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Screening the Vast PFAS Landscape: *In Vitro* Toxicokinetic Testing and LC-MS/MS Analysis

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U.S. Environmental Protection Agency
Office of Research and Development

Presentation for Waters Southeast Mass Spectrometry Users Meeting

Tuesday, July 21, 2020

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

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EPA Office of Research and Development

- Office of Research and Development (ORD) is the scientific research arm of the EPA
- Research is conducted by ORD's four Centers
- 10 facilities across the USA
- Research is conducted by a combination of Federal scientists, contract researchers, and postdoctoral, graduate student, and post-baccalaureate trainees

Research Triangle Park, NC

Athens, GA

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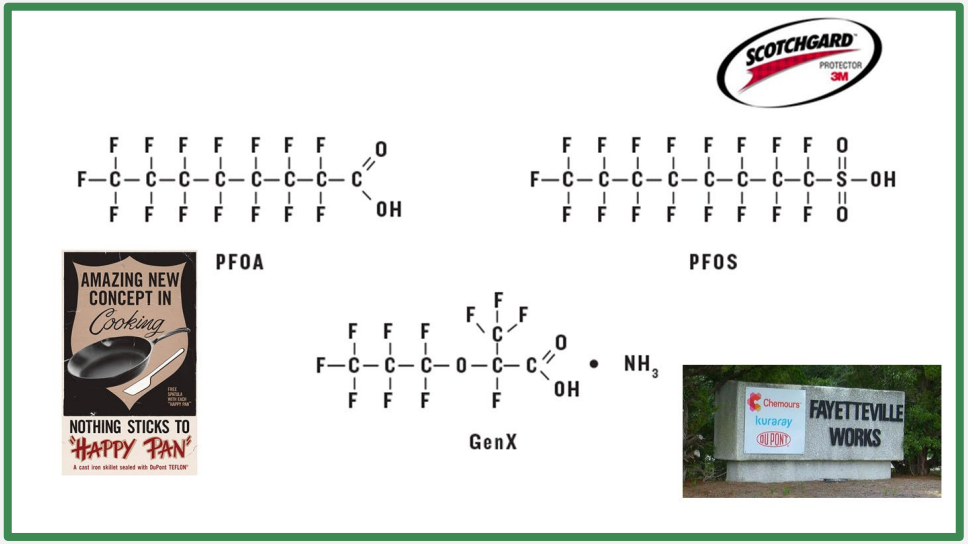
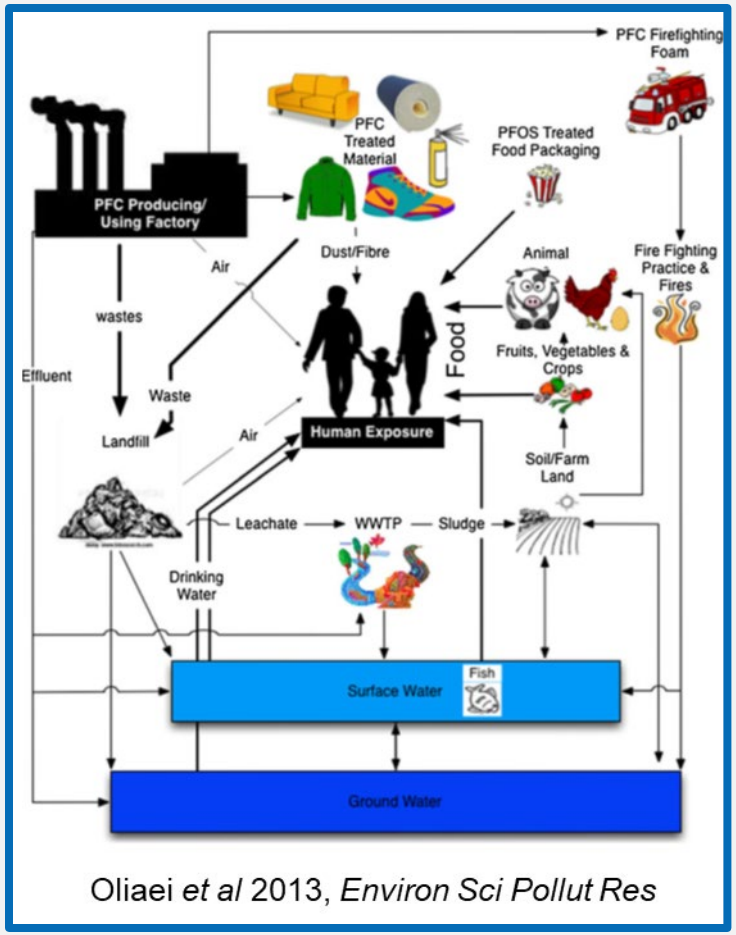
What are PFAS?

- **PFAS = per- and polyfluoroalkyl substances**
- Man-made chemicals used in industry and consumer products worldwide since the 1950s
- Repel water, resist heat, and protect surfaces





Oliaei *et al* 2013, *Environ Sci Pollut Res*

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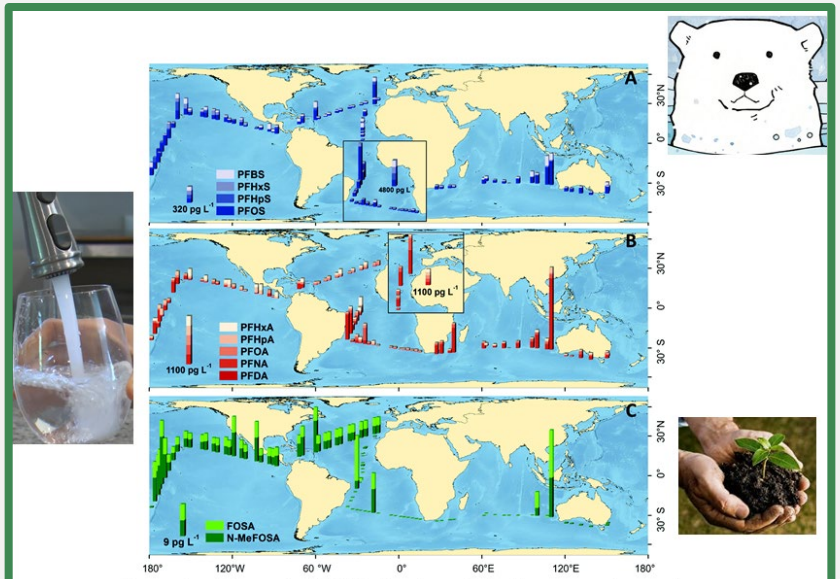
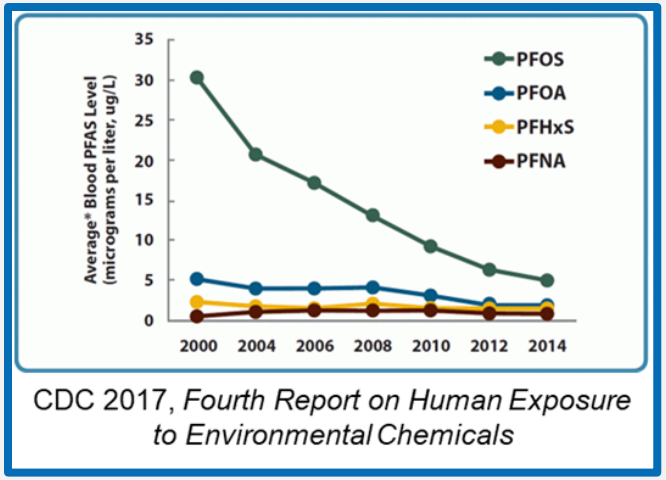
Why are PFAS receiving lots of attention?

- **Widespread occurrence**
  - PFAS in 97% of American population
  - Even in arctic polar bears
- **Persistence**
  - Carbon-fluorine bonds are some of the strongest
  - Little degradation in environment
- **Bioaccumulative**
  - Accumulate over time
  - Absorption > elimination



Gonzalez-Gaya et al 2019, *Environ. Sci.: Processes Impacts*



CDC 2017, *Fourth Report on Human Exposure to Environmental Chemicals*

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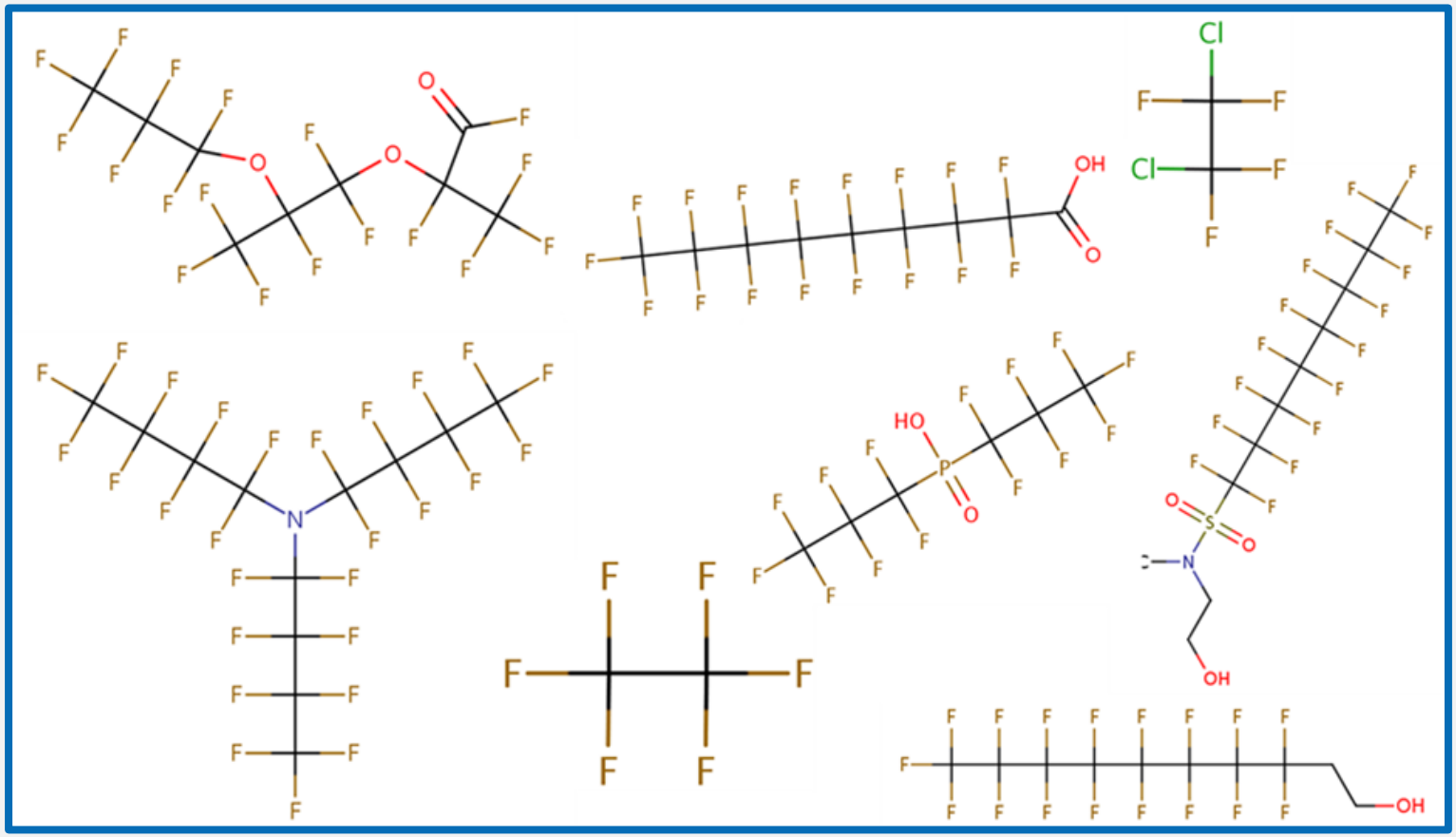
How many PFAS exist?

- More than 4700 PFAS recognized by OECD
- As industry continues to invent, the number will increase



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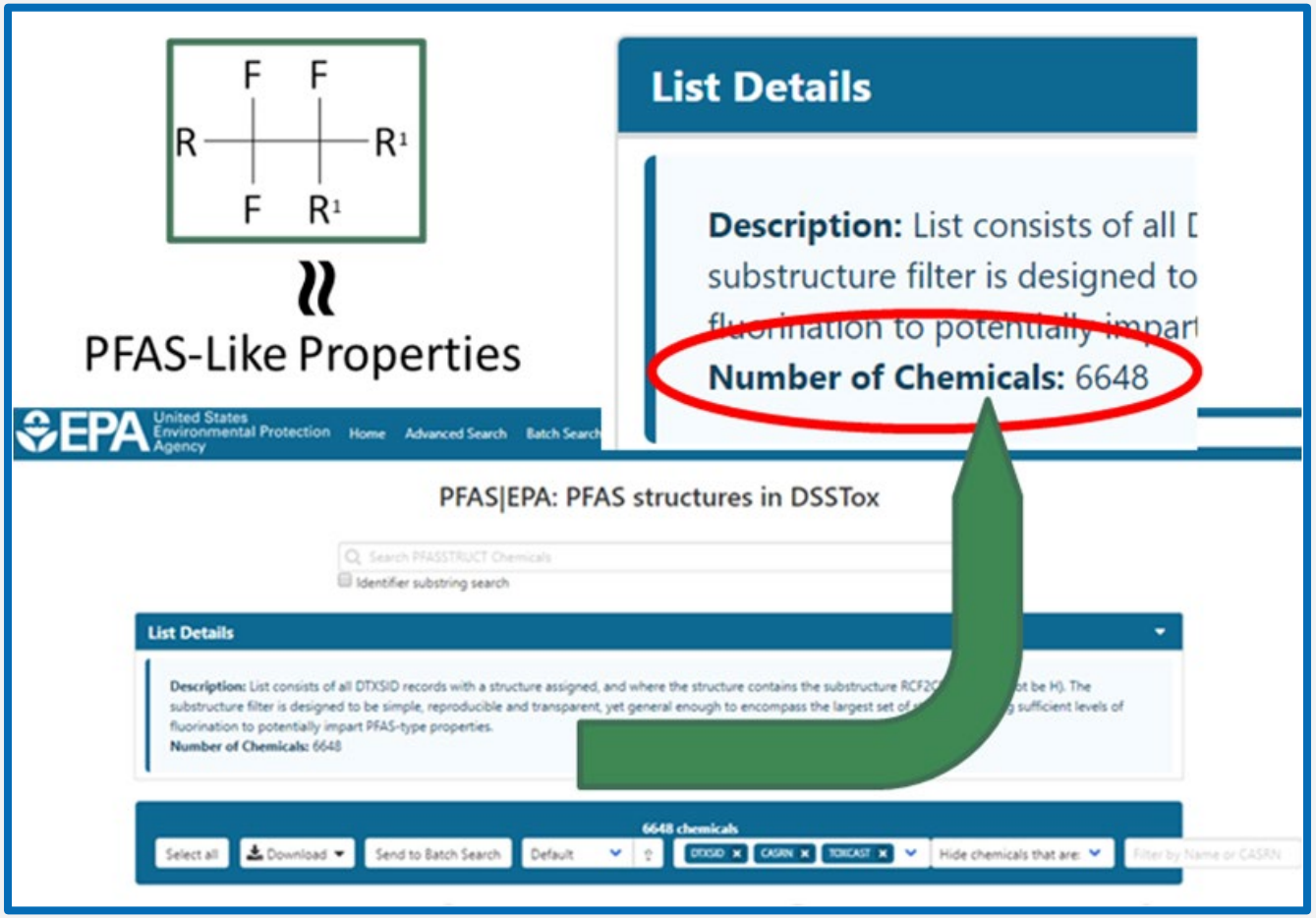

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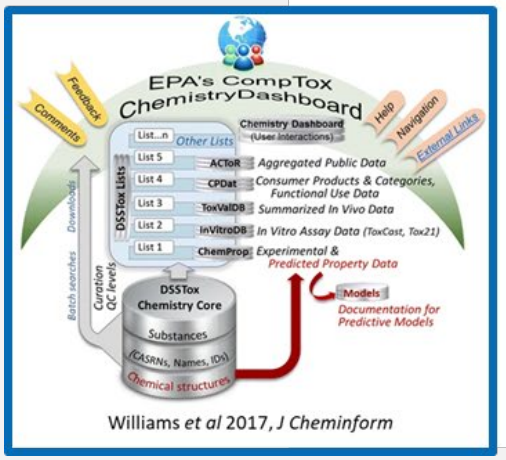
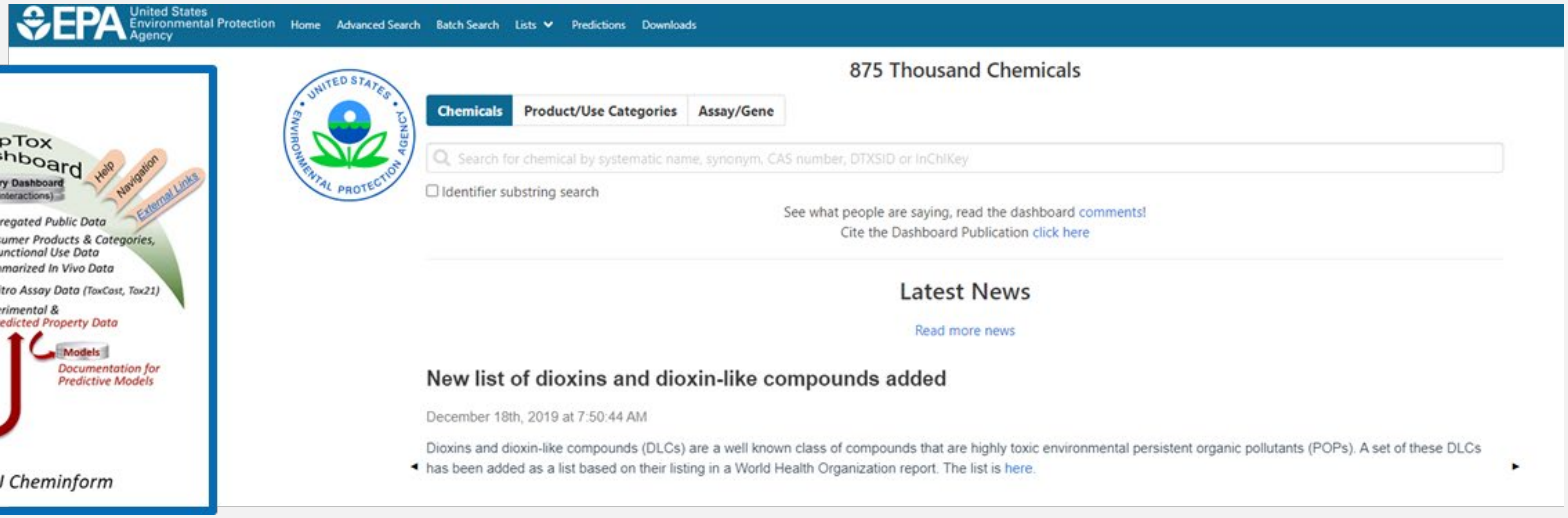
What's the CompTox Chemicals Dashboard?

- https://comptox.epa.gov/dashboard
- One-stop-shop for chemical, toxicological, and exposure information
- Almost 900,000 chemicals inventoried





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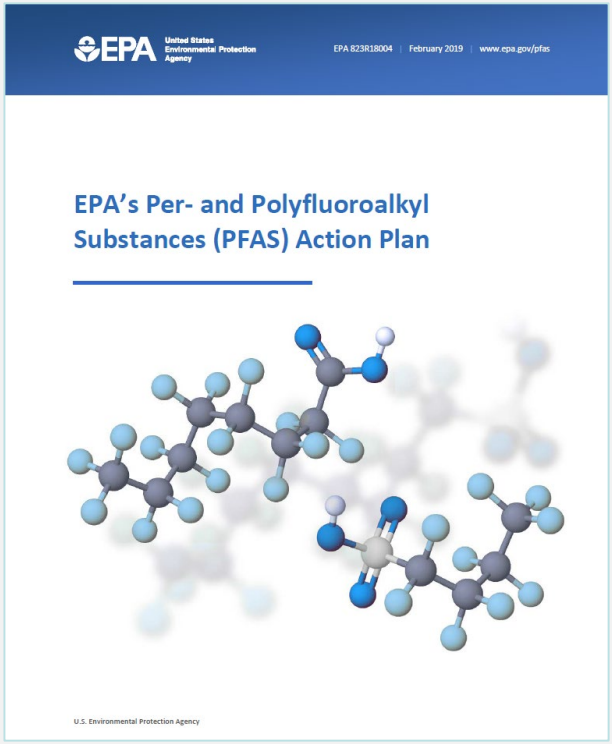
What is the EPA doing about PFAS?

- EPA PFAS Action Plan (2019)
  - Assist states, tribes, and communities address PFAS with short-term solutions and long-term strategies to address PFAS
- PFAS-Related Challenges
  - Developing/validating laboratory analytical methods for measuring PFAS
  - Assessing PFAS chemical toxicity
  - Developing standard toxicity values for PFAS chemicals
  - Characterizing potential human exposure pathways
  - Managing PFAS containing materials and waste
  - Testing drinking water treatment technologies
  - Identifying site remediation technologies



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Which PFAS are we interested in?

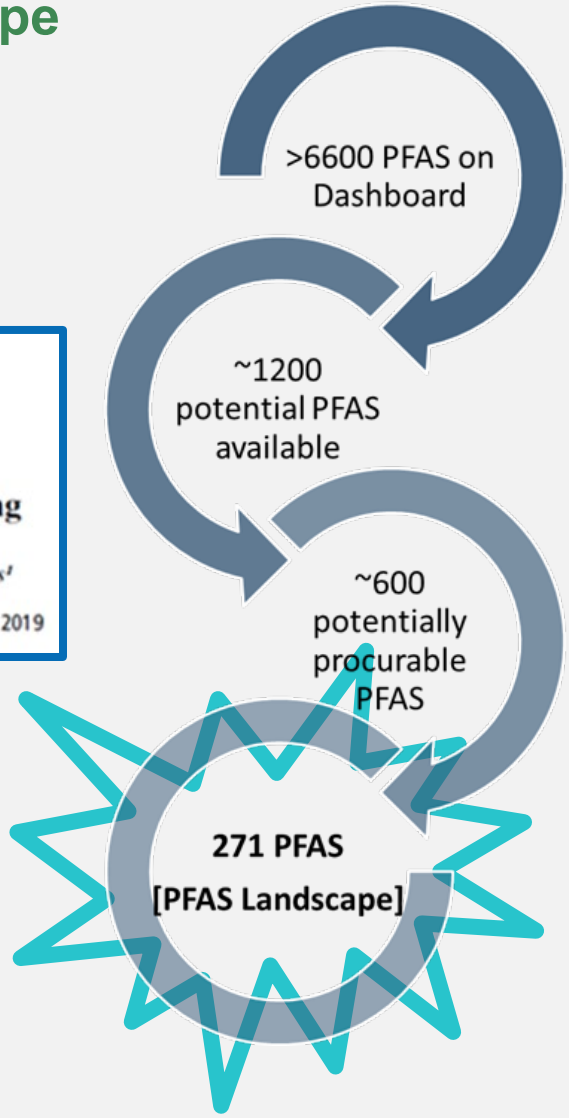
- PFAS Screening Library creation: **PFAS Landscape**
  - Maximize read-across
  - Capture structural diversity
- 1220 PFAS currently in TSCA inventory

Brief Communication

A Chemical Category-Based Prioritization Approach for Selecting 75 Per- and Polyfluoroalkyl Substances (PFAS) for Tiered Toxicity and Toxicokinetic Testing

Grace Patlewicz,[1] Ann M. Richard,[1] Antony J. Williams,[1] Christopher M. Grulke,[1] Reeder Sams,[1] Jason Lambert,[2] Pamela D. Noyes,[3] Michael J. DeVito,[4] Ronald N. Hines,[5] Mark Strynar,[6] Annette Guiseppi-Elie,[6] and Russell S. Thomas[1]

Environmental Health Perspectives 014501-1 127(1) January 2019



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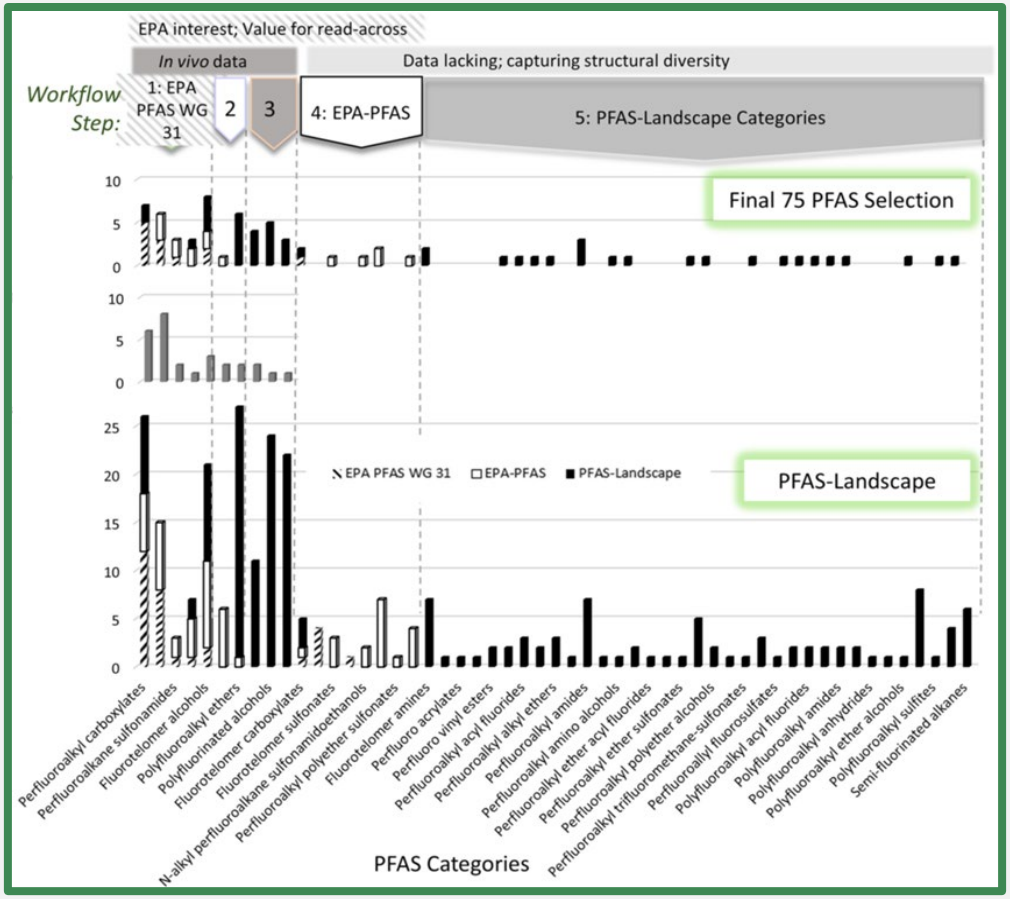
Which PFAS are we interested in?

- Initially, **75** PFAS selected from the PFAS Landscape, but the PFAS of interest now expands to near **200** unique structures and the Landscape up to **430** unique PFAS



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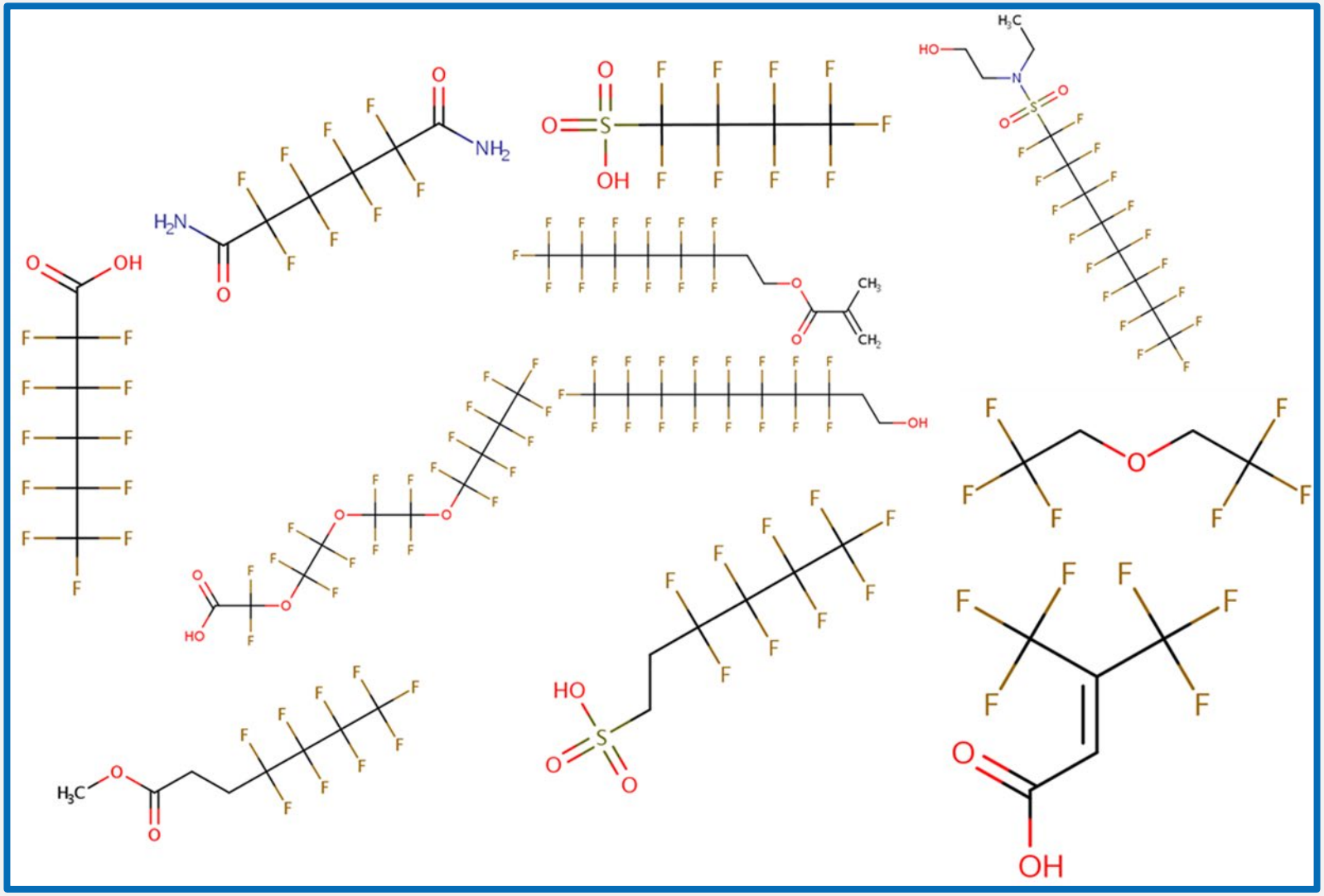
Which PFAS are being evaluated?



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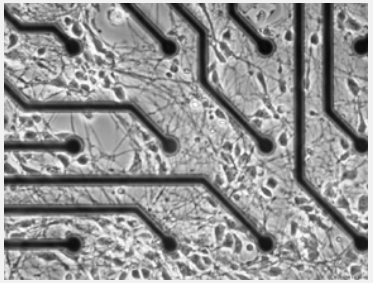
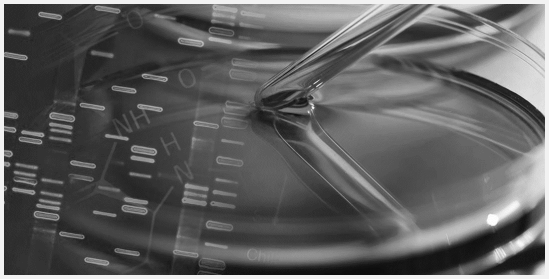
How are we examining these PFAS?

- A range of targeted and tiered high-throughput toxicity assays to serve as guide for potential human health risk

- New approach methodologies (NAMs) used
  - Alternative test methods and strategies to reduce, refine, and/or replace mammalian animals
  - *In vitro* tests/assays, *in chemico* assays, *in silico* algorithms

- Endpoints for PFAS work
  - Hepatotoxicity
  - Immunotoxicity
  - Developmental toxicity
  - Mitochondrial toxicity
  - ***In vitro* toxicokinetic assays**





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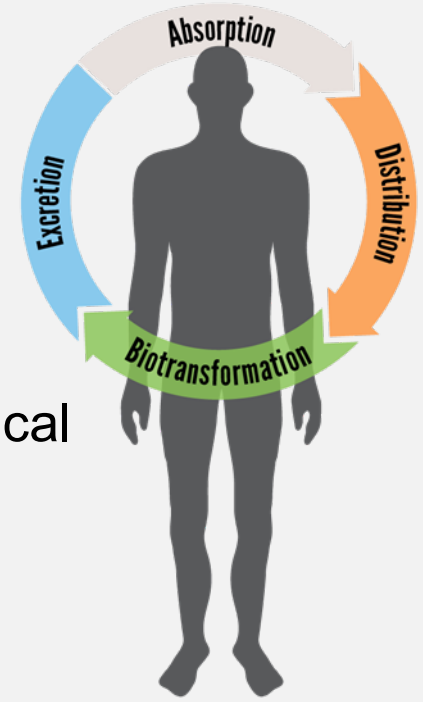
What are *in vitro* toxicokinetic assays?

- Toxicokinetics: the study of how a substance gets into the body and what happens to it in the body
  - Can be used to look at how chemicals move throughout the body and lead to harmful effects
  - Often viewed as a function of dose over time
- Kinetic data can inform…
  - Bioavailability (degree of a substance to enter circulation when introduced to body)
  - Bioaccumulation potential (absorption >> excretion)
  - Metabolite formation (transformation of original chemical new entity; can lead to bioactivation or detoxification)



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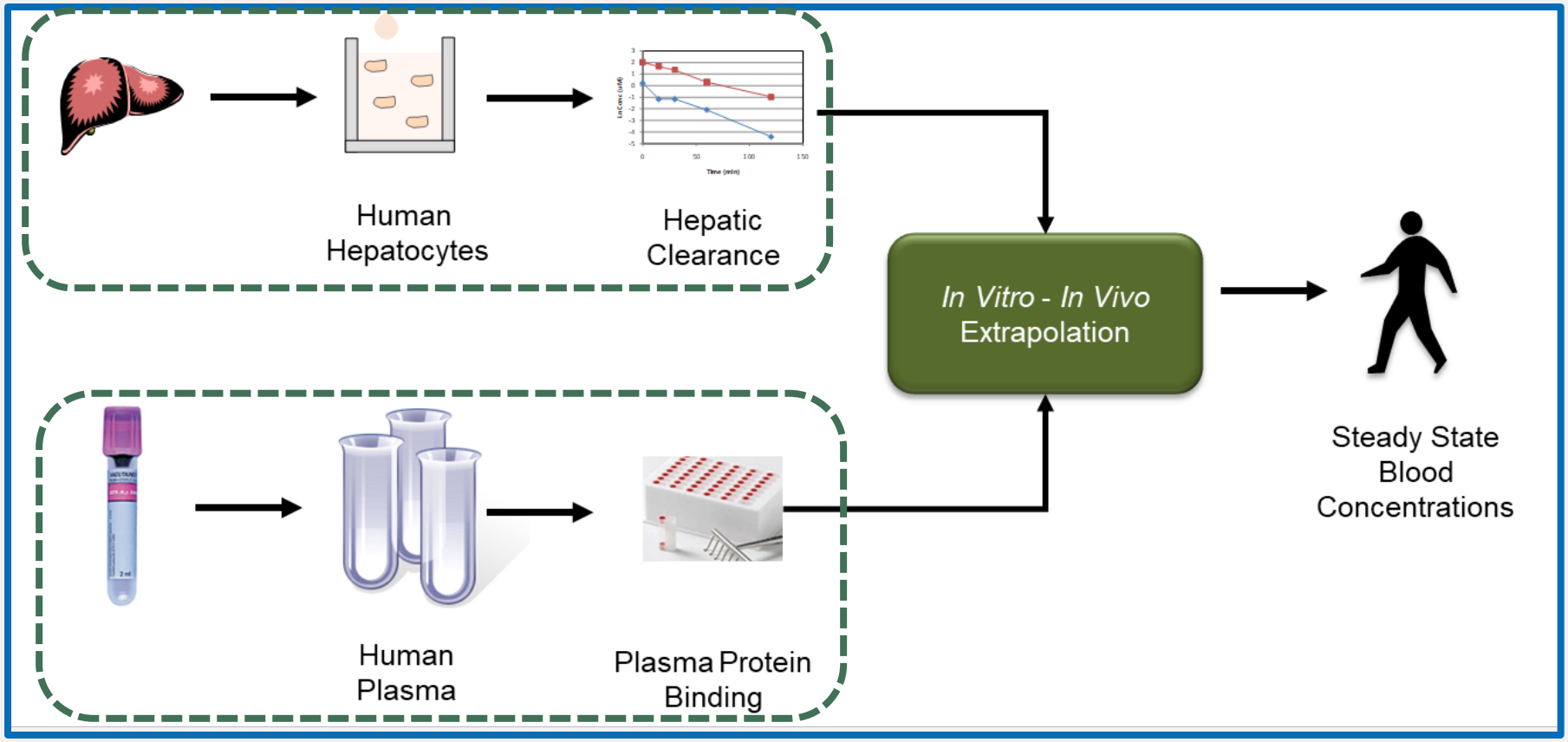
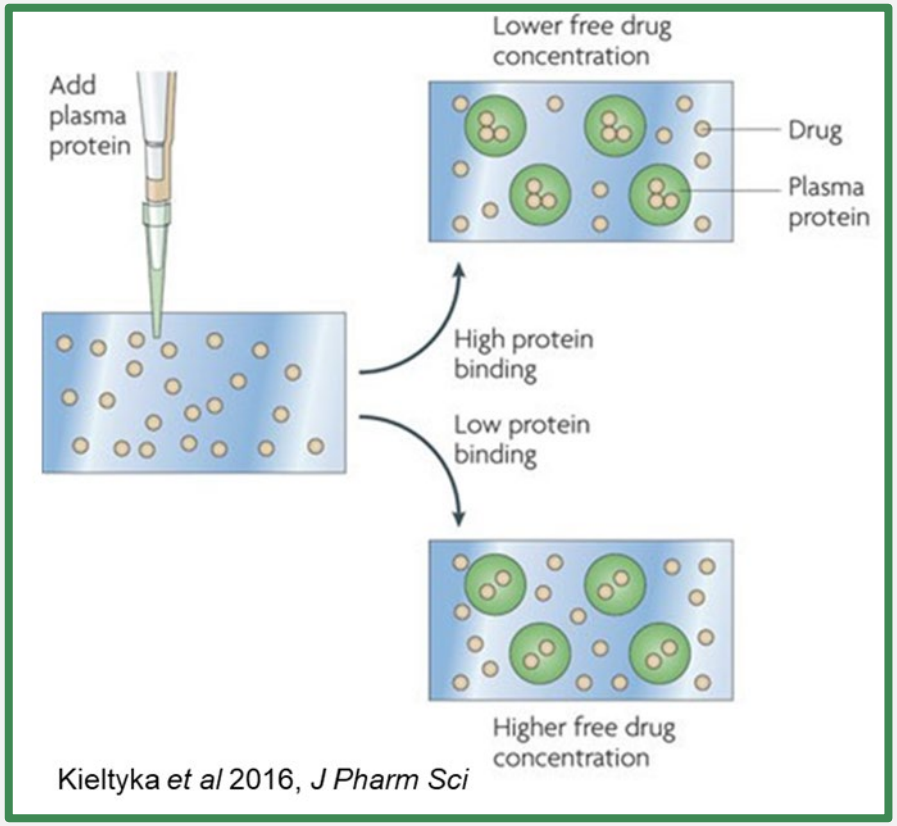


What is plasma protein binding?

- Assay to assess the free (unbound) fraction of chemical to proteins within the blood
  - F_u
  - Unbound molecules permeate through cell membranes to reach 'target'
  - Determine by equilibrium dialysis, ultrafiltration, and/or ultracentrifugation
- Ultracentrifugation assay used
  - Human plasma (10-donor pool, mixed sex) centrifuged to separate aqueous fraction from albumin, lipoproteins, and fatty acids
  - Mixtures of up to 4 PFAS (10 μM) were included with each plasma sample, run in triplicate



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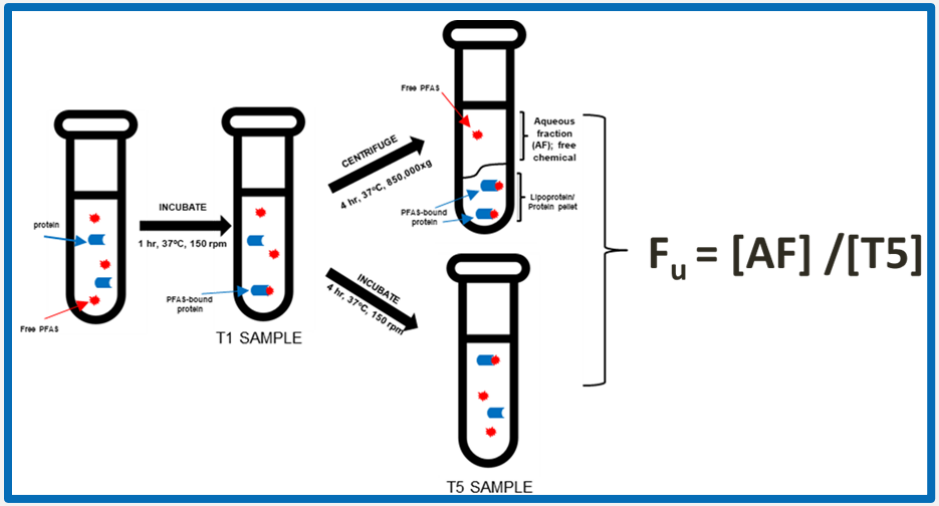
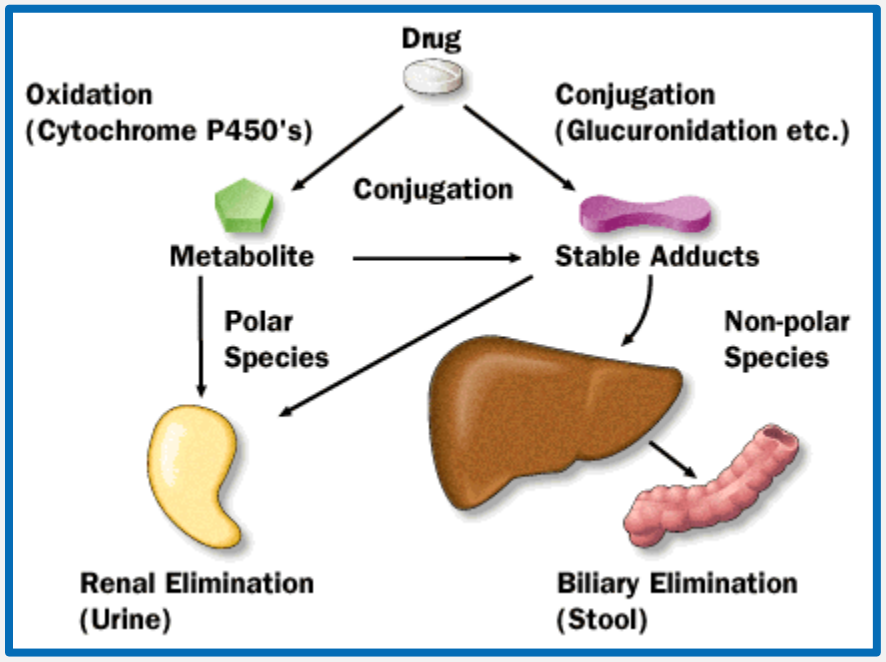
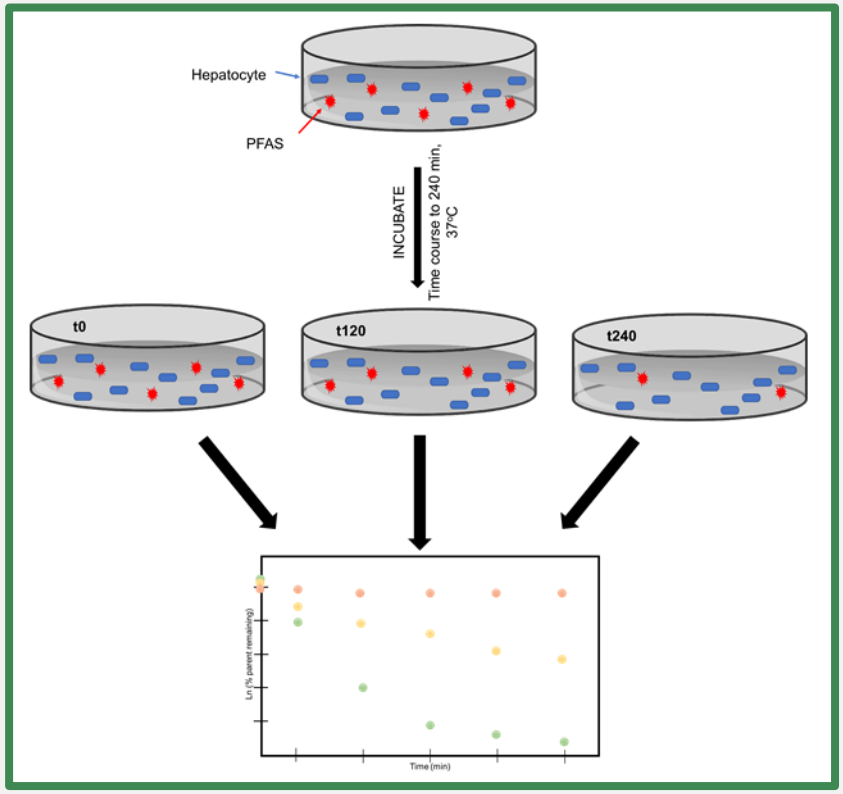


What is *in vitro* hepatic clearance?



- Liver is the major site of drug metabolism in body (hepatic)
- Hepatic clearance ($CL_{hepatic}$) is measure of the rate of elimination of a chemical from the liver
- Models to study metabolism:
  - Human liver microsomes
  - Recombinantly expressed enzymes
  - **Hepatocytes** contain full complement of hepatic drug metabolizing enzymes

What is *in vitro* hepatic clearance?



For the PFAS work…

- Substrate depletion approach using primary human hepatocytes (50-donor pool, mixed sex) at 1 μM PFAS concentration
- Time course: 0, 15, 30, 60, 90, 120, and 240 min with non-linear regression fit
- Work completed by collaborator at National Toxicology Program (NIEHS) [David Crizer]

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How do we analyze these assay samples?

- Both assays require concentration determination of parent PFAS
- EPA has a range of analytical capabilities (single quads, triple quads, high resolution mass spec)

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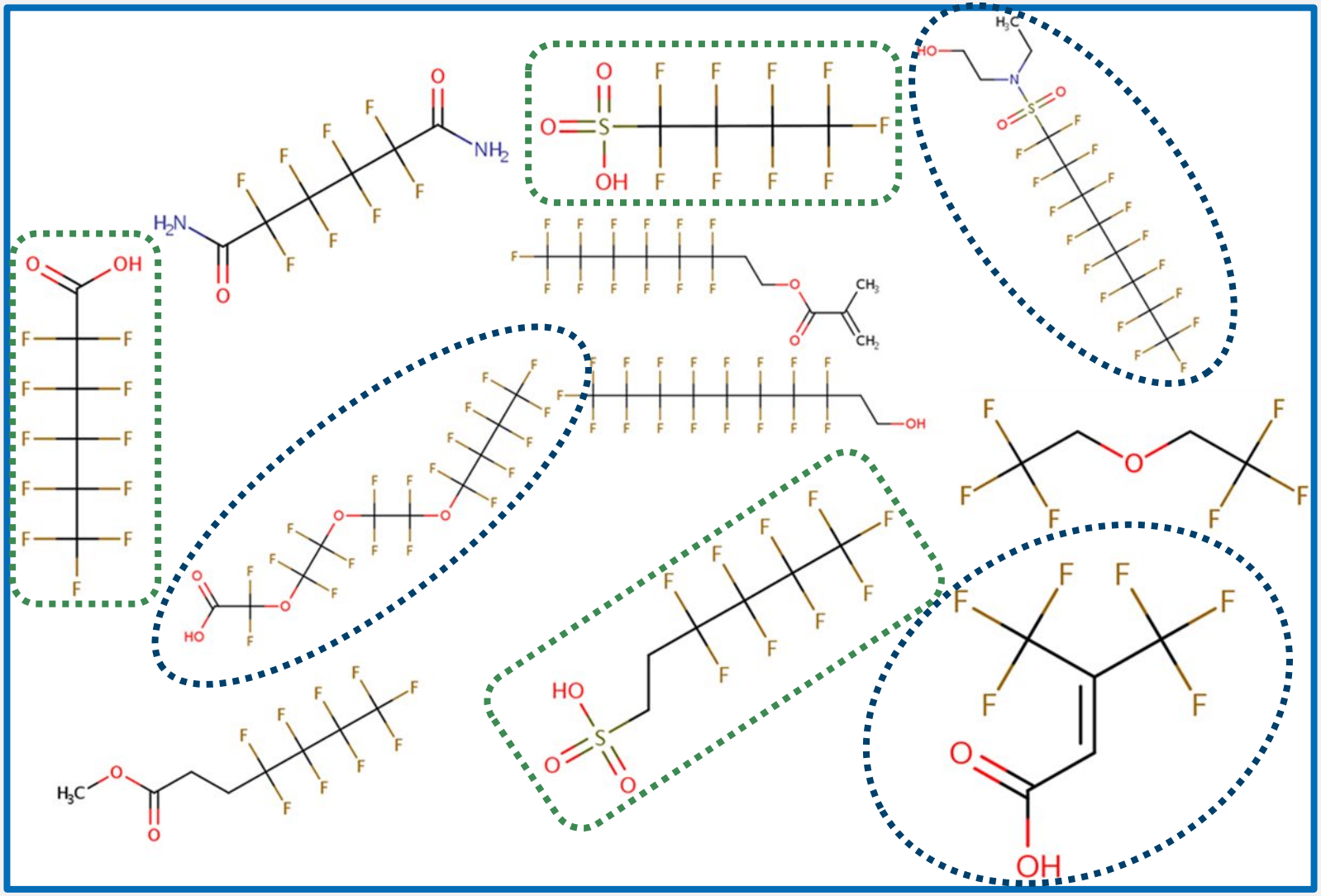
Which PFAS of interest are LC-able?



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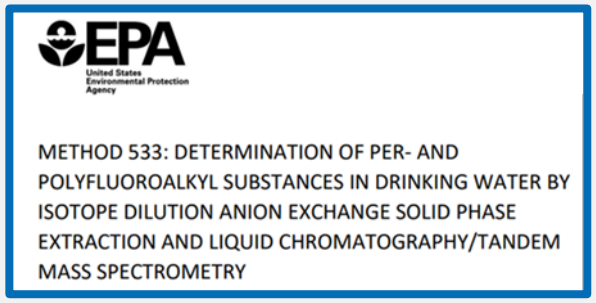
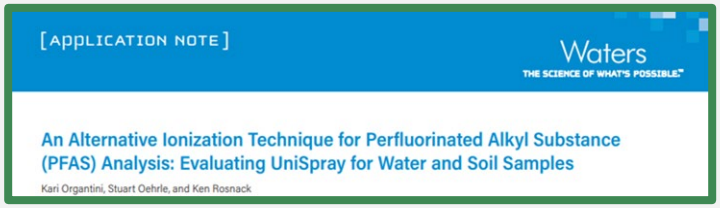
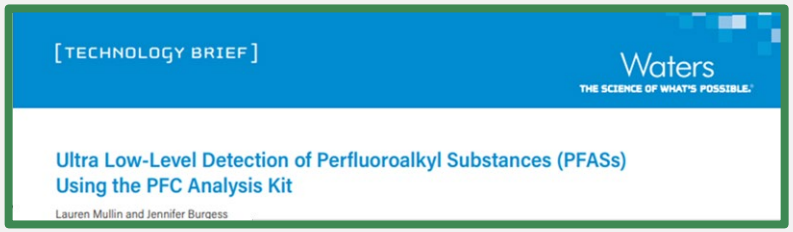
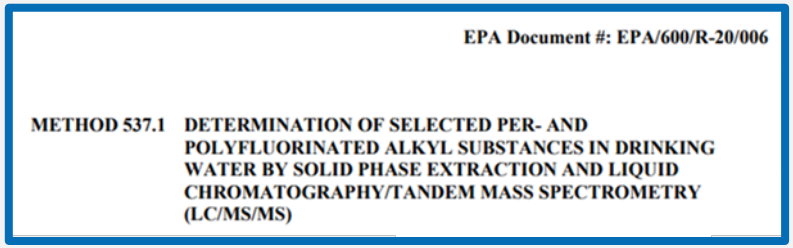
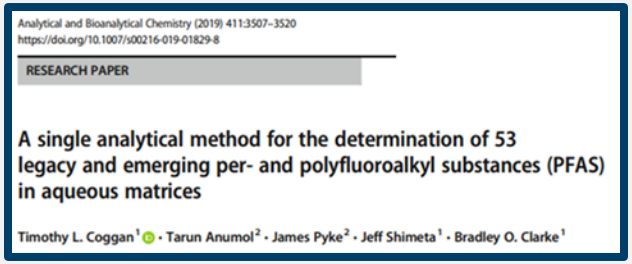
What guided our LC-MS/MS method development journey?

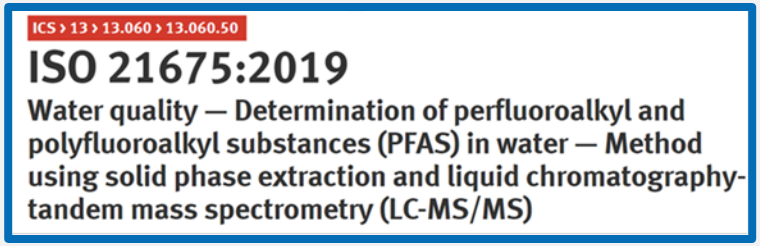


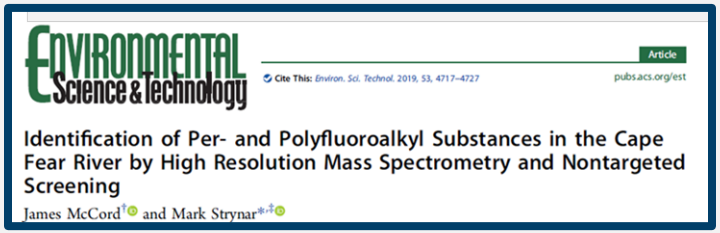












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What LC-MS/MS settings were used?

ACQUITY UPLC I-Class FTN

- Equipped with Waters PFC Kit
- CORTECS T3 2.7 µM 3.0x100 mm
- Column Temp: 55°C
- Flow Rate: 0.6 mL/min
- Run Time: 6.5 min
- Mobile Phase A: 95:5 water: acetonitrile with 2.5 mM ammonium acetate
- Mobile Phase B: 5:95 water: acetonitrile with 2.5 mM ammonium acetate

Xevo TQ-S Micro

- MRM transitions determined
- Acquisition Polarity: ESI+ and ESI-
- Capillary Voltage: 0.4 kV
- Source Temperature: 150°C
- Desolvation Temperature: 500°C
- Desolvation Gas Flow: 1000 L/hr
- Cone Gas Flow: 150 L/hr

- 19 mass-labelled PFAS (Wellington Laboratories, MPFAC-24ES) was included for quantitation

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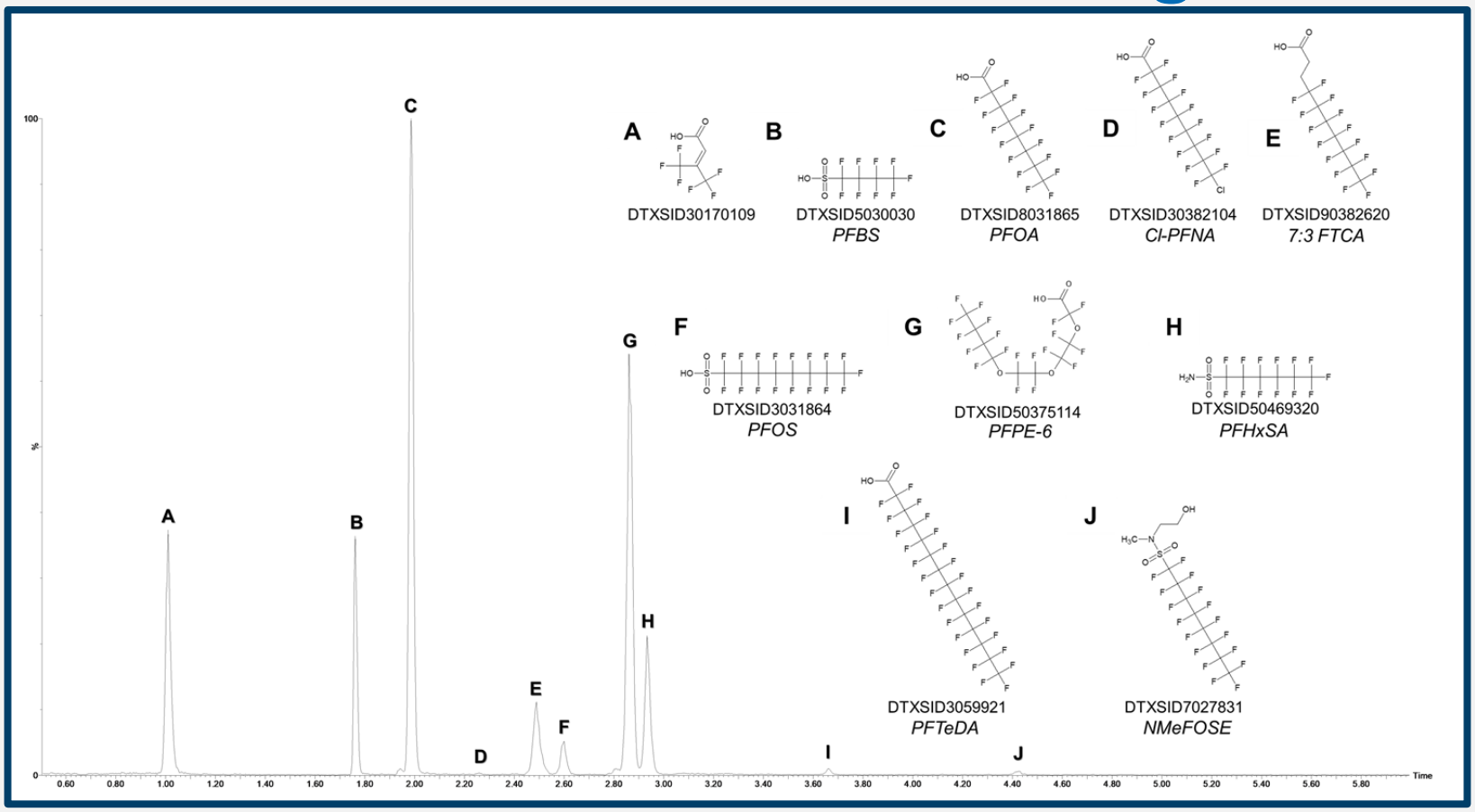
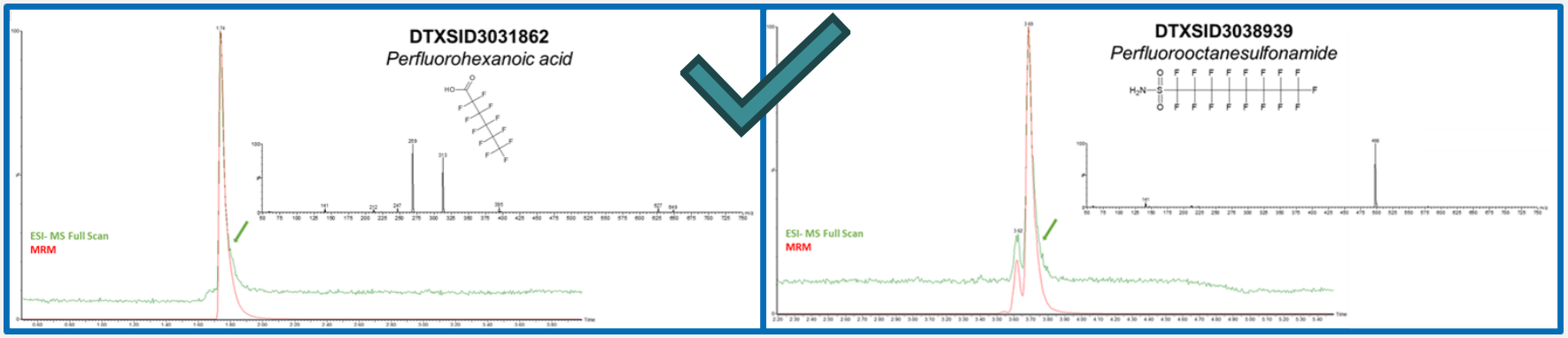
How's the method working?



- Mixture of PFAS run at 100 ppb
- Most have estimated LOQ < 50 ppt
- Most PFAS were analyzed in ESI negative; others were monitored as acetate adducts, fragmented in-source

What about DMSO stock quality?

- DMSO is a common solvent used for *in vitro* assays
- RADAR = MRM (MS/MS) + MS full scan
  - Monitor for any interferences and impurities
  - Application Note: 720005033EN
- Created scoring system for quality of stocks



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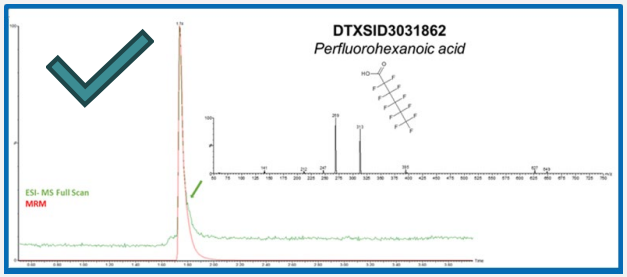

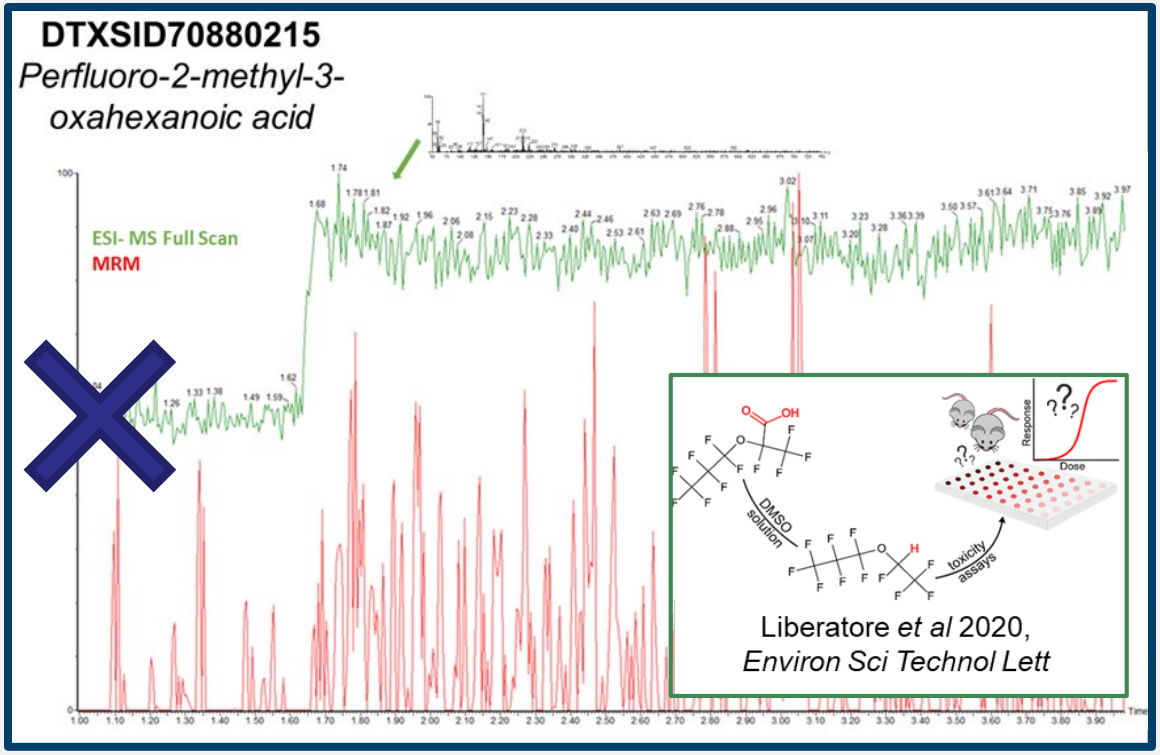

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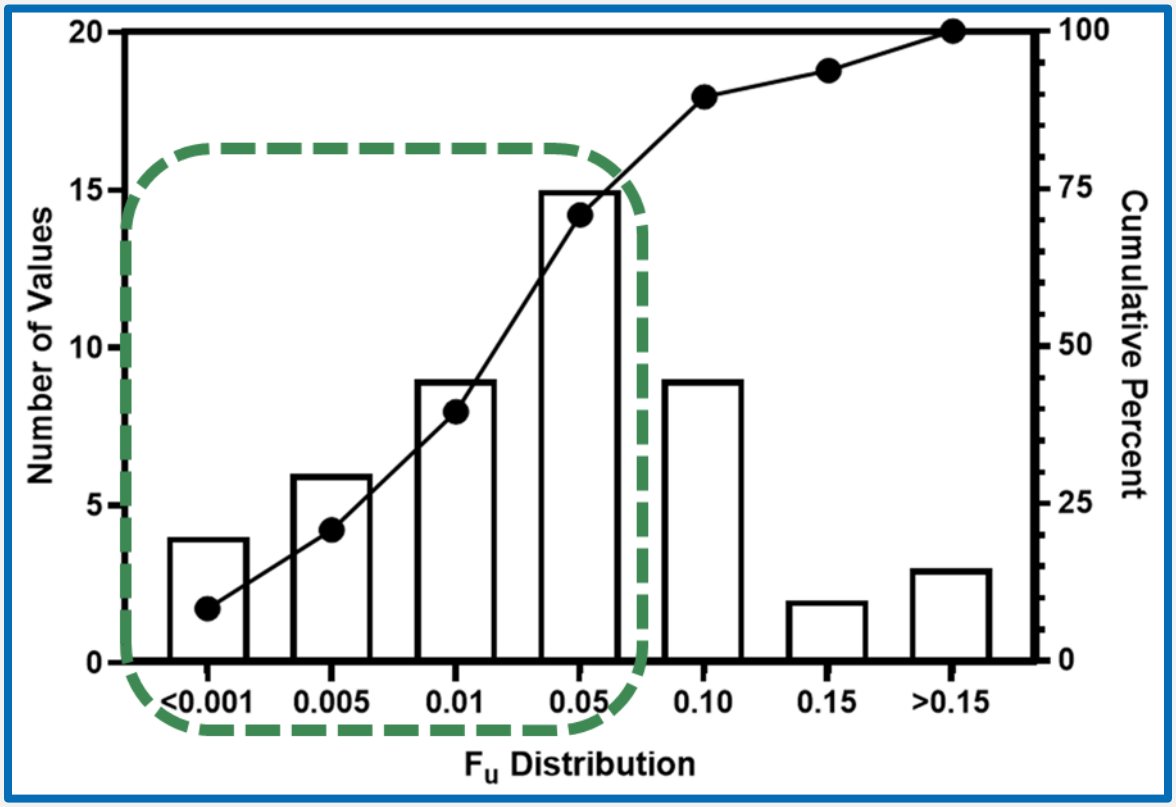
What did we find from the plasma protein binding assay?

50 LC-able PFAS have determined fraction unbound data

F_u ↓ binding to plasma proteins ↑



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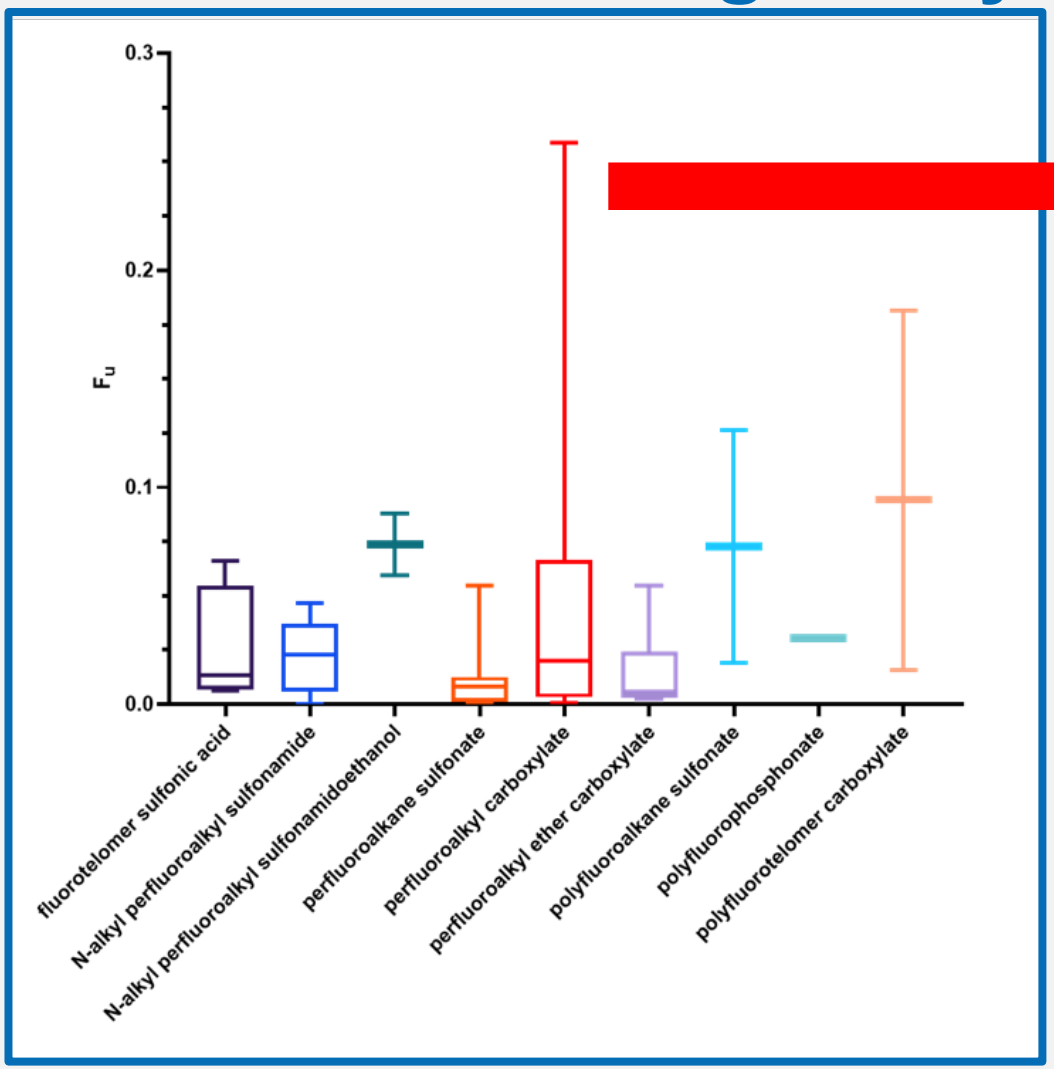
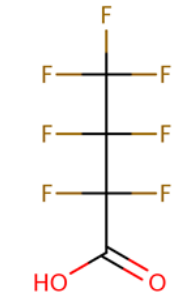
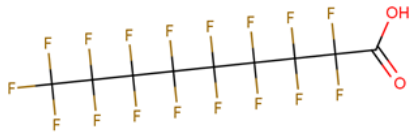




PFAS	Avg Fu
Perfluoropropanoic acid	0.2586
Perfluorobutanoic acid	0.0939
Perfluoropentanoic acid	0.0440
Perfluorohexanoic acid	0.0076
Perfluorooctanoic acid	0.0034
Perfluorononanoic acid	0.0015

chain length ↓ F_u



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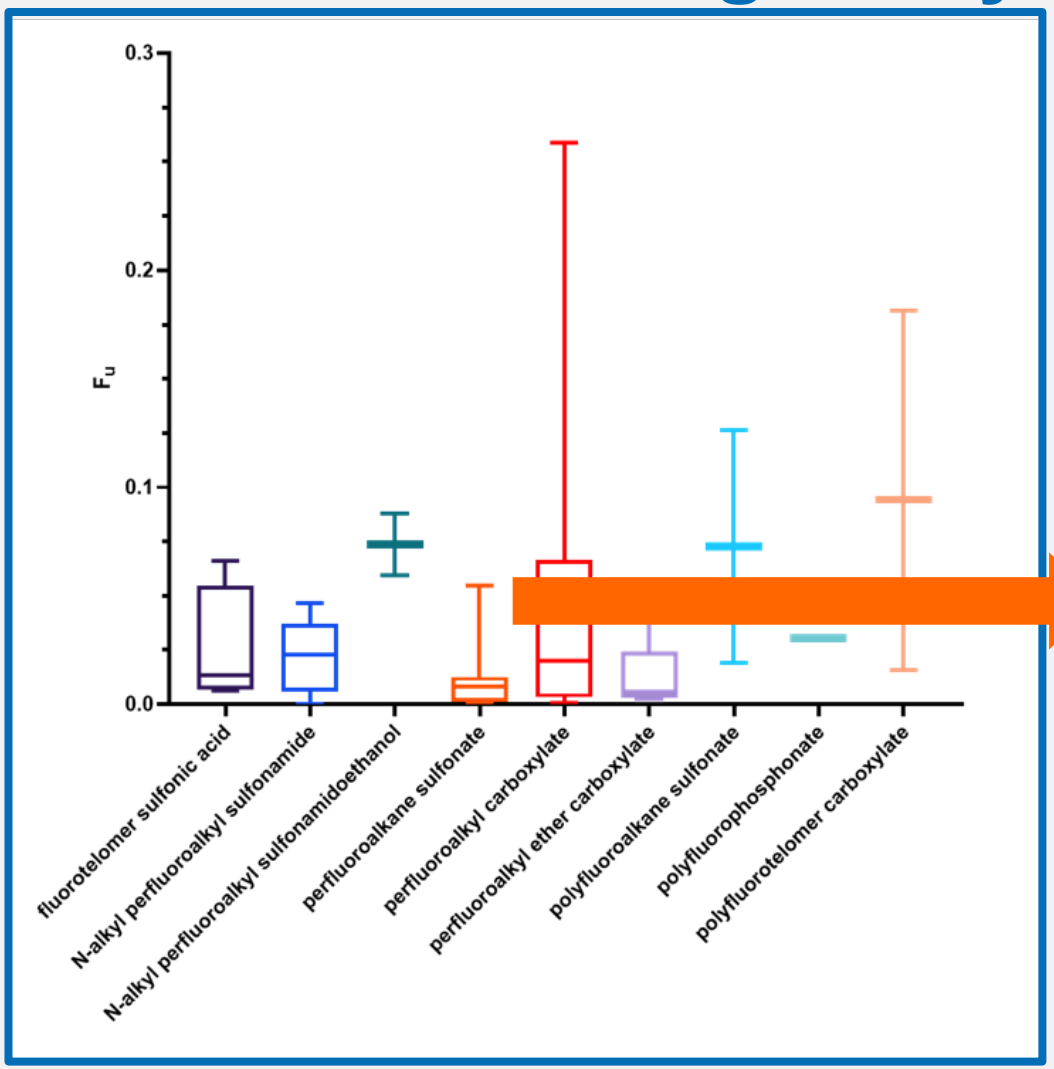
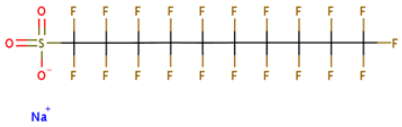
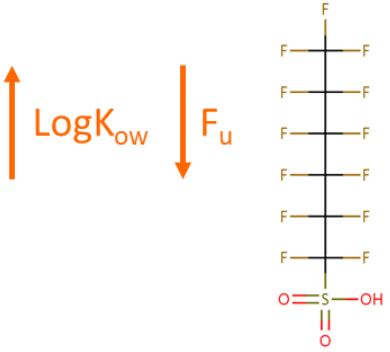




PFAS	Avg Fu	LogK$_{ow}$
Perfluorohexanesulfonic acid	0.0008	3.47
Potassium perfluorooctanesulfonate	0.0040	3.39
Perfluorobutanesulfonic acid	0.0128	2.57
Sodium perfluorodecanesulfonate	0.0546	1.83



* This is based on Dashboard Average Predicted LogK$_{ow}$

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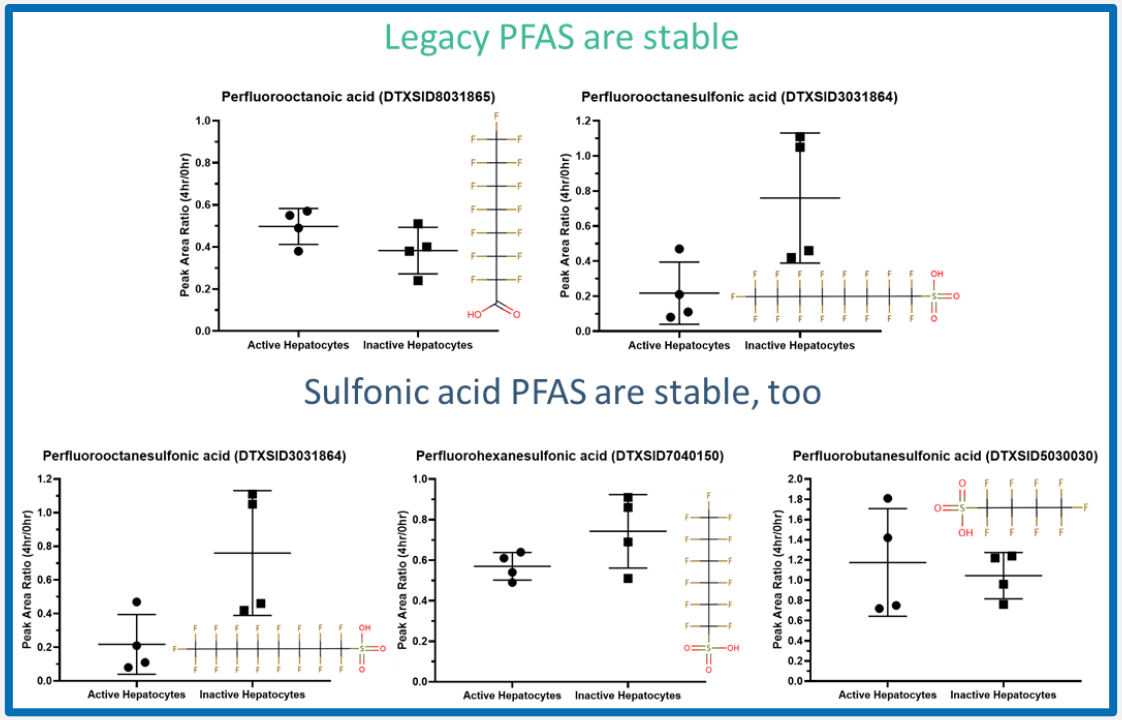
Any observations from the hepatic clearance assay?

- More than 20 LC-able PFAS assessed

1. *In vitro hepatic clearance screen*
   - 0 and 4 hr time points for active and inactive hepatocytes
   - Compared time ratios to examine for clearance potential



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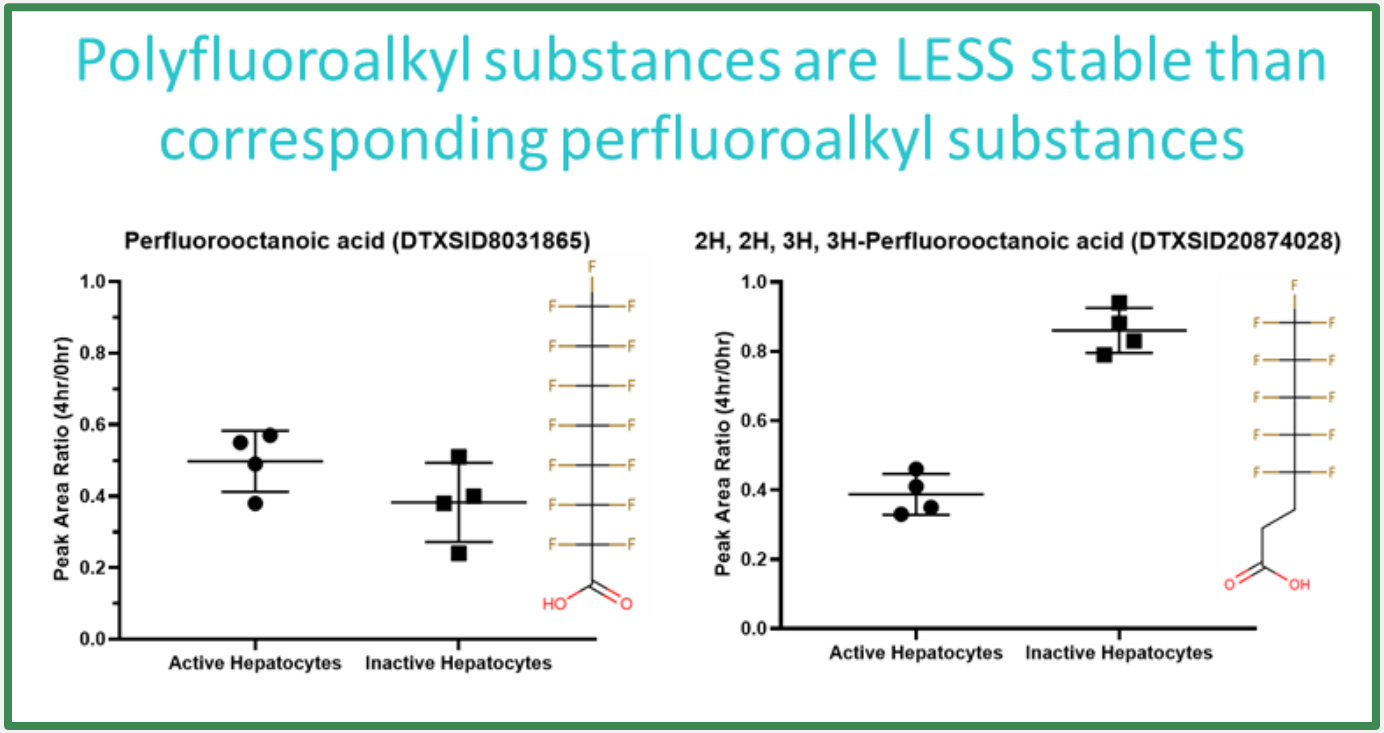
- More than 20 LC-able PFAS assessed

1. *In vitro hepatic clearance screen*
   - 0 and 4 hr time points for active and inactive hepatocytes
   - Compared time ratios to examine for clearance potential



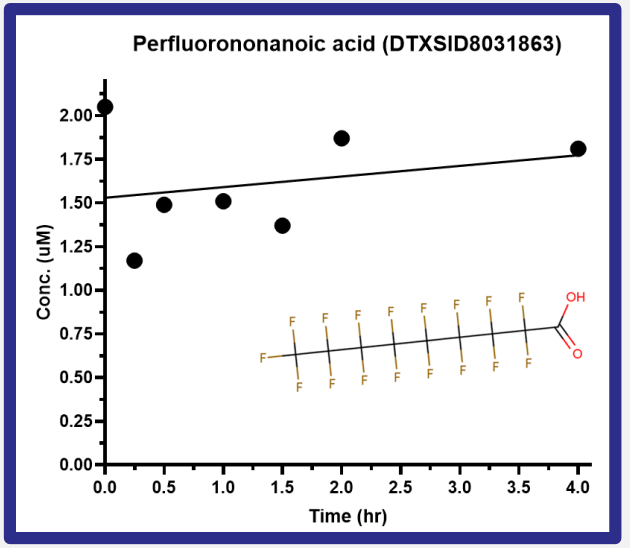
Any observations from the hepatic clearance assay?

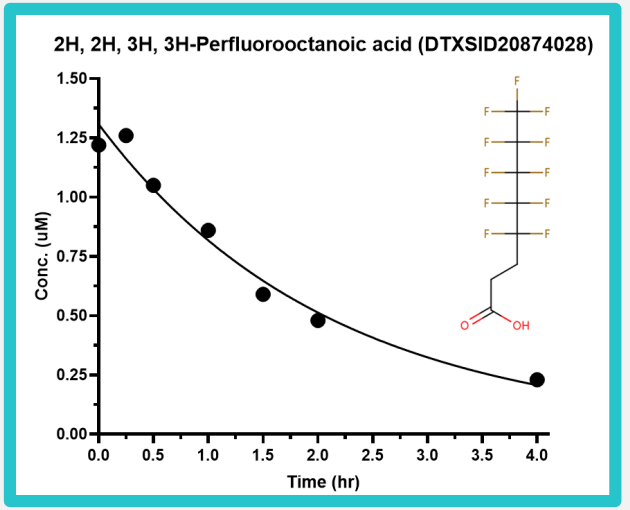
- More than 20 LC-able PFAS assessed

2. *Metabolic stability time course*
   - 0, 0.25, 0.50, 1, 1.5, 2, 4 hr time points
   - Non-linear fit to determine half-life ($T_{1/2}$)

Clearance rate increasing (faster)

Compound Name	Half-life (min)	Clearance (µL/min/million cells)
Perfluorobutanoic acid	44769343	1.55E-05
Potassium perfluorohexanesulfonate	21340366	3.25E-05
Perfluorohexanoic acid	237257	2.92E-03
Ammonium perfluorooctanoate	88735	7.81E-03
Potassium perfluorobutanesulfonate	2300	3.01E-01
Perfluorononanoic acid	1155	6.00E-01
Perfluorooctanesulfonic acid	990	7.00E-01
Perfluoro(4-methoxybutanoic) acid	346.5	2.00E+00
2H,2H,3H,3H-Perfluorooctanoic acid	101.4	6.83E+00
N-Ethylperfluorooctanesulfonamide	57	1.22E+01
3-(Perfluoro-2-butyl)propane-1,2-diol	35.87	1.93E+01
Perfluoro-3,6,9-trioxatridecanoic acid	29.71	2.33E+01
Nonafluoropentanamide	25.45	2.72E+01
3,3-Bis(trifluoromethyl)-2-propenoic acid	19.77	3.51E+01
4:2 Fluorotelomer sulfonic acid	17.5	3.96E+01
Octafluoroadipamide	12.8	5.41E+01
Perfluoropentanamide	10.63	6.52E+01
N-Methylperfluorooctanesulfonamide	10.17	6.81E+01
2,2,3,3,4,4-Hexafluorobutanoic acid	4.209	1.65E+02
Perfluorooctanesulfonamide	2.789	2.48E+02





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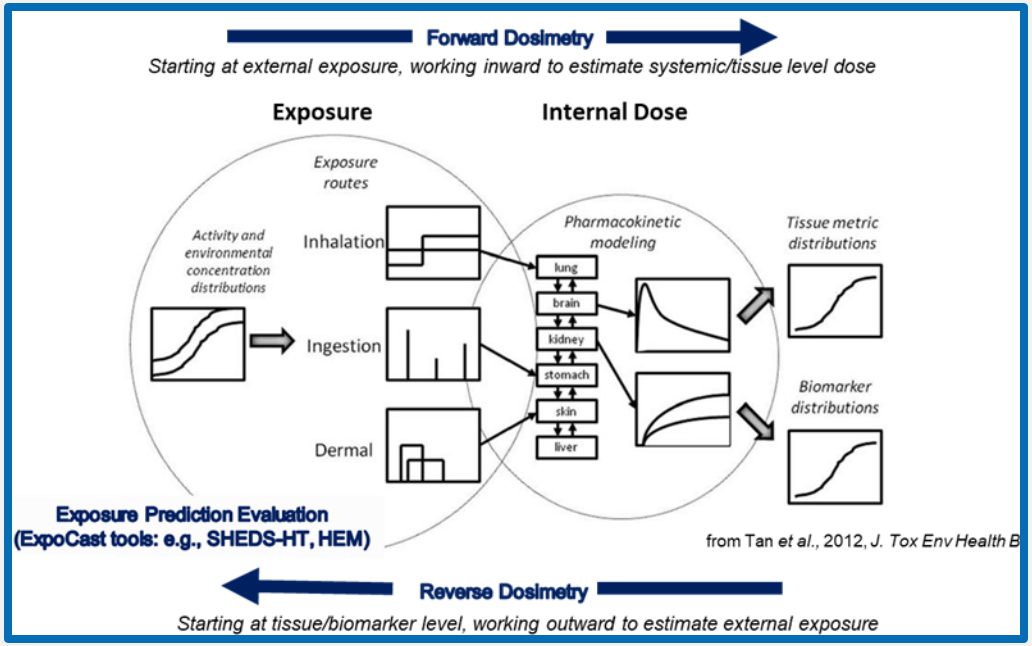
What is IVIVE?

- *In vitro-in vivo* extrapolation = IVIVE
  - Model approach that allows *in vitro* data to be extrapolated to estimate corresponding *in vivo* effects
  - Start at tissue/biomarker level → estimate external exposure
- **Steady-state concentration (C_{ss})**
  - Concentration of compound in body that stays consistent
  - This takes into account plasma protein binding and hepatic clearance data



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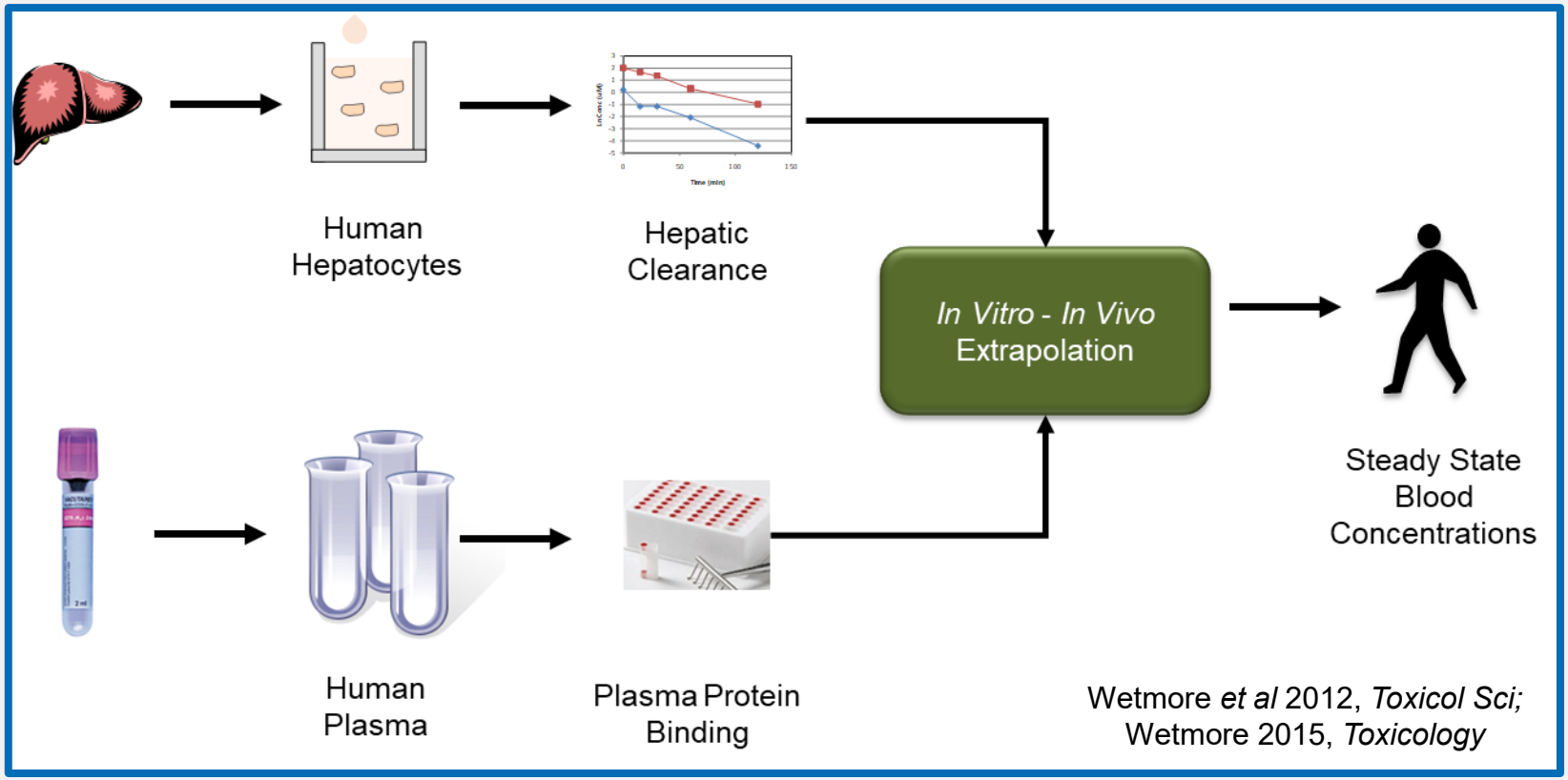
What is IVIVE?



Wetmore *et al* 2012, *Toxicol Sci;*
Wetmore 2015, *Toxicology*

What is IVIVE?

In Vitro - In Vivo Extrapolation

Steady State Blood Concentrations

$$[Conc]_{SS} = \frac{\text{Dose Rate} * \text{Body Weight}}{CL_{WholeBody}}$$

$$CL_{WholeBody} = CL_R + CL_H$$

$$CL_R = F_U * GFR$$

where GFR ≈ 6.7 L/hr

Assumptions

Exposure at 1 µg/kg/day

Linear kinetics

100% oral bioavailability

$$CL_H = \frac{F_U * Q_L * CL_{Int}}{Q_L + F_U * CL_{Int}}$$

where Q_L ≈ 90 L/hr

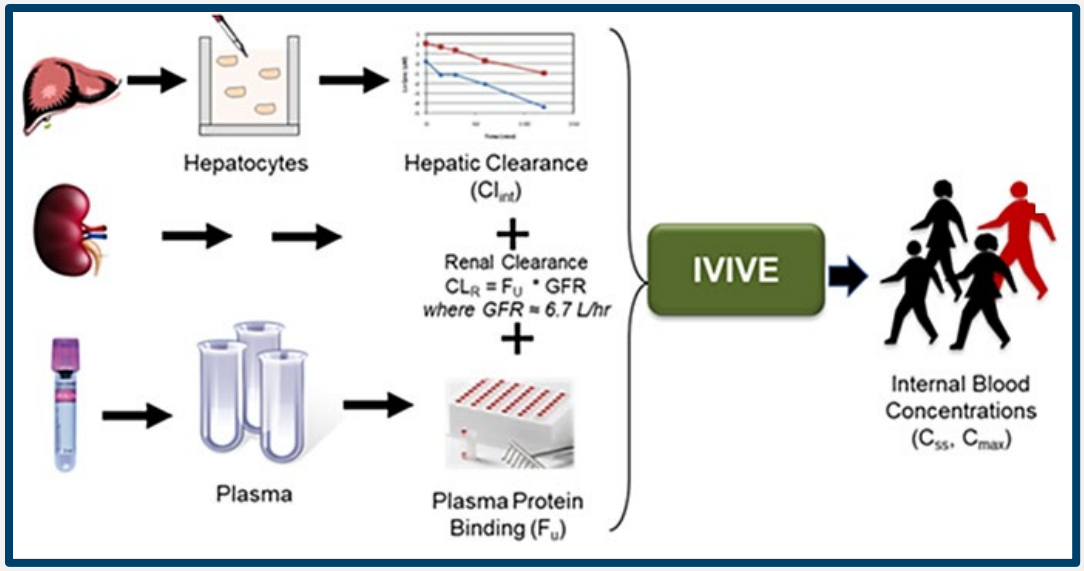
Wetmore *et al* 2012, *Toxicol Sci;*
Wetmore 2015, *Toxicology*

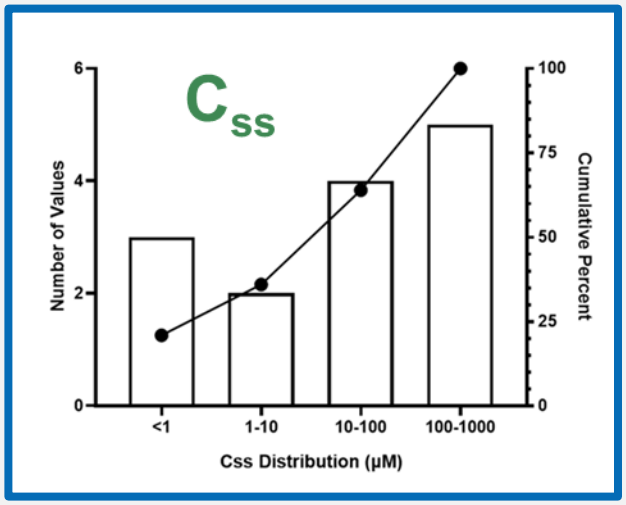
$$CL_{Int} = HPGL * V_L * CL_{invitro}$$

where HPGL ≈ 137 million cells/g

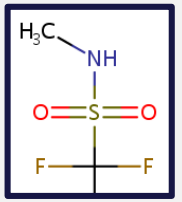
V_L ≈ 1820 g

What did IVIVE show with PFAS data?





Compound Name	F_u	Cl_{renal} (L/hr)	$Cl_{hepatic}$ (L/hr)	Css (μM)
Potassium perfluorohexanesulfonate	0.0011	0.0075	3.82E-07	894.5132
Ammonium perfluorooctanoate	0.0014	0.0094	1.16E-04	713.7360
Perfluorononanoic acid	0.0013	0.0088	8.33E-03	368.6974
Perfluorohexanoic acid	0.0076	0.0507	2.33E-04	183.6569
Potassium perfluorobutanesulfonate	0.0087	0.0581	2.75E-02	101.5252
Perfluorooctanesulfonic acid	0.0073	0.0490	5.38E-02	57.1902
Perfluoro(4-methoxybutanoic) acid	0.0142	0.0950	2.97E-01	26.7545
Perfluorobutanoic acid	0.1032	0.6927	1.68E-05	19.8299
2H,2H,3H,3H-Perfluorooctanoic acid	0.0072	0.0483	5.15E-01	15.2577
Perfluoro-3,6,9-trioxatridecanoic acid	0.0026	0.0176	6.38E-01	7.9748
4:2 Fluorotelomer sulfonic acid	0.0142	0.0951	5.55E+00	1.5874
N-Ethylperfluorooctanesulfonamide	0.0464	0.3110	5.57E+00	0.9485
N-Methylperfluorooctanesulfonamide	0.0113	0.0757	7.43E+00	0.7633
Perfluorooctanesulfonamide	0.0229	0.1536	3.60E+01	0.1630



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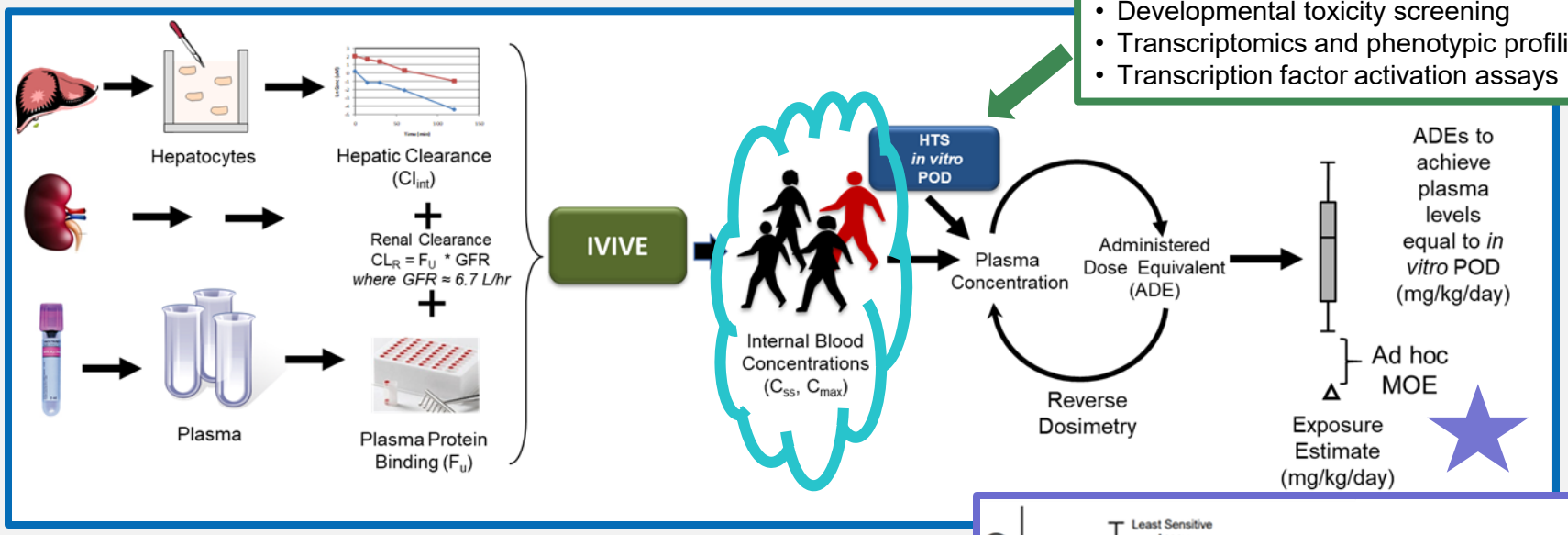
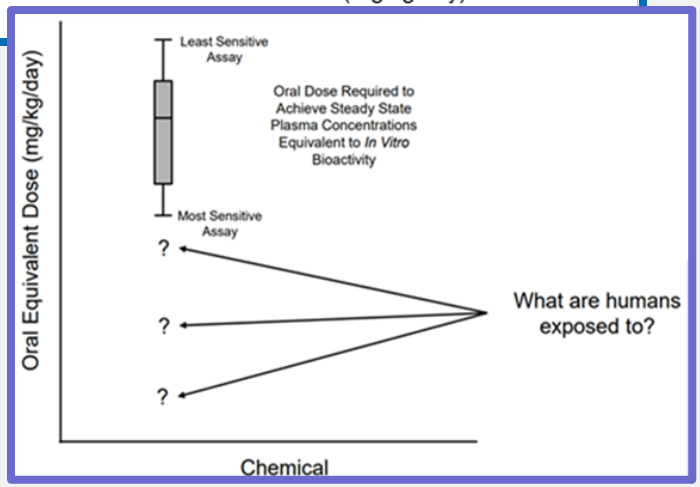
What is the big picture of this PFAS toxicity effort?

High Throughput Screening Assays
- Developmental neurotoxicity
- Developmental toxicity screening
- Transcriptomics and phenotypic profiling
- Transcription factor activation assays



Generated PFAS data along with human exposure information will assist in informing human health risk assessment and subsequent testing



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Summary of findings

- Experimental *in vitro* toxicokinetic data (F_u and $Cl_{hepatic}$) are being measured on over 120 PFAS for use in IVIVE modeling
- Multi-residual LC-MS/MS method developed to analyze more than 60 unique PFAS
- Plasma protein binding data indicate high binding rates, with 75% exhibiting F_u values from 0.001 – 0.05
- Assuming an external exposure of 1 μg/kg/day, C_{ss} predictions ranged from 0.16-895 μM, with a median value of 23.29 μM
- These C_{ss} estimates eventually will be combined with other high-throughput screening data to help identify PFAS risk to humans
- Continuing data generation for additional PFAS and toxicokinetic assays for bioavailability, metabolite identification, and renal reuptake

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