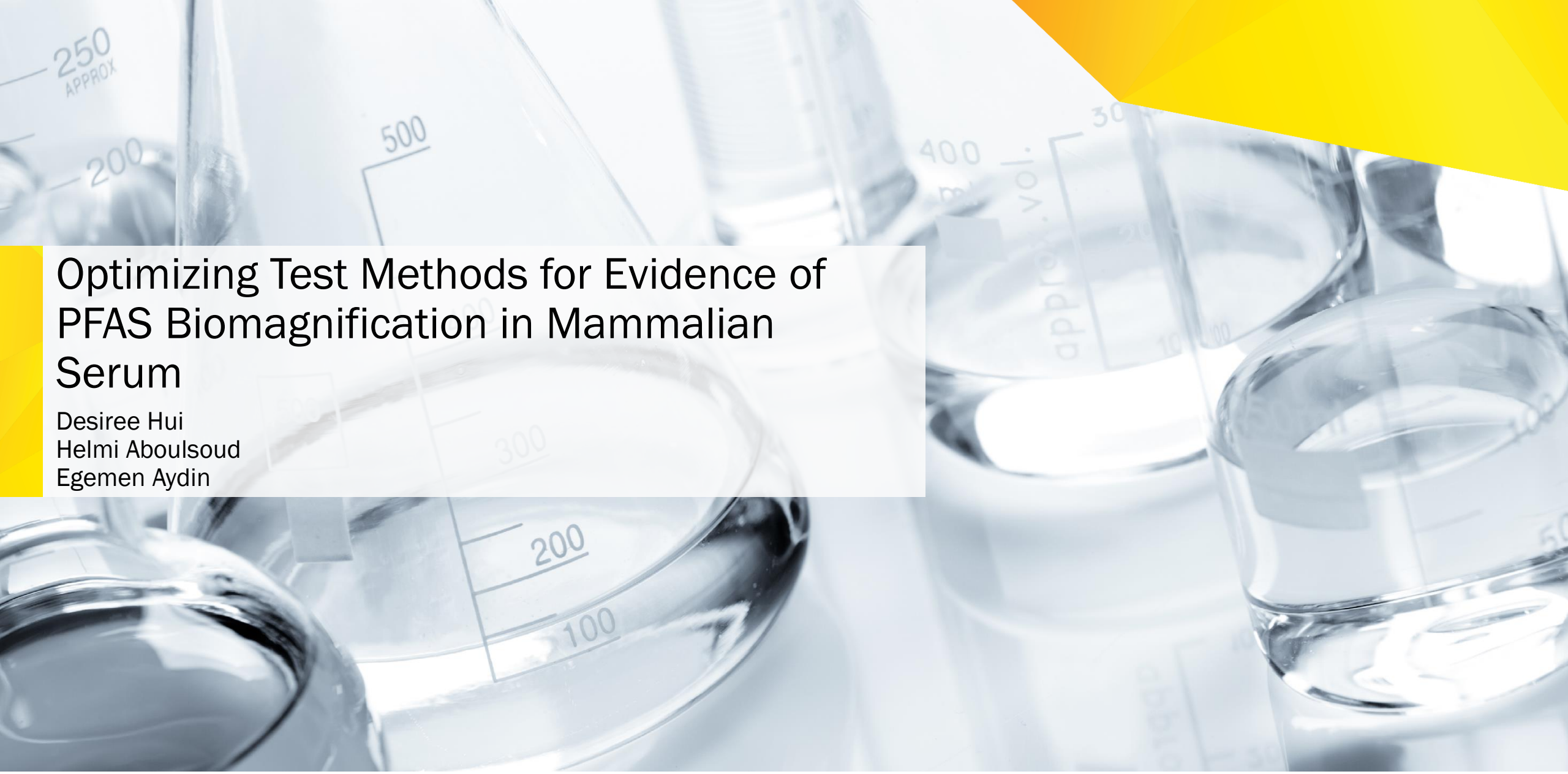

Optimizing Test Methods for Evidence of PFAS Biomagnification in Mammalian Serum

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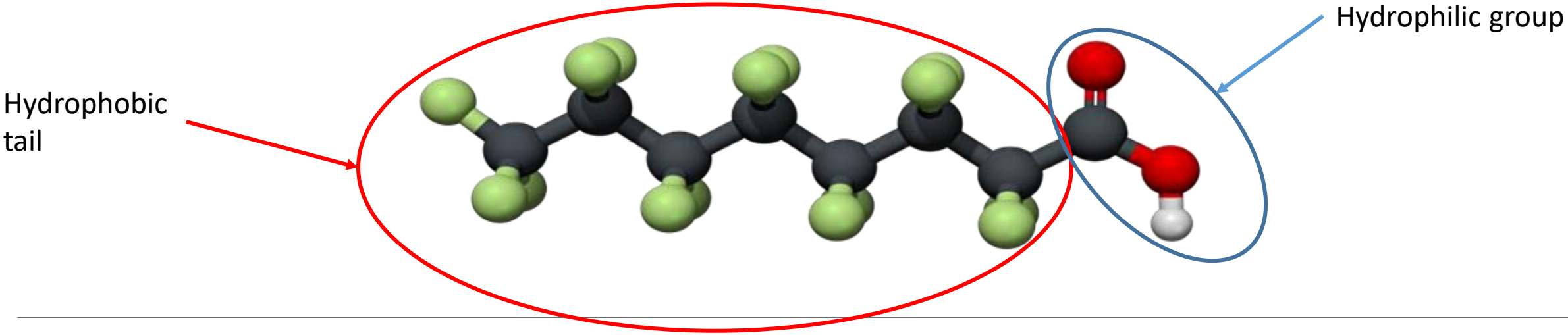
Overview

- PFAS Chemistry
- Types and Sources of PFAS
- Human Health
- Method and Parameter Comparison
- Quantification
- Whole Blood versus Serum versus Plasma
- Project
- Biomonitoring to date
- Results

PFAS Chemistry

- Perfluorinated: all carbons are saturated with fluorine
- Polyfluorinated: not all carbons are saturated with fluorine
- Water and stain repellent
- Surfactant
- Chemical and heat resistant



Types and Sources of PFAS

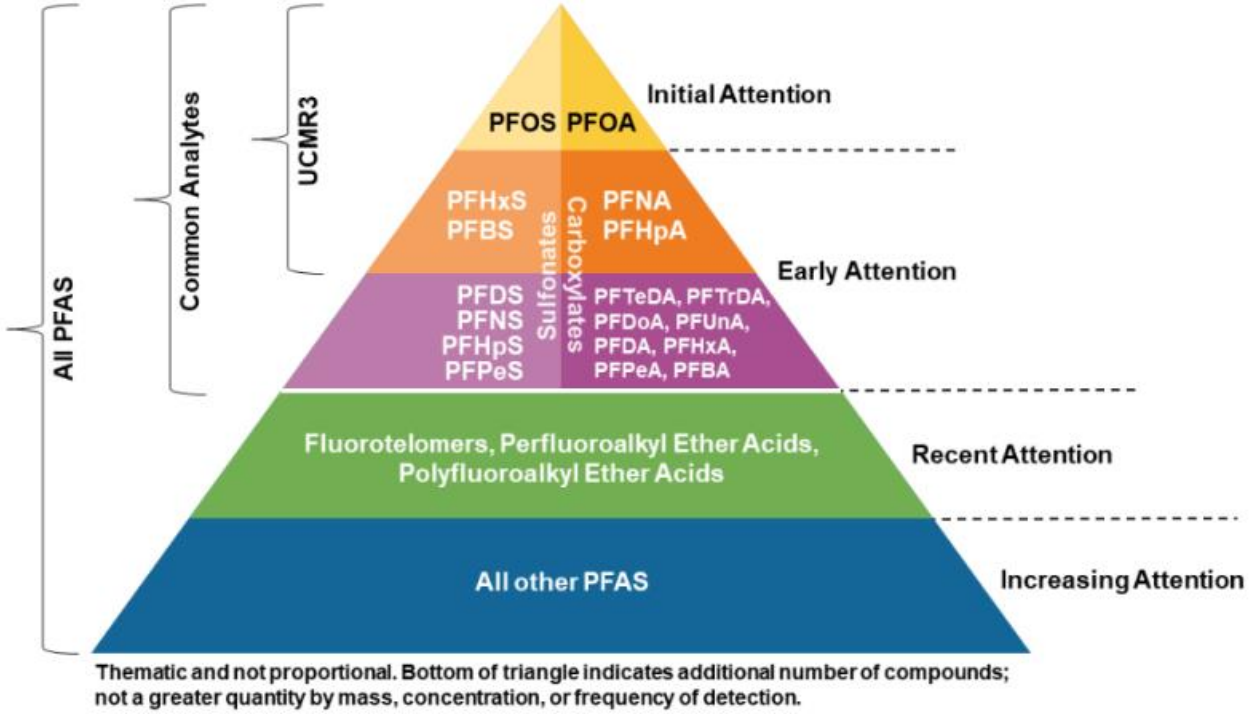
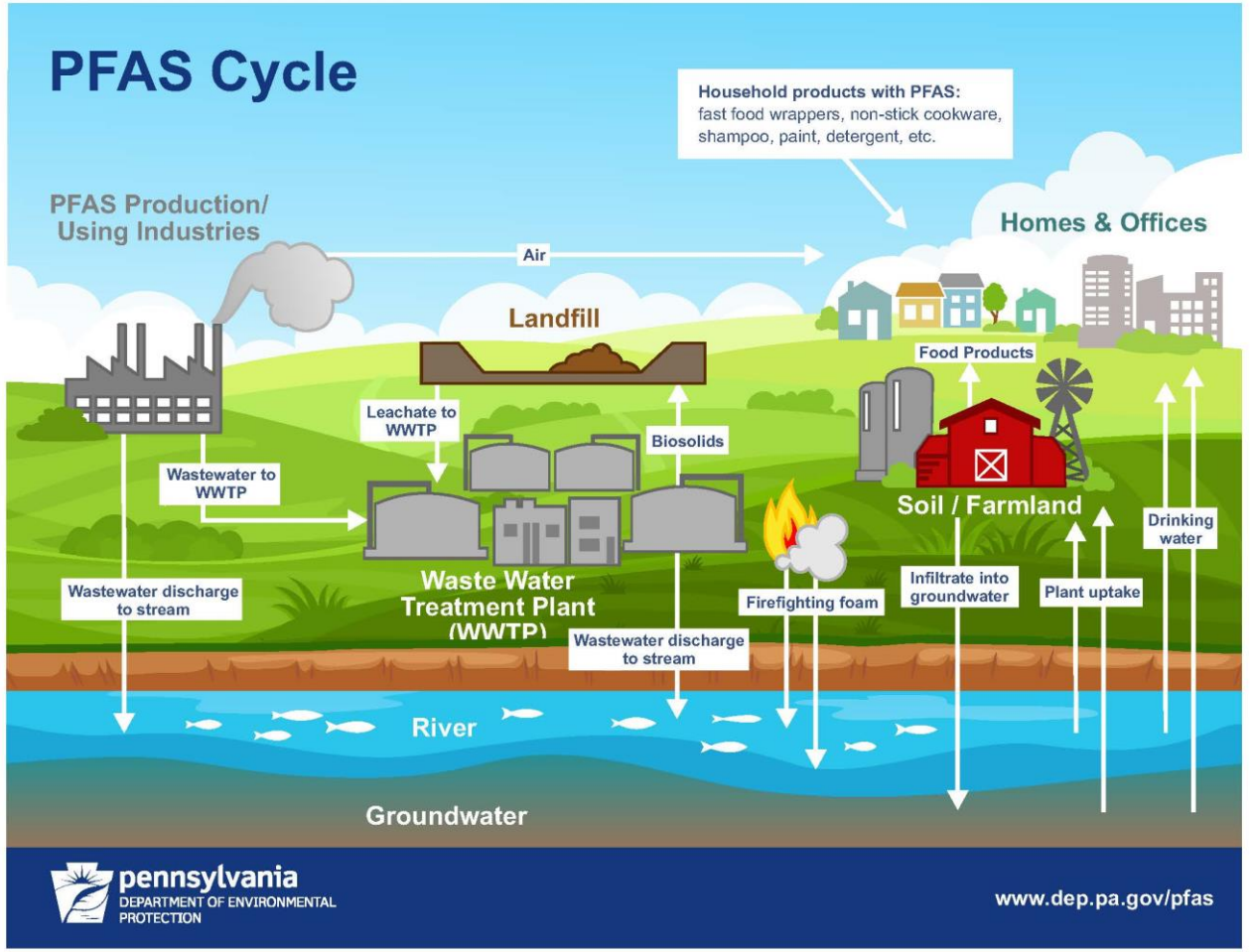


Image: ITRC Fact Sheet, 2017

Image: Pennsylvania PFAS Action Team Report, 2019.

Sources and transport



Environmental Concerns

- Persistency
  - Very resistant to transformation reactions
- Long distance transport via adsorption on particles
- Accumulation in tissues
  - Bioaccumulation
  - Biomagnification
- Ecotoxicological effects

Human Health Concerns

- Persistence of PFAS in the Human Body
  - PFAS compounds can persist in the human body from several years to decades.
  - There have been estimates of PFOS and PFOA in the body for:
    - PFOS (Perfluorooctane sulfonic acid) – estimated half life of ~ 5 – 7 years
    - PFOA (Perfluorooctanoic acid) – estimated half life of 3 – 5 years
- Tendency for bioaccumulation
- Biomonitoring

EPA method comparison

EPA 537.1	EPA 533	EPA 1633
Drinking water	Drinking water	All matrices other than drinking water
Cl_2 quencher: Trizma	Cl_2 quencher: NH_4Ac	No preservative
18 compounds	25 compounds	40 compounds
SPE SDVB	SPE WAX	SPE WAX
Hold Time: 14 days	Hold Time: 28 days	Hold Time: 28 days
Internal Standard	Isotope dilution	Isotope dilution
No clean up	No clean up	Carbon clean up
No recovery correction	Surrogate recovery correction	Surrogate recovery correction
Branched + linear isomers	Branched + linear isomers	Branched + linear isomers

Parameter comparison

Parameter	Method 537.1	Method 533	Method 1633
PFBA		x	x
PFPeA		x	x
PFHxA	x	x	x
PFHpA	x	x	x
PFOA	x	x	x
PFNA	x	x	x
PFDA	x	x	x
PFUnA	x	x	x
PFDoA	x	x	x
PFTrDA	x		x
PFTeDA	x		x
PFBS	x	x	x
PFPeS		x	x
PFHxS	x	x	x
PFHpS		x	x
PFOS	x	x	x
PFNS			x
PFDS			x
PFDoS			x
PFOSA			x

Parameter	Method 537.1	Method 533	Method 1633
N-MeFOSAA	x		x
N-EtFOSAA	x		x
ADONA	x	x	x
HFPO-DA	x	x	x
9Cl-PF3ONS	x	x	x
11Cl-PF3OUdS	x	x	x
4:2-FTS		x	x
6:2-FTS		x	x
8:2-FTS		x	x
NFDHA		x	x
PFEESA		x	x
PFMBA		x	x
PFMPA		x	x
NMeFOSA			x
NEtFOSA			x
N-MeFOSE			x
N-EtFOSE			x
3:3 FTCA			x
5:3 FTCA			x
7:3 FTCA			x

Quantification

- Isotope dilution used in EPA Methods 1633 and 533.
- Why is this important for PFAS analysis?
  - In analytical chemistry we use these isotopically labeled standards as internal standards to correct for variations in sample preparation and instrument conditions.
- Benefit?
  - This provides more accurate quantification by compensating for losses during sample preparation.

Next steps

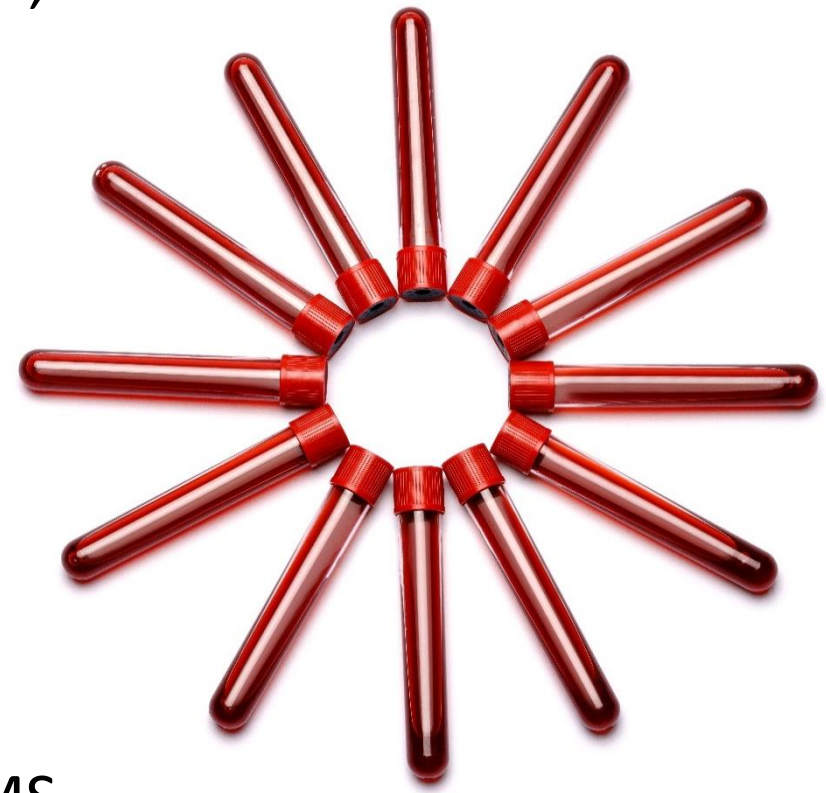
- ✓ Method parameters according to 1633 selected
- ✓ Quantification by isotope dilution selected

- What matrix?

  Whole blood? Serum? Plasma?

Whole blood, Serum, and Plasma Definitions

- Whole blood – contains all components of blood, including red and white blood cells, platelets and plasma.
- Serum – liquid part of the blood that remains after coagulation, devoid of blood cells and clotting factors.
- Plasma – the liquid component of the blood contains water, salts, enzymes, antibodies and other proteins.



All three of the above can be analyzed by LC-MSMS.

Whole blood, Serum, and Plasma - Comparison

- Whole blood –
  - Advantage: Represents the total body burden of PFAS since it includes all blood components
  - Challenge: Complex matrix which requires extensive sample preparation and cleanup.
- Plasma –
  - Advantage: Contains proteins and other components that can bind PFAS, providing comprehensive view of PFAS distribution in blood.
  - Challenges: May require additional steps to separate plasma from blood cells adding an additional level of complexity to sample preparation.

Whole blood, Serum, and Plasma - Comparison

- Serum –
  - Advantage: Widely used in PFAS biomonitoring studies due to its relatively simpler matrix compared to whole blood.
  - Reflects the bioavailable fraction of PFAS that circulates in the body providing important information about exposure levels.
  - There are many established methods existing for PFAS analysis in Serum.

After reviewing the advantages and challenges associated with these three matrices we decided to proceed with serum.

Biomonitoring Compounds of Interest

1. PFOS (Perfluorooctane sulfonic acid)
   - Sources: firefighting foams, stain repellents, other consumer products
2. PFOA (Perfluorooctanoic acid)
   - Sources: non stick coatings, Teflon manufacturing
3. PFHxS (Perfluorohexane sulfonic acid)
   - Sources: firefighting foams, stain repellents

With regulatory guidelines in place the levels of these compounds has been found to be decreasing.

PFAS in Serum

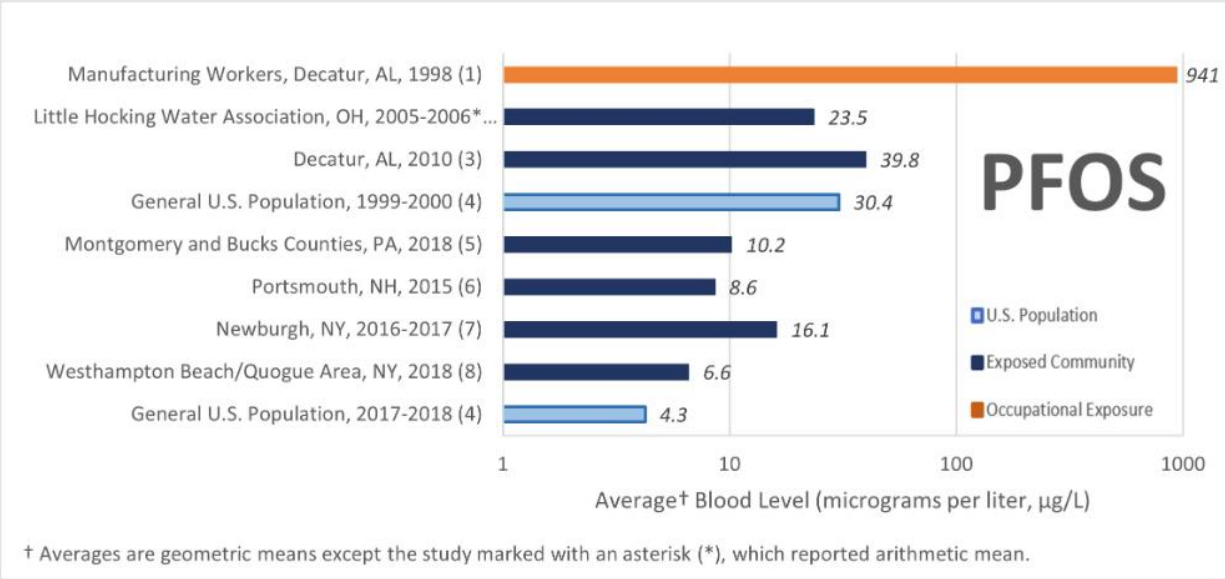
- Study of PFAS compounds found in blood serum of human and canine subjects.
- Possible exposure could occur from:
  - Protective turnout gear
    - Jacket, pants, boots, respirator, helmet, etc
  - AFFF – aqueous film forming foam
- Changes to gear and products containing less PFAS could be monitored by continued testing.

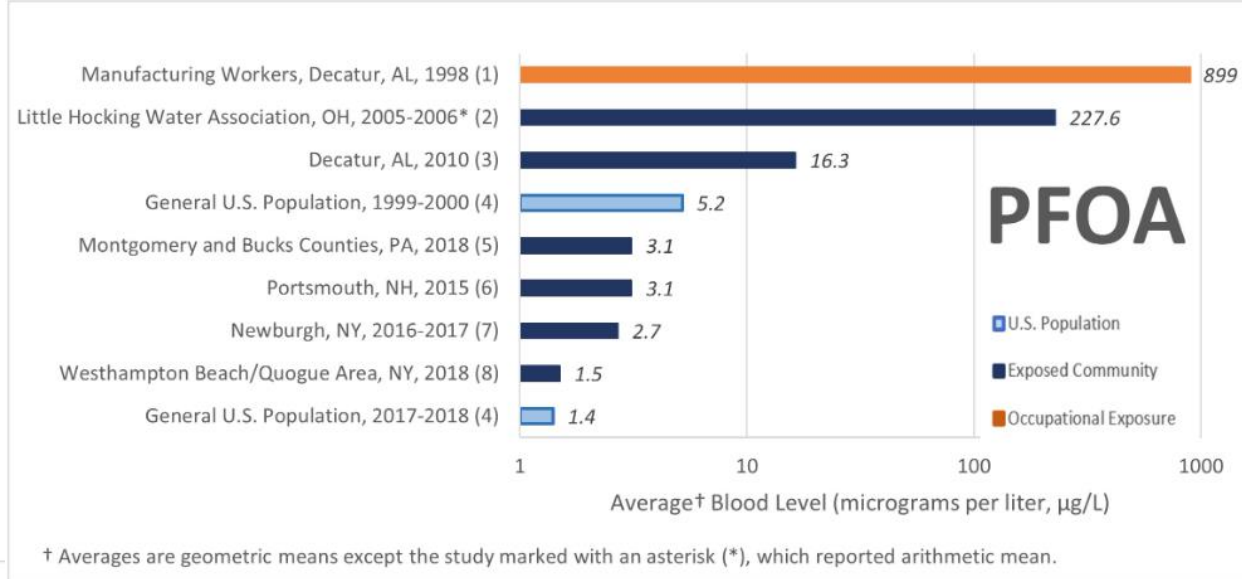
Willow
Accelerant Detection Canine, Calgary Fire Department

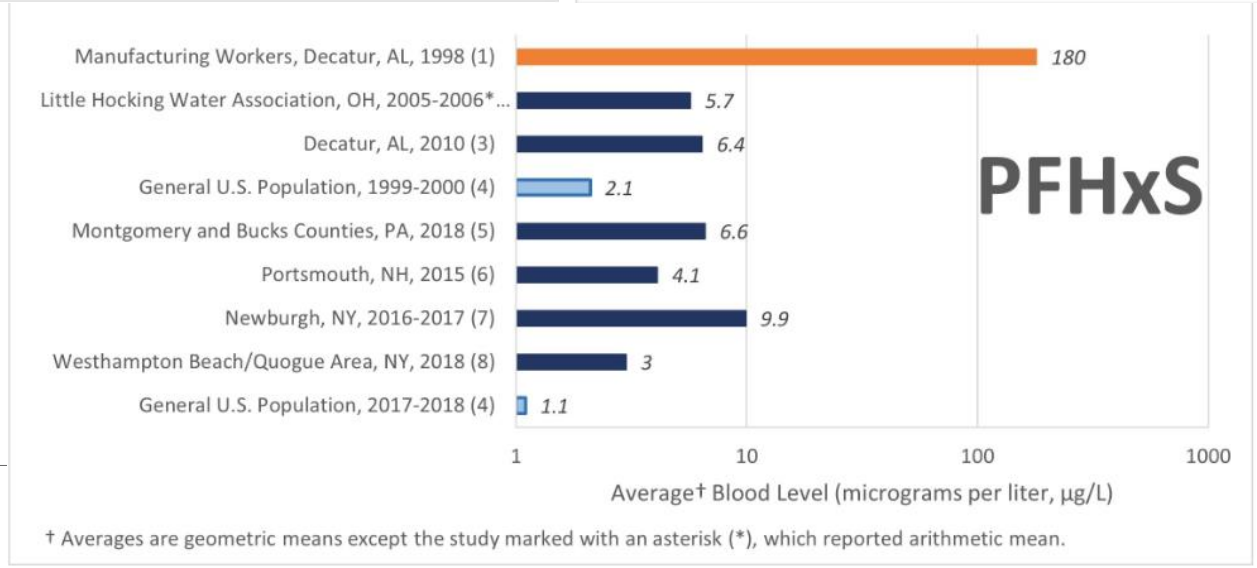
NHANES Method

- National Health and Nutrition Examination Survey
  - Since 1999
  - Health and nutrition metrics including exposure to certain environmental chemicals like PFAS.
  - Data collection is performed by interviews and examinations.
  - LC-MSMS used for the quantification of PFAS compounds in serum samples along with isotopically labeled standards.

ATSDR biomonitoring data







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

Canadian Health Measures Survey Results

Average concentrations of select perfluoroalkyl substances in Canadians aged 20 to 79, Canadian Health Measures Survey, Cycle 1 (2007 to 2009), Cycle 2 (2009 to 2011) and Cycle 5 (2016 and 2017), micrograms per litre

	Cycle 1 (2007 to 2009)	Cycle 2 (2009 to 2011)	Cycle 5 (2016 and 2017)
Perfluorooctane sulfonate	8.9	6.9	3.40
Perfluorooctanoic acid	2.5	2.3	1.30
Perfluorohexane sulfonate	2.3	1.7	0.98

https://www150.statcan.gc.ca/n1/daily-quotidien/191113/cg-a002-eng.htm

Results – Lab participants

Parameter	1 Final Conc. (ng/mL)	2 Final Conc. (ng/mL)	3 (Des) Final Conc. (ng/mL)
01. PFBA	0	0	0
02. PFPeA	0.1554	0.0750	0.1236
03. PFHxA	0	0	0
04. PFHpA	0.0042	0	0
05.PFOA	**0.2466**	**0.3630**	**0.1788**
06. PFNA	0	0.1692	0.0582
07. PFDA	0.0216	0.0270	0.0096
08. PFUdA	0	0.1320	0.0780
09. PFDoA	0	0	0
10. PFTrDA	0	0.0168	0
11. PFTeDA	0	0	0
12. PFBS	0.0144	0	0.0246
13. PFPeS	0	0	0
14. PFHxS	**0**	**0**	**0**
15. PFHpS	0	0	0
16. PFOS	**0.5640**	**0.3780**	**0.1044**
17. PFNS	0	0	0
18. PFDS	0	0	0
19. PFDoS	0	0	0
20. 4:2-FTS	0.0150	0.0156	0.0264

Parameter	1 Final Conc. (ng/mL)	2 Final Conc. (ng/mL)	3 (Des) Final Conc. (ng/mL)
21. 6:2-FTS	0	0	0
22. 8:2-FTS	0	0	0
23. PFOSA	0	0	0
24. NMeFOSA	0	0	0
25. NEtFOSA	**0**	**0**	**0**
26. N-MeFOSAA	0	0	0
27. N-EtFOSAA	0	0	0
28. NMeFOSE	0	0	0
29. NEtFOSE	0	0	0
30. HFPO-DA	0	0	0
31. ADONA	0	0	0
32. 9Cl-PF3ONS	0	0	0
33. 11Cl-PF3OUdS	0	0	0
34. 3:3 FTCA	**0**	**0**	**0**
35. 5:3 FTCA	0	0	0
36. 7:3 FTCA	**0**	**0**	**0**
37. PFEESA	0	0	0
38. PFMPA	0	0	0
39. PFMBA	0	0	0
40. NFDHA	0	0	0

Surrogate recoveries were all within the EPA 1633 guidelines with an average of 77%.

Results – CFD participants

	Willow	CFD-001	CFD-002	CFD-003	CFD-004	CFD-005	CFD-006	CFD-007	CFD-008
Age range		45-54	45-54	25-34	65+	35 - 44	35-44	25-34	45-54
Gender assigned at birth		Female	Male	Male	Male	Male	Male	Male	Male
Length of Service in the Fire industry		16+ years	16+ years	0-5 years	16+ years	11-15 years	16+ years	0-5 years	16+ years
Parameter	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**
01. PFBA	0	0	0	0	0	0	0	0	0
02. PFPeA	0	0.0426	0.1884	0	0	0	0	0	0
03. PFHxA	0	0	0	0	0	0	0	0	0
04. PFHpA	0.0006	0	0	0	0	0	0	0	0
05.PFOA	**0.0492**	**0.2952**	**0.2166**	**0.2064**	**0.2049**	**0.1799**	**0.2002**	**0.1524**	**0.2569**
06. PFNA	0	0.0630	0.0618	0.0608	0.0577	0.0744	0.0504	0.0775	0.0863
07. PFDA	0.015	0.0240	0.0264	0	0	0	0	0	0
08. PFUdA	0	0.1056	0	0	0	0	0	0	0
09. PFDoA	0	0	0	0	0	0	0	0	0
10. PFTrDA	0	0.0060	0	0	0	0	0	0	0
11. PFTeDA	0	0	0	0	0	0	0	0	0
12. PFBS	0.1074	0	0.0540	0	0	0	0	0	0
13. PFPeS	0	0	0	0	0	0	0	0	0
14. PFHxS	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
15. PFHpS	0	0	0	0	0	0	0	0	0
16. PFOS	**0.2790**	**0.2460**	**0.1146**	**0.3390**	**0.3479**	**0.2049**	**1.3510**	**0.6666**	**0.9048**
17. PFNS	0	0	0	0	0	0	0	0	0
18. PFDS	0	0	0	0	0	0	0	0	0
19. PFDoS	0	0	0	0	0	0	0	0	0
20. 4:2-FTS	0	0	0.0252	0.0255	0	0	0.0250	0	0

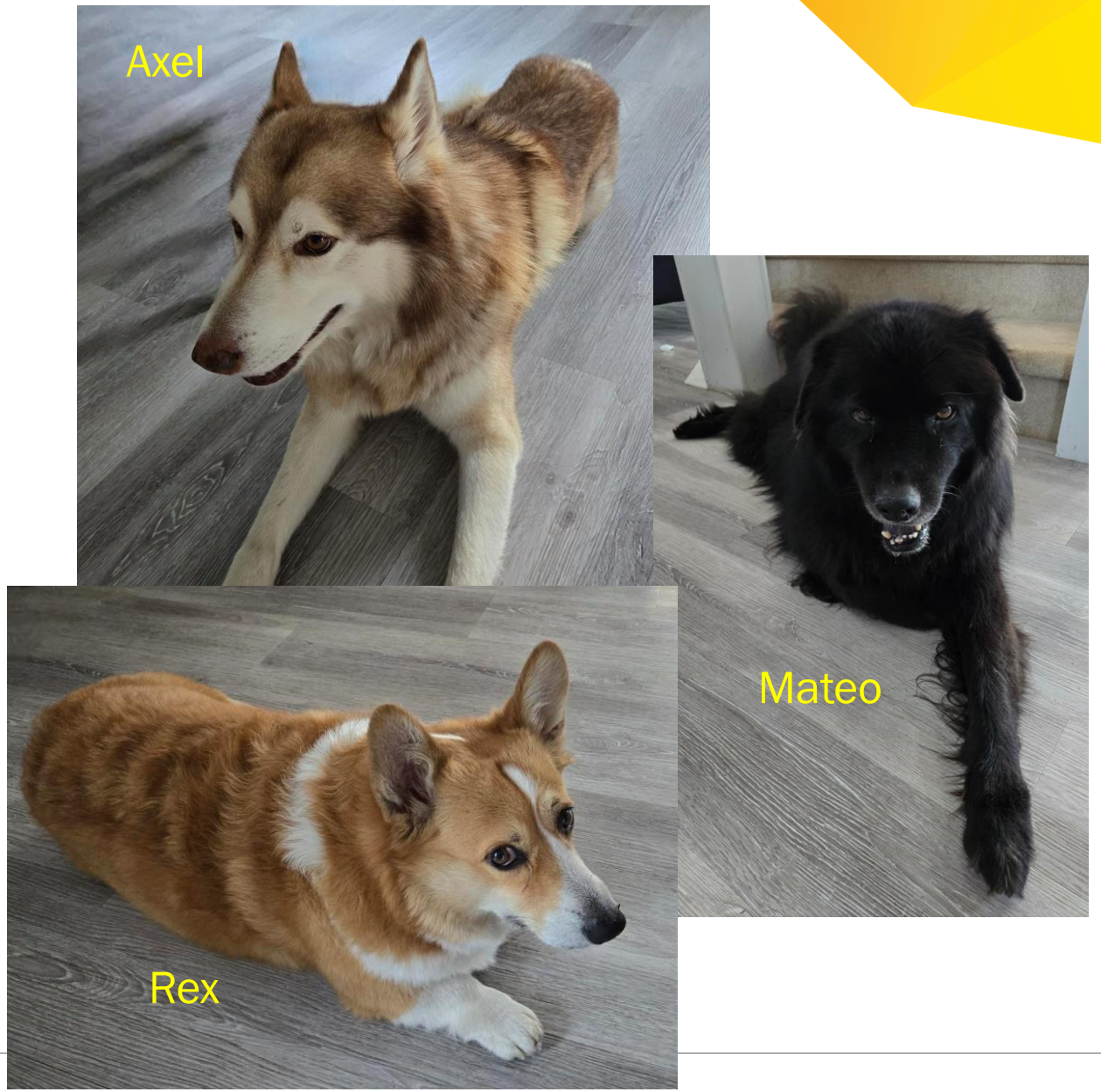
Surrogate recoveries were all within the EPA 1633 guidelines with an average of 85%.

Results – CFD participants

	Willow	CFD-001	CFD-002	CFD-003	CFD-004	CFD-005	CFD-006	CFD-007	CFD-008
Age range		45-54	45-54	25-34	65+	35 - 44	35-44	25-34	45-54
Gender assigned at birth		Female	Male	Male	Male	Male	Male	Male	Male
Length of Service in the Fire industry		16+ years	16+ years	0-5 years	16+ years	11-15 years	16+ years	0-5 years	16+ years
Parameter	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**	**Final Conc. (ng/mL)**
21. 6:2-FTS	0	0	0	0	0	0	0	0	0
22. 8:2-FTS	0	0	0	0	0	0	0	0	0
23. PFOSA	0	0	0	0	0	0	0	0	0
24. NMeFOSA	0	0	0	0	0	0	0	0	0
25. NEtFOSA	0	0	0	0	0	0	0	0	0
26. N-MeFOSAA	0	0	0	0	0	0	0	0	0
27. N-EtFOSAA	0	0	0	0	0	0	0	0	0
28. NMeFOSE	0	0	0	0	0	0	0	0	0
29. NEtFOSE	0	0	0	0	0	0	0	0	0
30. HFPO-DA	0	0	0.0342	0	0	0.0364	0	0.0244	0
31. ADONA	0	0	0	0	0	0	0	0	0
32. 9Cl-PF3ONS	0	0	0	0	0	0	0	0	0
33. 11Cl-PF3OUdS	0	0	0	0	0	0	0	0	0
34. 3:3 FTCA	0	0	0	0	0	0	0	0	0
35. 5:3 FTCA	0	0	0	0	0	0	0	0	0
36. 7:3 FTCA	0	0	0	0	0	0	0	0	0
37. PFEESA	0	0	0	0	0	0	0	0	0
38. PFMPA	0	0	0	0	0	0	0	0	0
39. PFMBA	0	0	0	0	0	0	0	0	0
40. NFDHA	0	0	0	0	0	0	0	0	0

Results – Canine Participants

- Serum donations obtained during routine checkups from Axel, Rex and Mateo allowed us to obtain further insight using canine results!



Results - Canine Participants

Parameter	Willow Final Conc. ng/mL	Axel Final Conc. ng/mL	Axel Duplicate Final Conc. ng/mL	Mateo Final Conc. ng/mL	Rex Final Conc. ng/mL
Age	7	8	8	8	5
01. PFBA	0	0	0	0	0
02. PFPeA	0	0	0	0	0
03. PFHxA	0	0	0	0	0
04. PFHpA	0.0006	0	0	0	0
05.PFOA	**0.0492**	**0.0597**	**0.0671**	**0.2418**	**0.1661**
06. PFNA	0	0.0227	0.0279	0.0567	0.0491
07. PFDA	0.0150	0.0314	0.0236	0.0628	0.0305
08. PFUdA	0	0	0	0	0
09. PFDoA	0	0	0	0	0
10. PFTrDA	0	0	0.0065	0	0
11. PFTeDA	0	0	0	0	0
12. PFBS	0.1074	0	0	0	0
13. PFPeS	0	0	0	0	
14. PFHxS	**0**	**0.1156**	**0.1203**	**0.1136**	**0.0932**
15. PFHpS	0	0	0	0	0
16. PFOS	**0.2790**	**0.5650**	**0.5535**	**0.4679**	**0.4618**
17. PFNS	0	0	0	0	0
18. PFDS	0	0	0	0	0
19. PFDoS	0	0	0	0	0
20. 4:2-FTS	0	0	0	0	0

Parameter	Willow Final Conc. ng/mL	Axel Final Conc. ng/mL	Axel Duplicate Final Conc. ng/mL	Mateo Final Conc. ng/mL	Rex Final Conc. ng/mL
21. 6:2-FTS	0	0	0	0	0
22. 8:2-FTS	0	0	0	0	0
23. PFOSA	0	0	0	0	0
24. NMeFOSA	0	0	0	0	0
25. NEtFOSA	0	0	0	0	0
26. N-MeFOSAA	0	0	0	0	0
27. N-EtFOSAA	0	0	0	0	0
28. NMeFOSE	0	0	0	0	0
29. NEtFOSE	0	0	0	0	0
30. HFPO-DA	0	0	0	0	0
31. ADONA	0	0	0	0	0
32. 9Cl-PF3ONS	0	0	0	0	0
33. 11Cl-PF3OUdS	0	0	0	0	0
34. 3:3 FTCA	0	0	0	0	0
35. 5:3 FTCA	0	0	0	0	0
36. 7:3 FTCA	0	0	0	0	0
37. PFEESA	0	0	0	0	0
38. PFMPA	0	0	0	0	0
39. PFMBA	0	0	0	0	0
40. NFDHA	0	0	0	0	0

Surrogate recoveries were all within the EPA 1633 guidelines with an average of 104%.

Summary

- We still have multiple participants submitting data.
- We have a questionnaire that will also provide more insight on our results.
- Average numbers at this time:

Parameter	Canine Average (ng/mL)	Lab Average (ng/mL)	CFD Average (ng/mL
05.PFOA	0.1168	0.2628	0.2141
14. PFHxS	0.0885	0	0
16. PFOS	0.4654	0.3488	0.5219

- Many thanks to the Calgary Fire Department, Alberta Precision Labs, and the Animal Clinic of Calgary for the participation in this study!
- Keep a look out for the final results!

Thank you!

Questions?

Desiree Hui - dhui@agatlabs.com