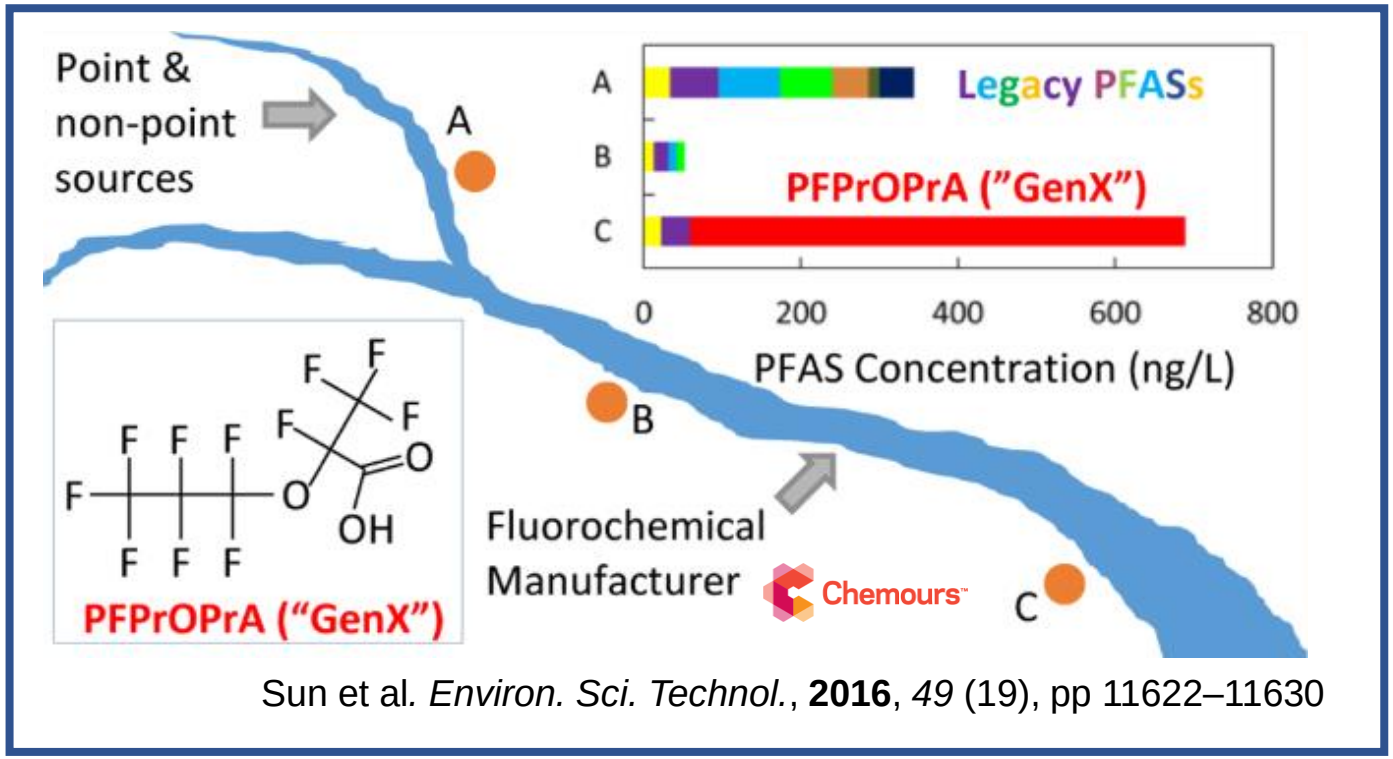


High Priority Species:
Unique to Downstream,
Likely Fluorinated

Identifying emerging PFAS point sources

Cape Fear River, NC

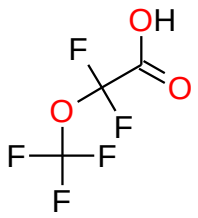




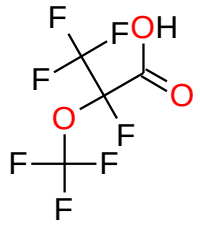
Sun et al. *Environ. Sci. Technol.*, **2016**, *49* (19), pp 11622–11630

Novel PFAS in the CFR

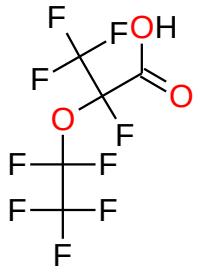
Monoether PFECAs



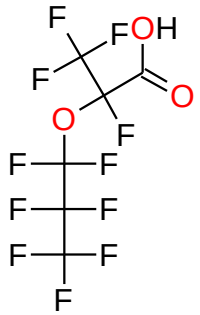
PFMOAA



PMPA

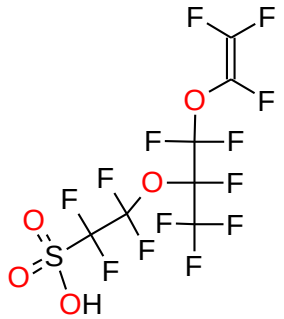


PEPA

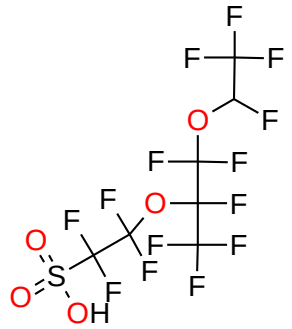


GenX; HFPO-DA

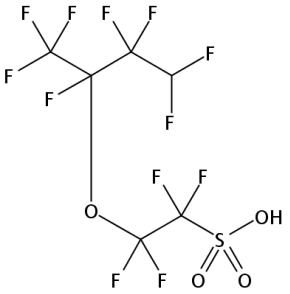
PFESAs



Nafion BP1

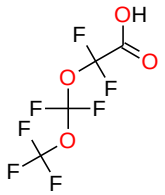


Nafion BP2

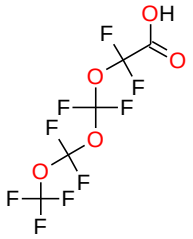


Nafion BP6

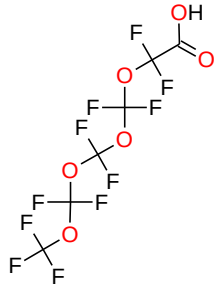
Polyether PFECAs



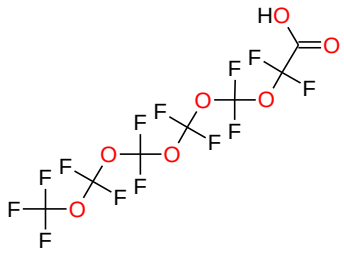
PFO_2HxA



PFO_3OA

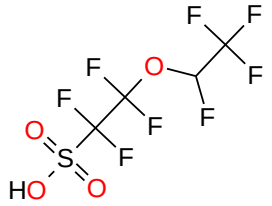


PFO_4DA

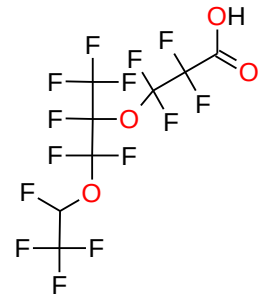


PFO_5DoDA

Other



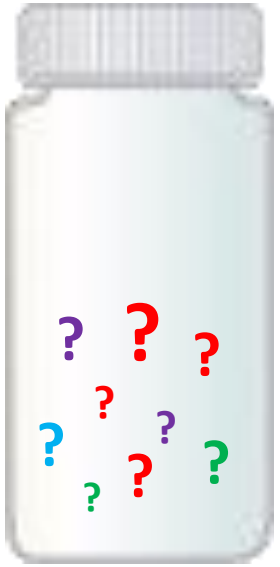
NVHOS



HydroEVE

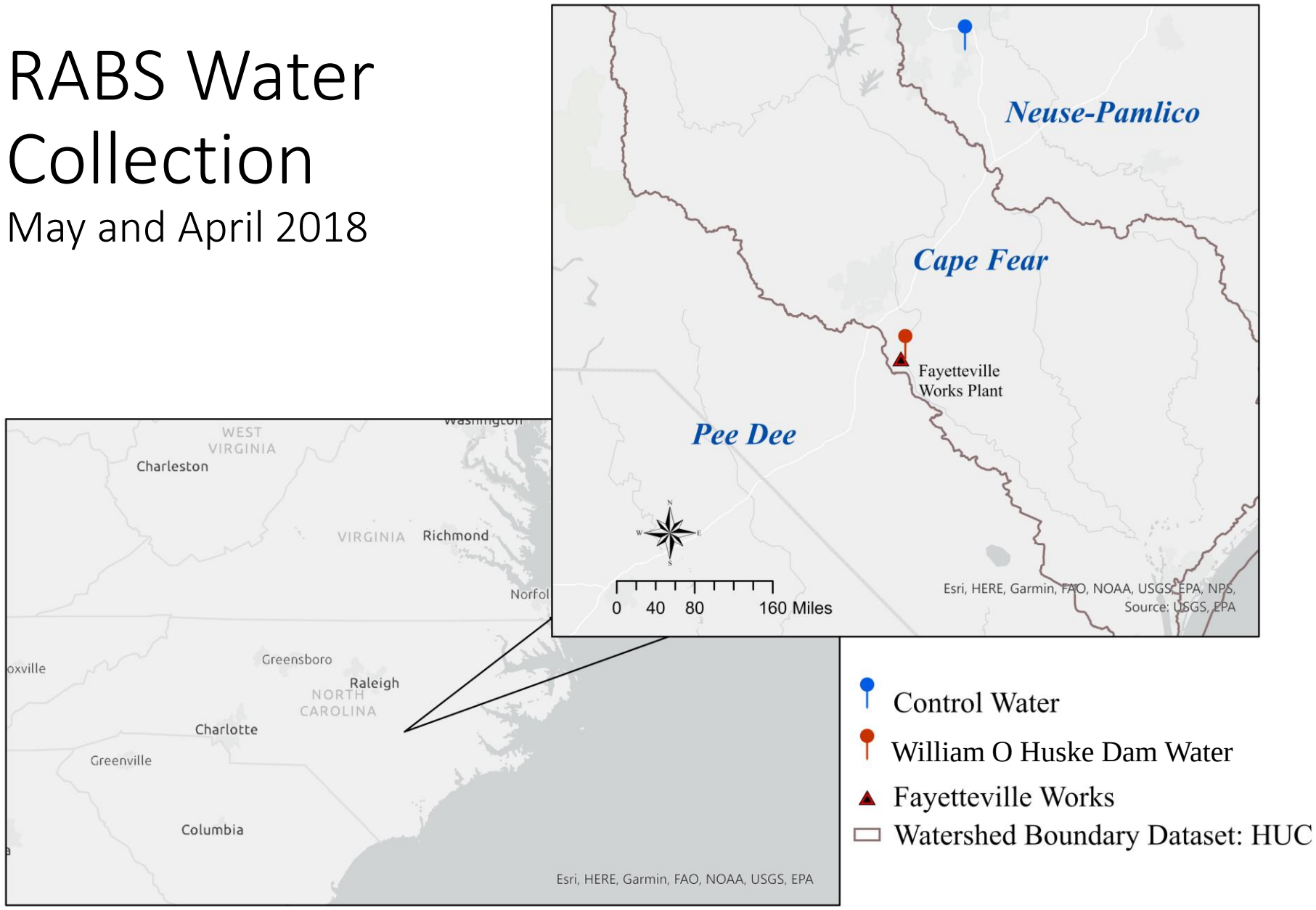
Determining the bioaccumulation of emerging PFAS

- Since many of these PFAS are emerging, standards often are not available to purchase for toxicology studies
- How do we prioritize synthesis and subsequent toxicological testing?
- Compounds with higher bioaccumulation can lead to higher toxicity due to longer residence time in tissues
- **Rapid Assessment Bioaccumulation Screening (RABS)**



RABS Water Collection

May and April 2018

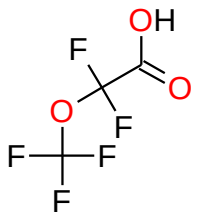


RABS Water Concentration

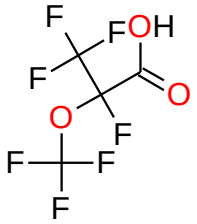
- Concentrated collected water approximately 1000X
- Targeting a known level of GenX since a standard became available shortly before the completion of this study

Novel PFAS in RABS Concentrate

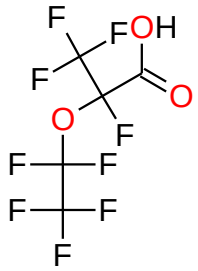
Monoether PFECAs



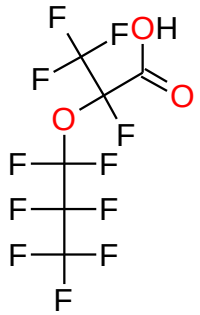
PFMOAA



PMPA



PEPA



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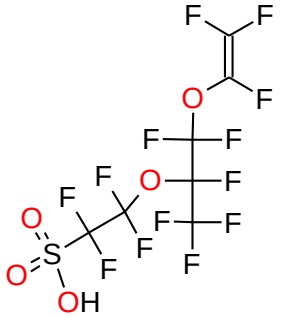
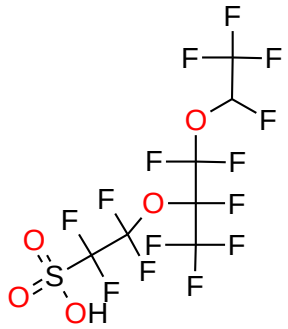
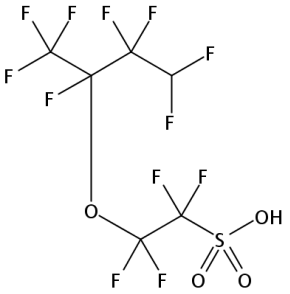


Nafion BP1

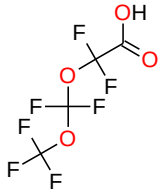


Nafion BP2

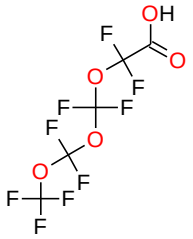


Nafion BP6

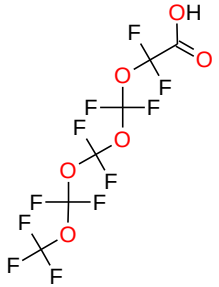
Polyether PFECAs



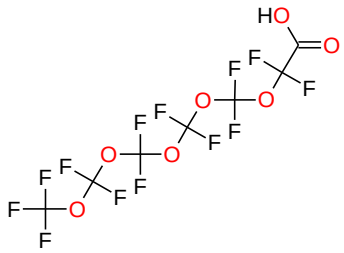
PFO_2HxA



PFO_3OA

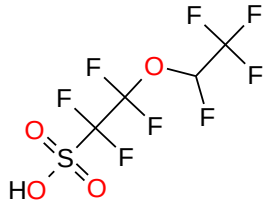


PFO_4DA

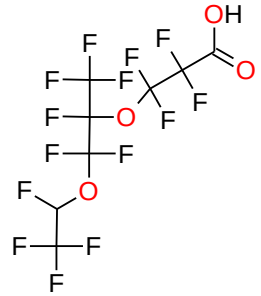


PFO_5DoDA

Other



NVHOS



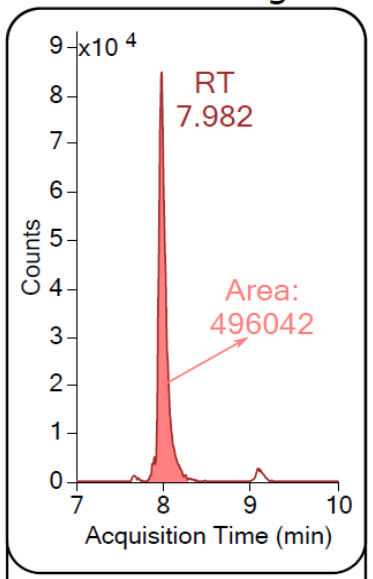
HydroEVE

Novel PFAS in RABS Concentrate

KEY:
Short chain PFAS

Long Chain PFAS

Ion Chromatogram



RT: 7.982

Area: 496042

Semi-Quantitation

PFMOAA

PFO_2HxA

GenX

PMPA

PFO_3OA

PEPA

NVHOS

PFO_4DA

Nafion BP2

HydroEVE

Nafion BP1

PFO_5DoDA

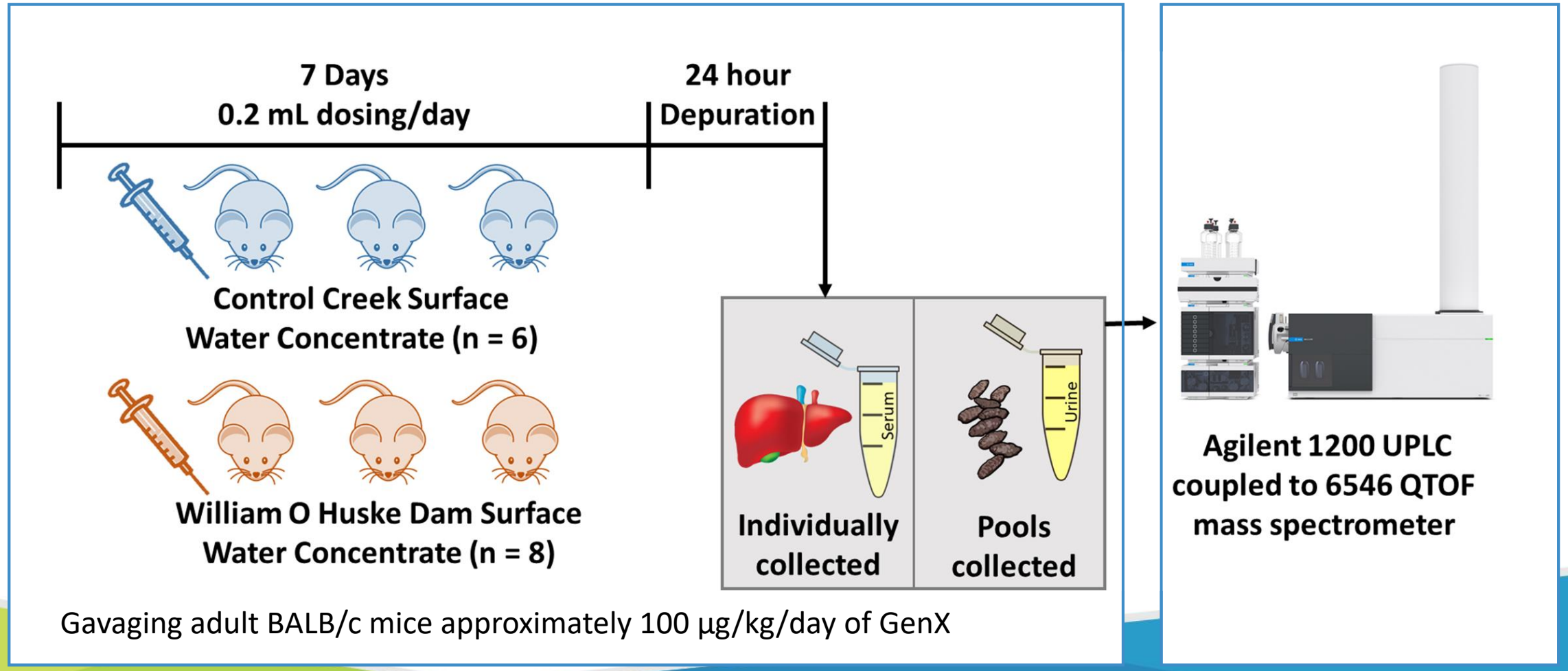
Nafion BP6

"High" PFAS
(Approx. 4X GenX signal)

100 µg/kg/day of GenX

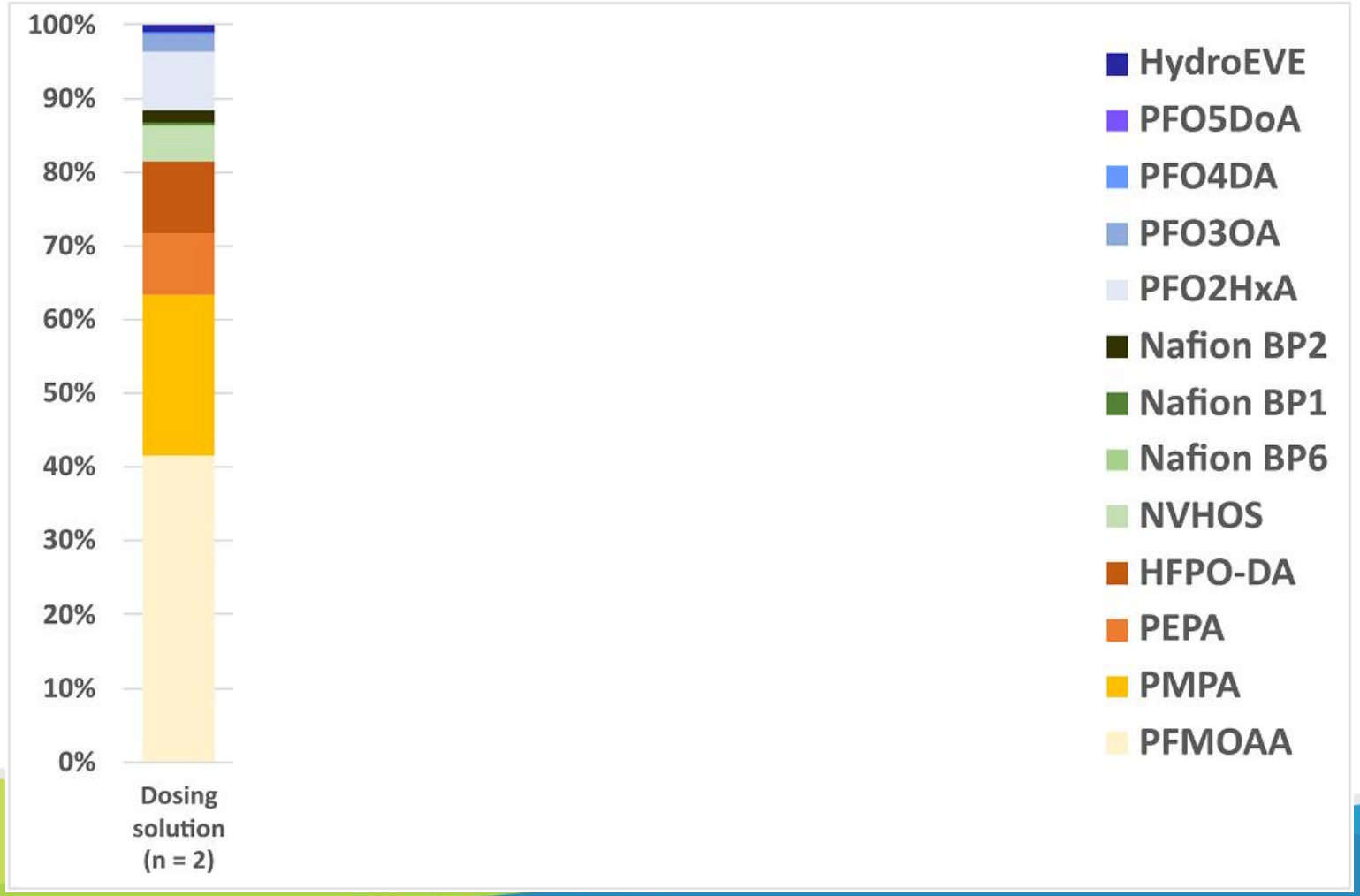
"Low" PFAS
(Approx. 1/20th GenX signal)

RABS Dosing



Gavaging adult BALB/c mice approximately 100 µg/kg/day of GenX

Determining the bioaccumulation of emerging PFAS



Determining the bioaccumulation of emerging PFAS

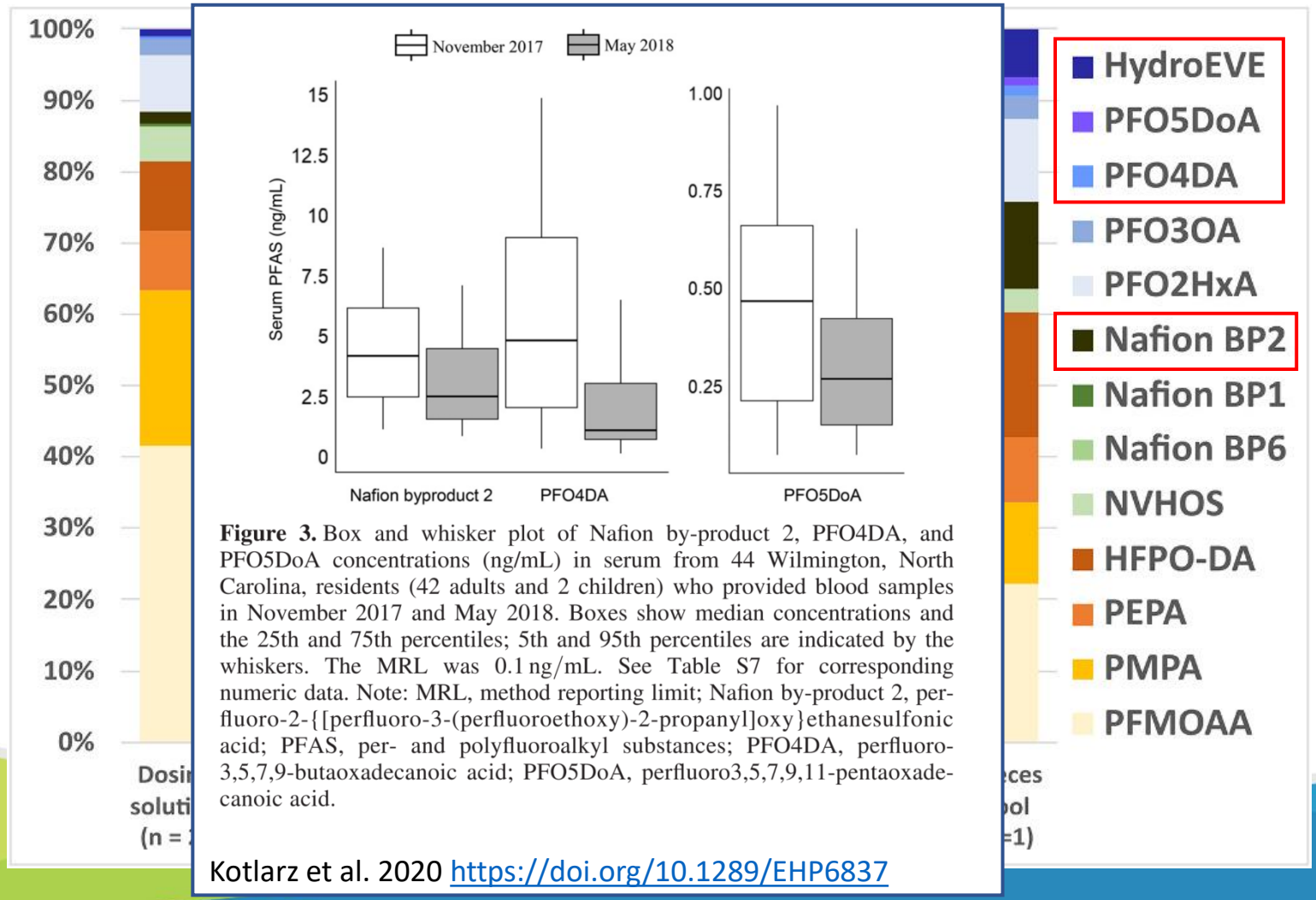


Figure 3. Box and whisker plot of Nafion by-product 2, PFO4DA, and PFO5DoA concentrations (ng/mL) in serum from 44 Wilmington, North Carolina, residents (42 adults and 2 children) who provided blood samples in November 2017 and May 2018. Boxes show median concentrations and the 25th and 75th percentiles; 5th and 95th percentiles are indicated by the whiskers. The MRL was 0.1 ng/mL. See Table S7 for corresponding numeric data. Note: MRL, method reporting limit; Nafion by-product 2, perfluoro-2-{[perfluoro-3-(perfluoroethoxy)-2-propanyl]oxy}ethanesulfonic acid; PFAS, per- and polyfluoroalkyl substances; PFO4DA, perfluoro-3,5,7,9-butaoxadecanoic acid; PFO5DoA, perfluoro3,5,7,9,11-pentaoxadecanoic acid.

Kotlarz et al. 2020 https://doi.org/10.1289/EHP6837

- Four PFAS of the dozen emerging PFAS were found to readily bioaccumulate in mice tissues
- Mice tissue results mirrored blood results from community members located near to the source of the surface water

Next Steps

- In the past year, several polyether PFECAs have been synthesized and purified and are now commercially available to purchase.
- Standardized toxicological studies are now under way for PFO_4DA and PFO_5DoDA.
- Can we expand upon the RABS study and complete a RABS for other PFAS mixtures?

Thank You

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