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Evaluating Per- and Polyfluoroalkyl Substances (PFAS) by *In Vitro* Toxicokinetic Data Generation with *In Vitro-In Vivo* Extrapolation (IVIVE)

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U.S. Environmental Protection Agency, Office of Research and Development, Center for Computational Toxicology and Exposure

Presentation for ASCCT 9th Annual Meeting

Tuesday, October 20, 2020

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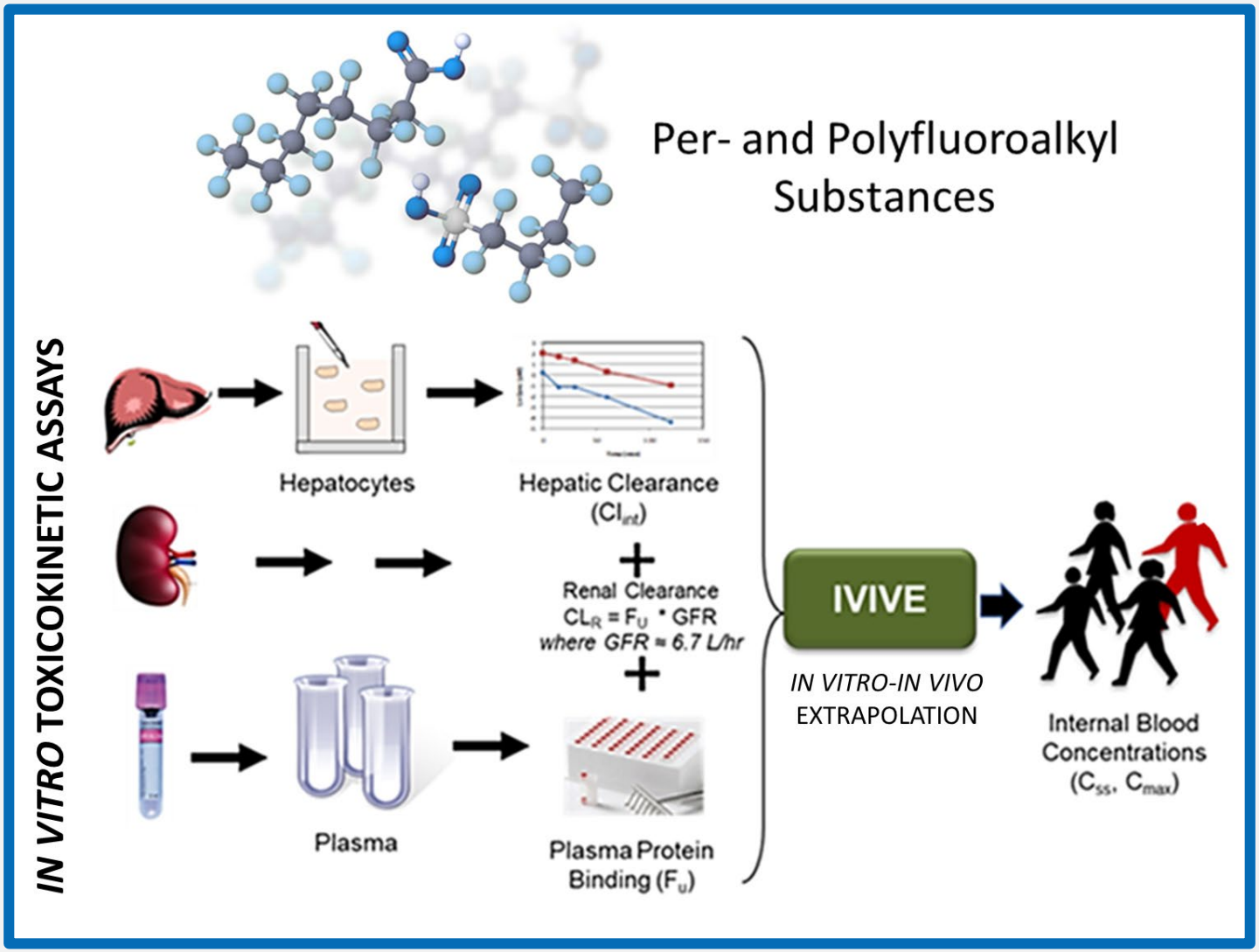

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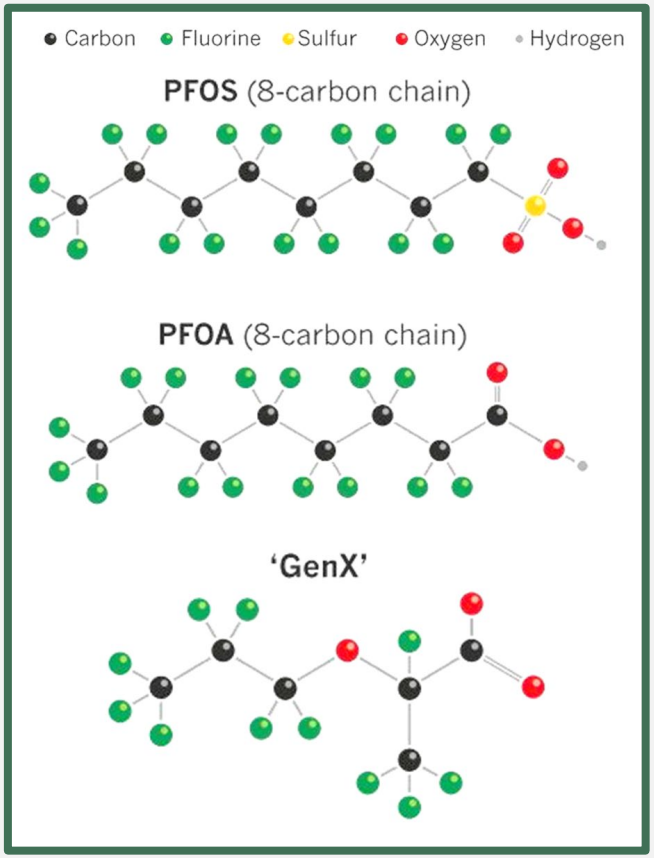
What are PFAS?

- **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



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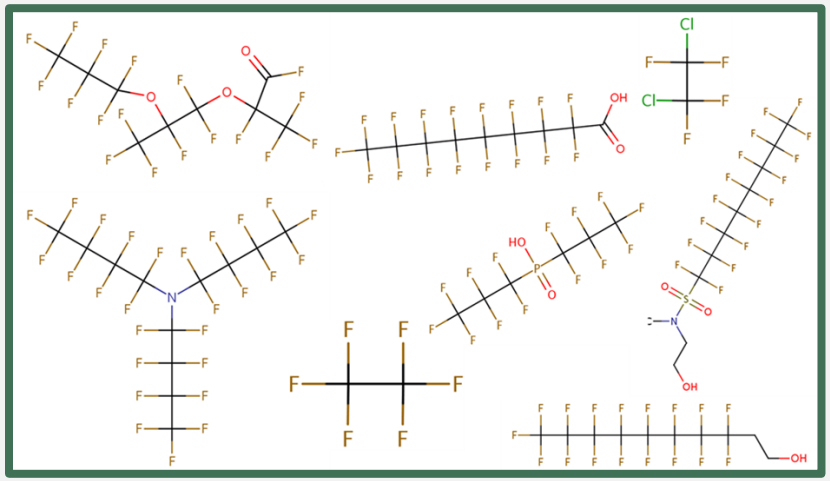
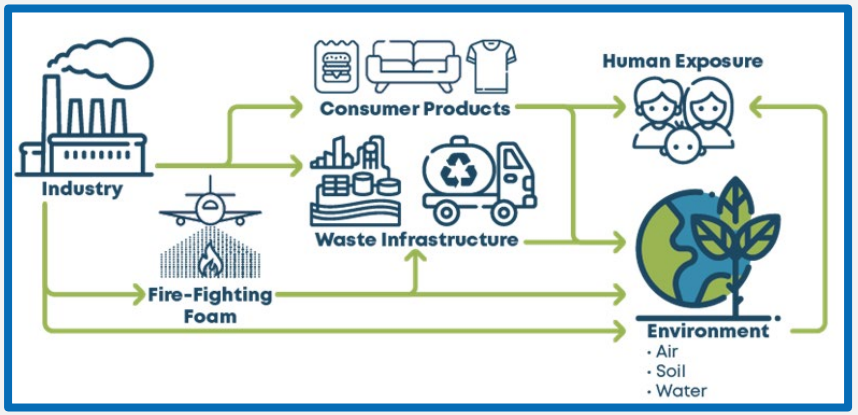
Why are PFAS receiving lots of attention?

- **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
- **Abundance**
  - 6,648 PFAS-like structures on EPA's Dashboard
  - 1,223 PFAS on TSCA inventory with 602 currently in use in USA





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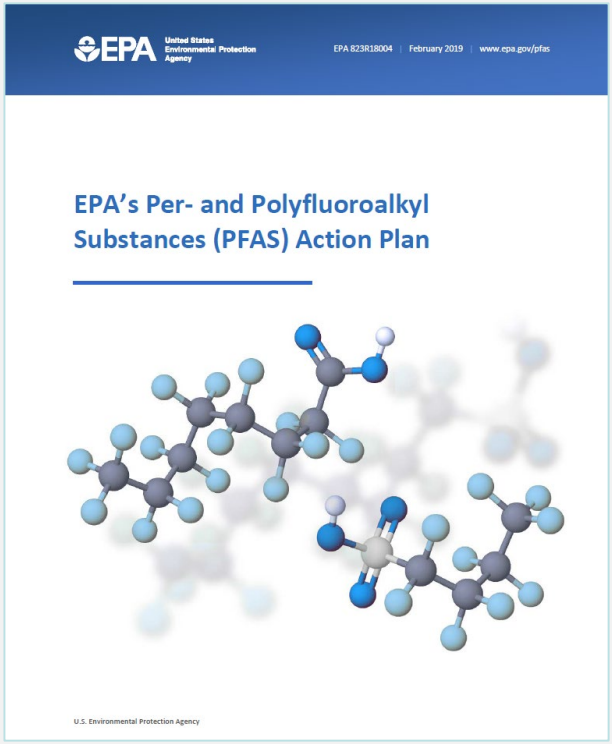
What is the EPA doing about PFAS?

- 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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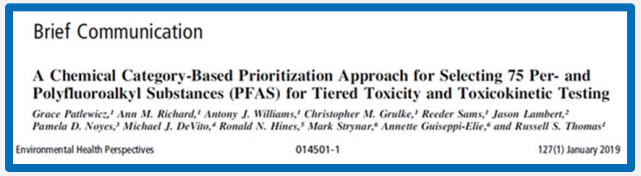
Which PFAS are we interested in?

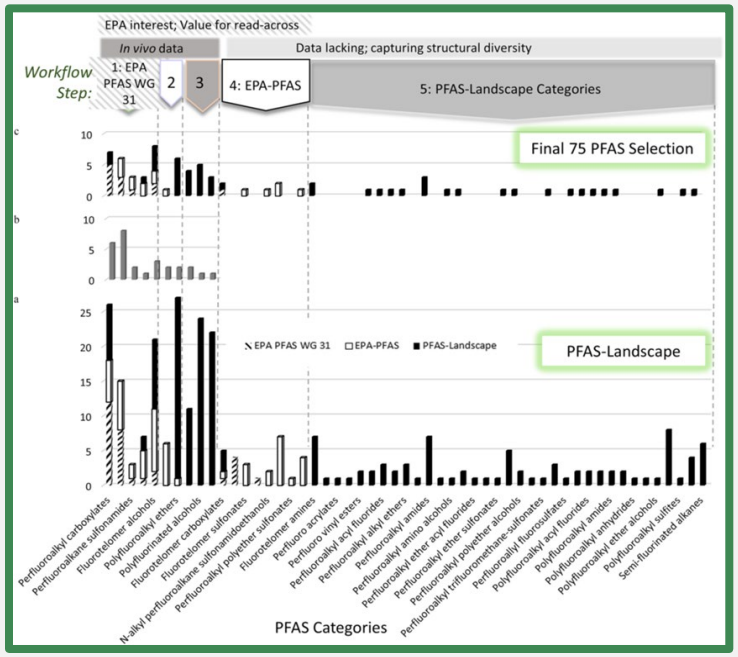
- PFAS Screening Library creation: **PFAS Landscape**
  - Maximize read-across
  - Capture structural diversity
- Initially, **75** PFAS selected from the PFAS Landscape
- Now, PFAS of interest for testing includes nearly **200** unique structures with **430** unique PFAS in the Landscape

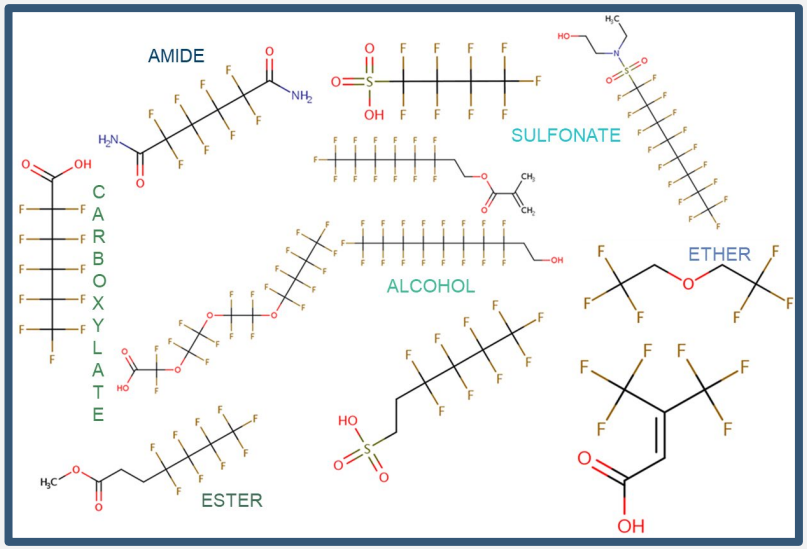

Brief Communication

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[4]

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





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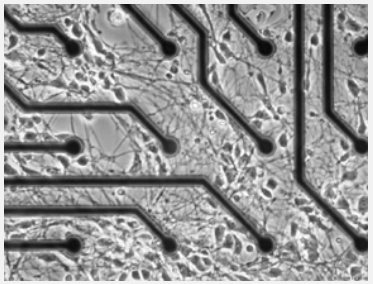
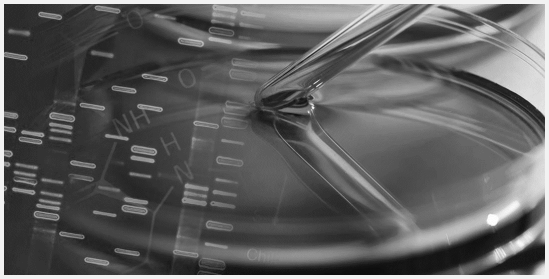
How are we examining these PFAS?

- 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

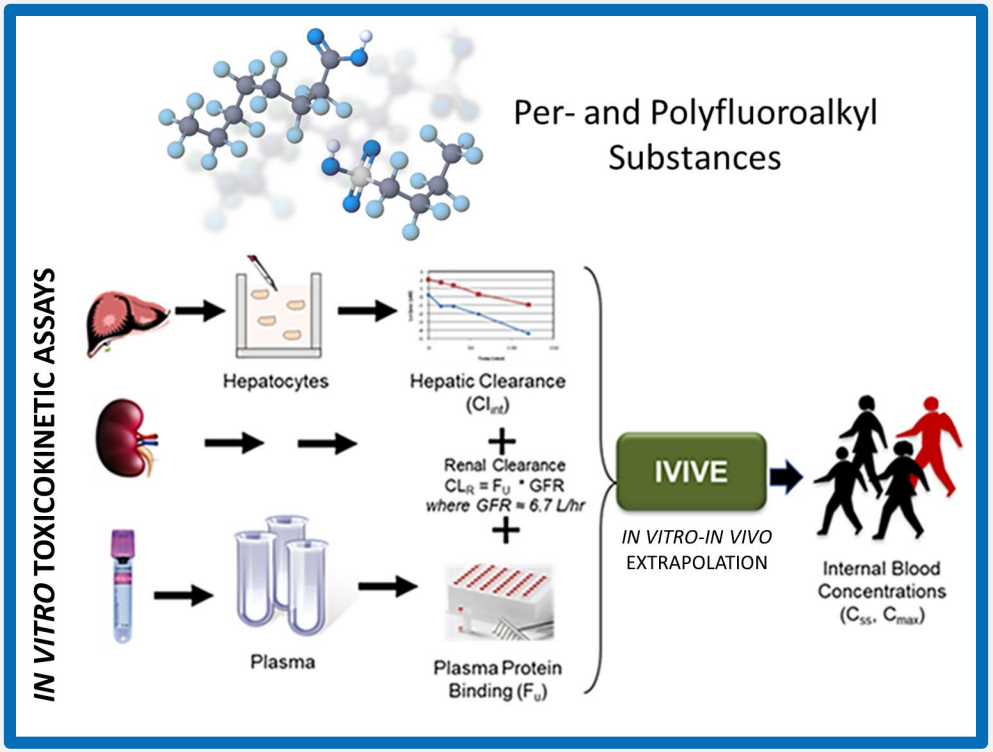
- Endpoints for PFAS work
  - Hepatotoxicity
  - Immunotoxicity
  - Developmental toxicity
  - Mitochondrial toxicity
  - ***In vitro* toxicokinetic assays**





What are *in vitro* toxicokinetic assays?

- Toxicokinetics (TK): 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



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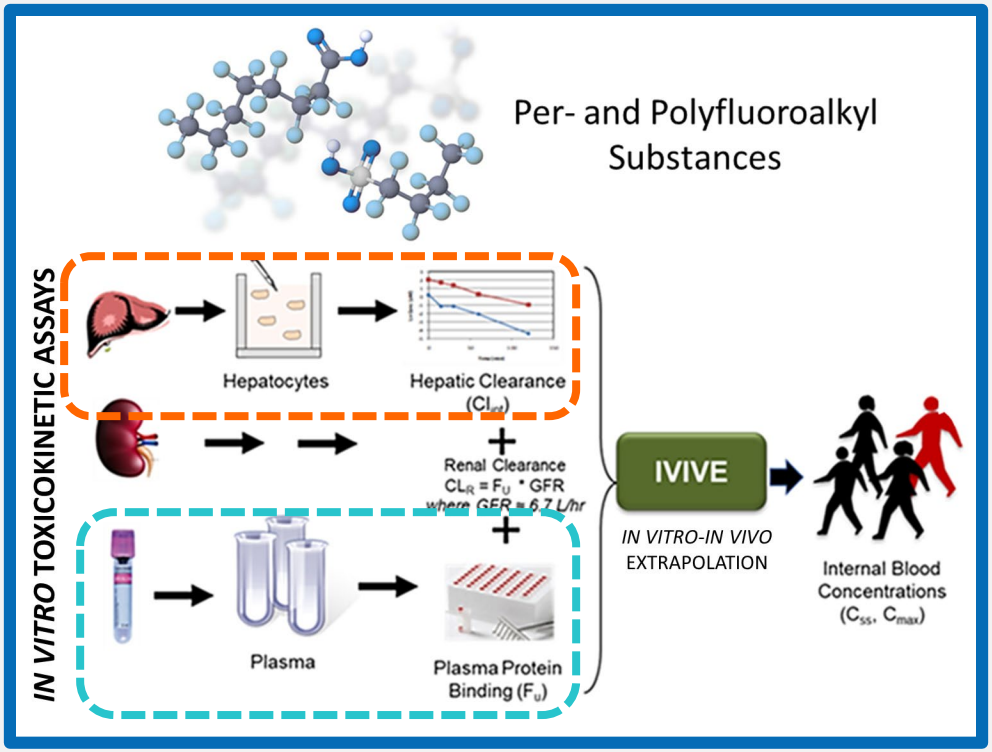
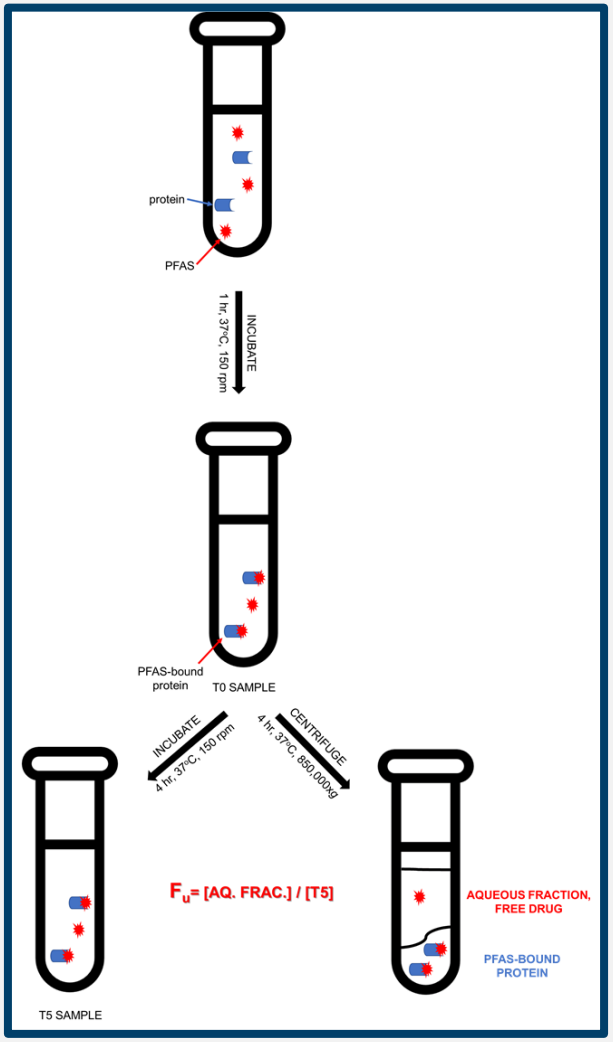


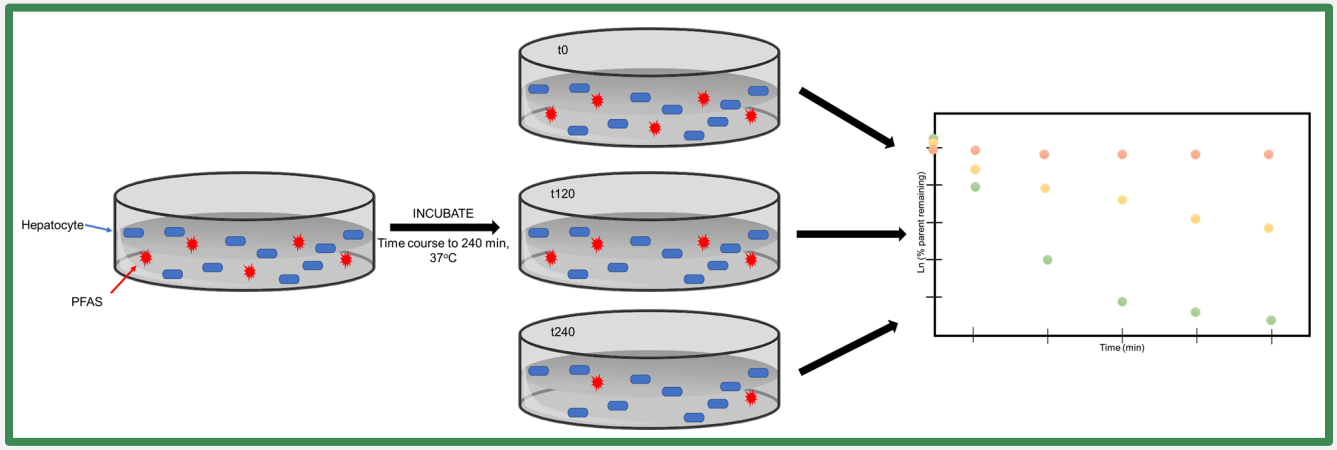
What is plasma protein binding?

- 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 for PFAS analysis
  - 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



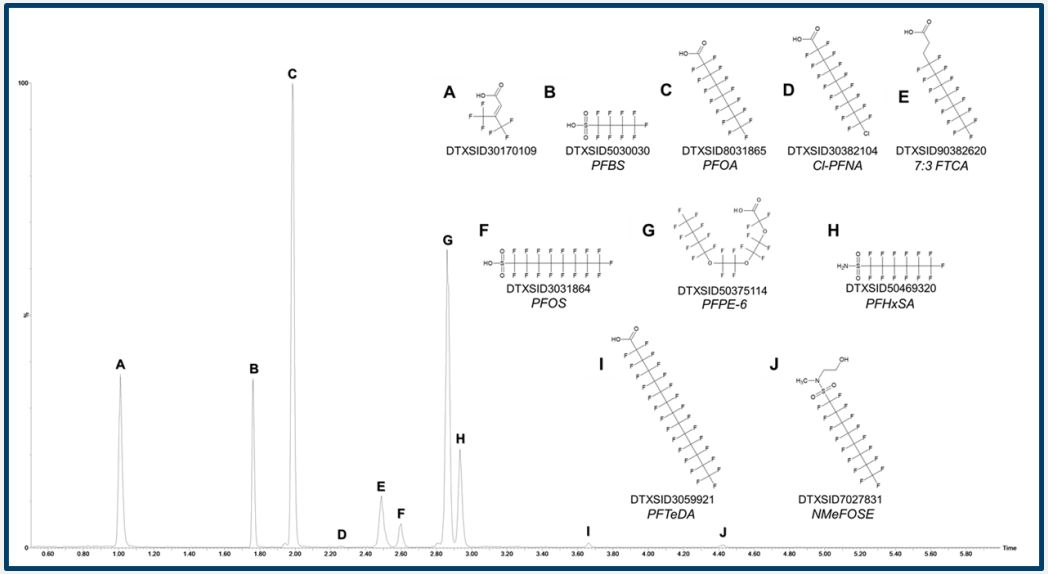
What is *in vitro* hepatic clearance?

- Hepatic clearance ($\mathbf{CL_H}$) is measure of the rate of elimination of a chemical from the liver
- Models to study metabolism include human liver microsomes, recombinantly expressed enzymes, and **hepatocytes**
- Substrate depletion approach utilized for PFAS work
  - 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 [**David Crizer**]



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 specs)
  - Ultra-high-performance liquid chromatography tandem mass spectrometer used



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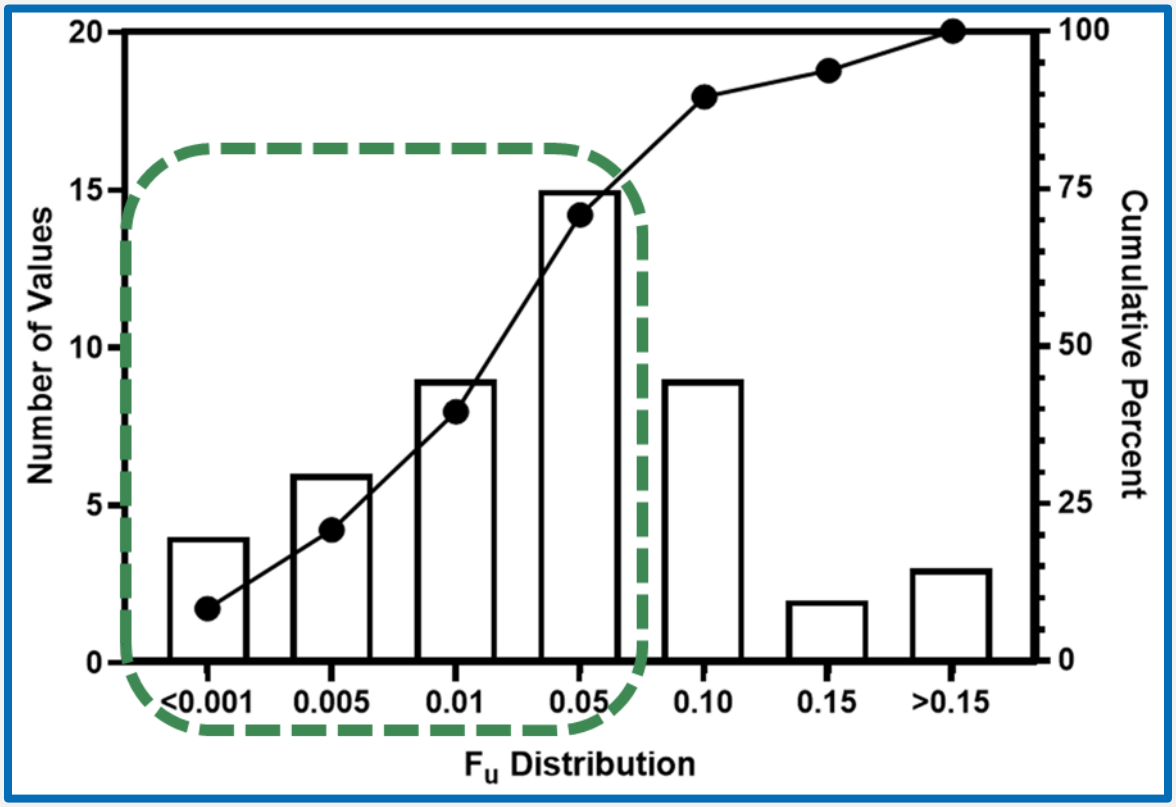
What did we find from the plasma protein binding assay?

50 LC-able PFAS have determined fraction unbound data

F_u ↓ binding to plasma proteins ↑



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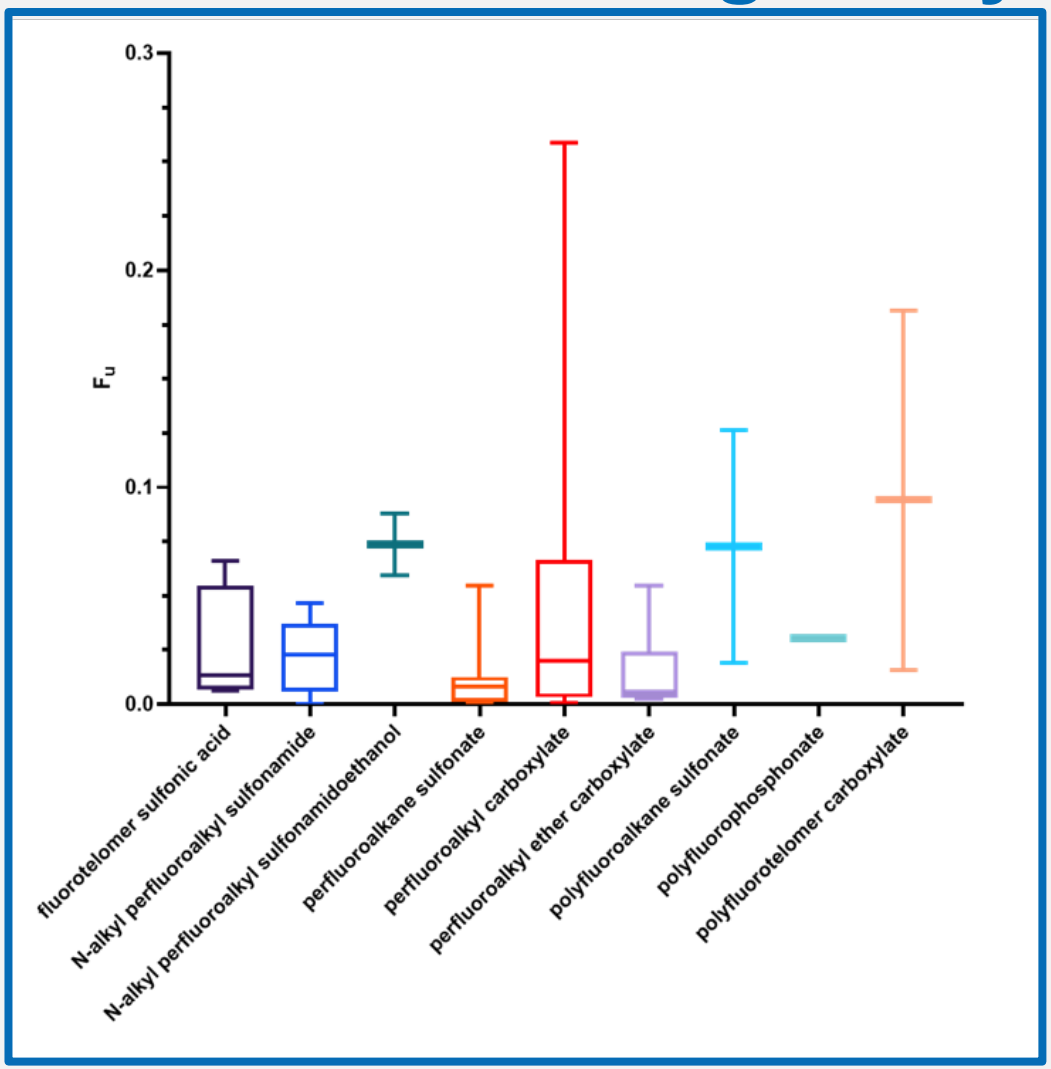

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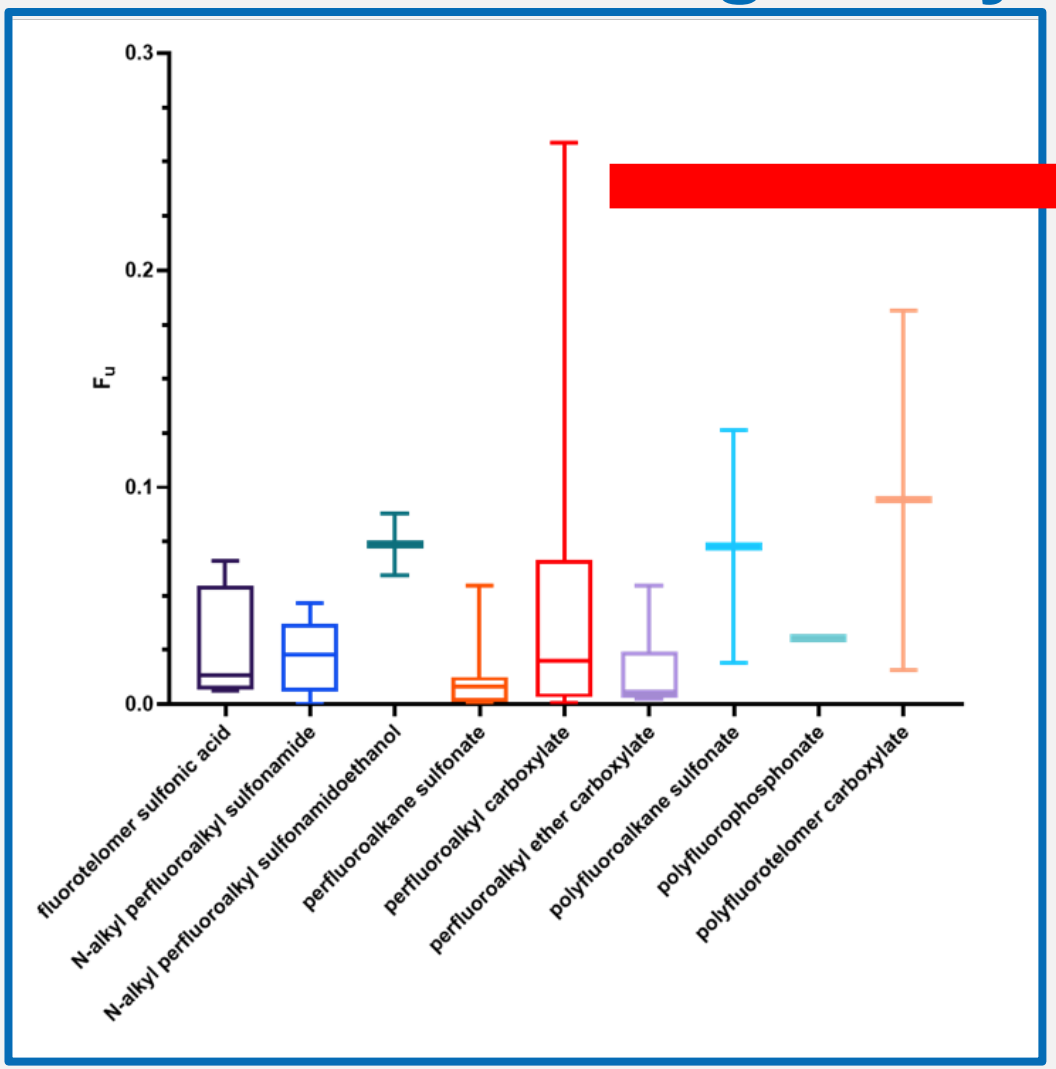
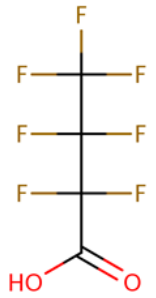
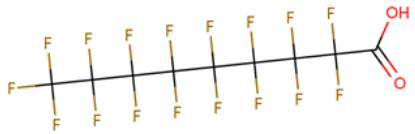




PFAS	Avg Fu
Perfluoropropanoic acid	0.2586
Perfluorobutanoic acid	0.0939
Perfluoropentanoic acid	0.0440
Perfluorohexanoic acid	0.0076
Perfluorooctanoic acid	0.0034
Perfluorononanoic acid	0.0015

↑ chain length ↓ F_u



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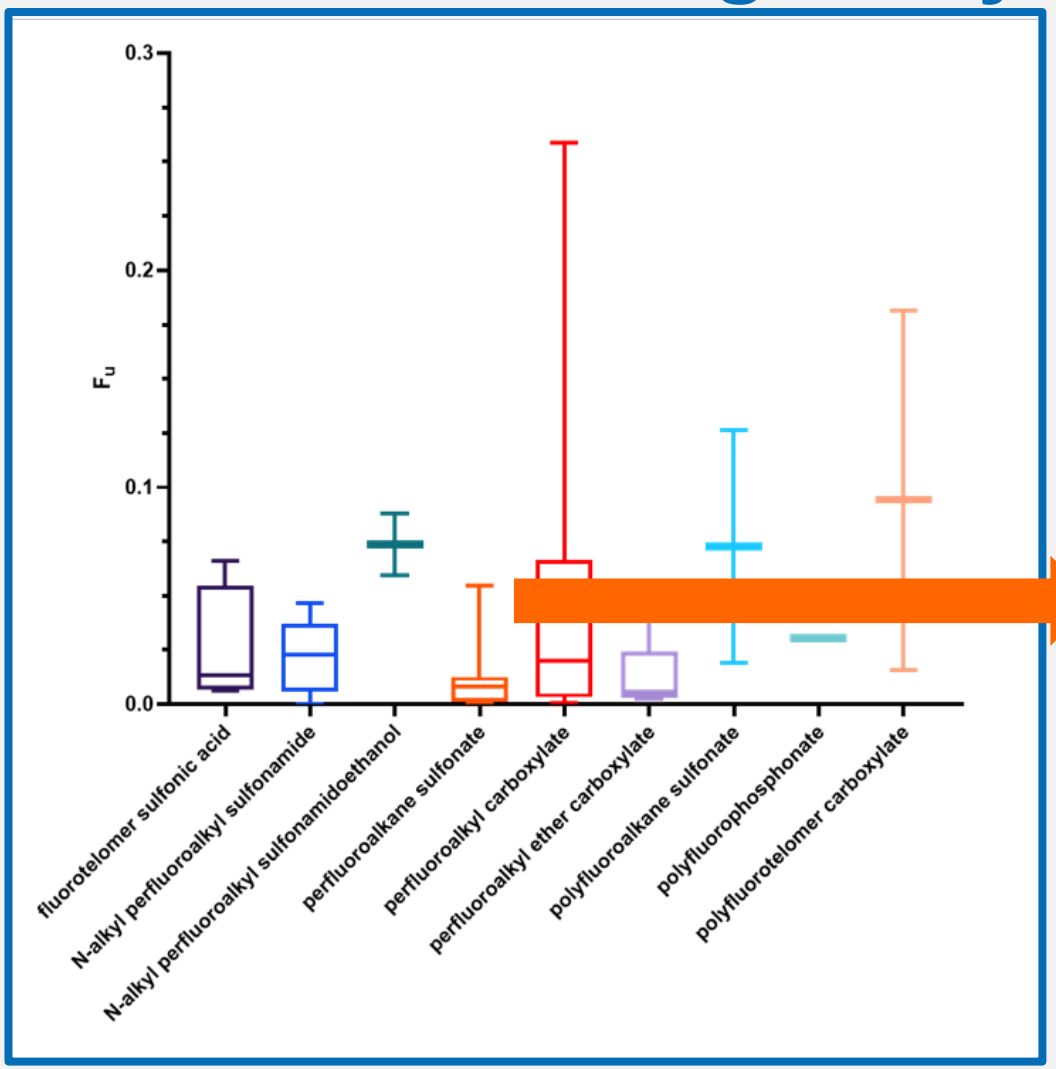
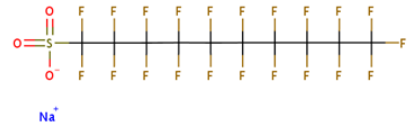
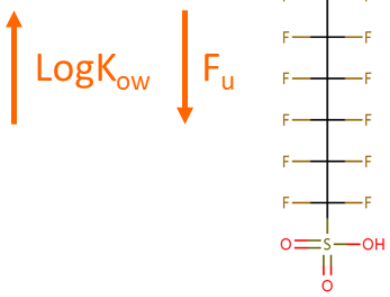




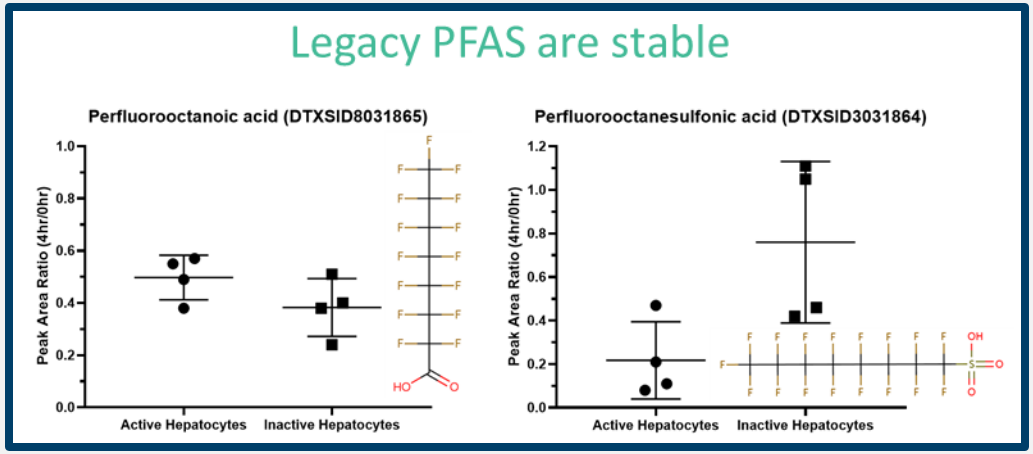
PFAS	Avg Fu	LogK$_{ow}$
Perfluorohexanesulfonic acid	0.0008	3.47
Potassium perfluorooctanesulfonate	0.0040	3.39
Perfluorobutanesulfonic acid	0.0128	2.57
Sodium perfluorodecanesulfonate	0.0546	1.83



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
   - Ratio of 1 indicates no loss over time



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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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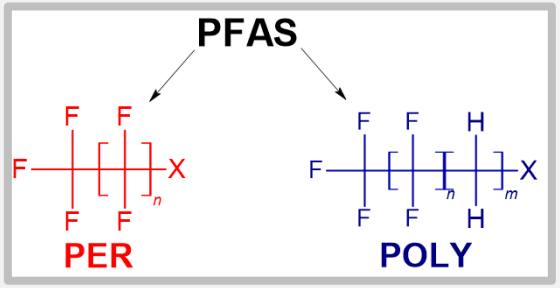

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Perfluoro(4-methoxybutanoic) acid	346.5	2.00E+00
2H,2H,3H,3H-Perfluorooctanoic acid	101.4	6.83E+00
N-Ethylperfluorooctanesulfonamide	57	1.22E+01
3-(Perfluoro-2-butyl)propane-1,2-diol	35.87	1.93E+01
Perfluoro-3,6,9-trioxatridecanoic acid	29.71	2.33E+01
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
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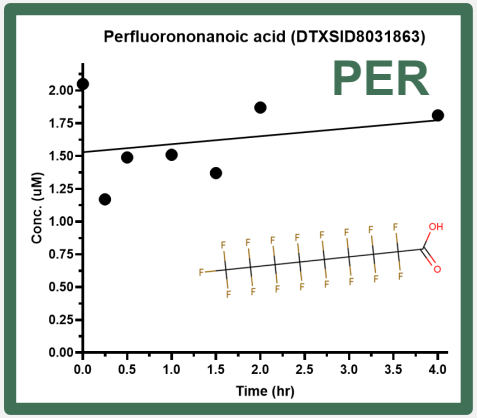

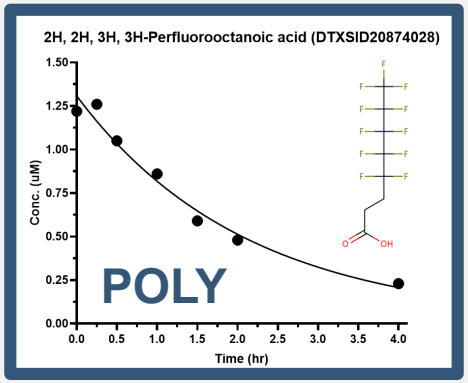

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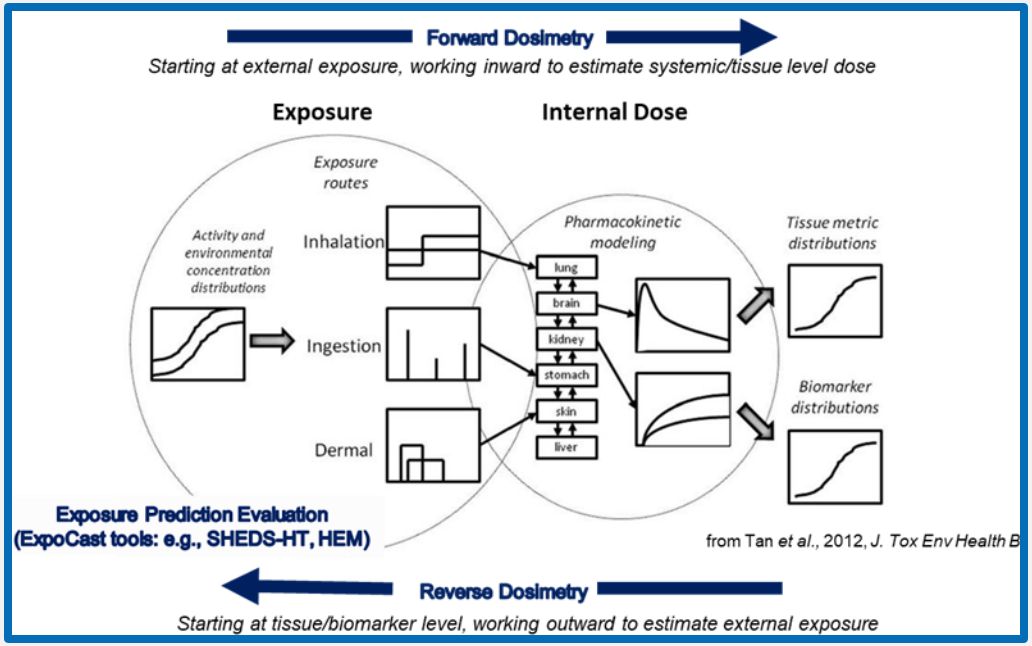
What is IVIVE?

- *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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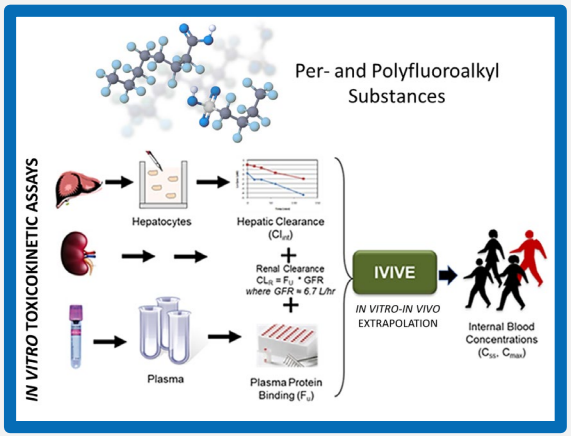


In Vitro - In Vivo Extrapolation → Steady State Blood Concentrations

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

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

$$CL_R = F_U * GFR$$
where GFR ≈ 6.7 L/hr

Assumptions

Exposure at 1 µg/kg/day

Linear kinetics

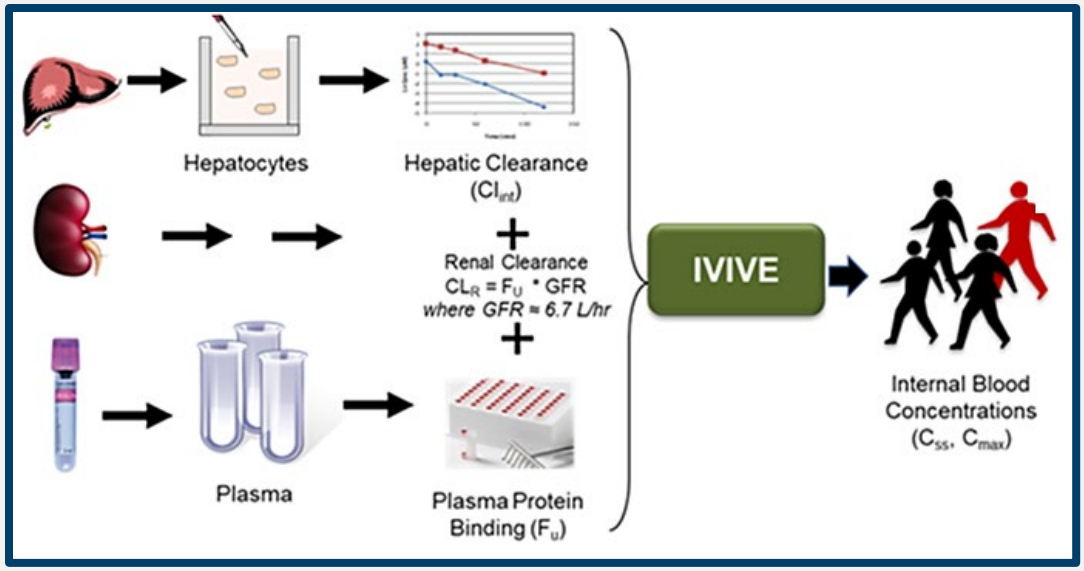
100% oral bioavailability

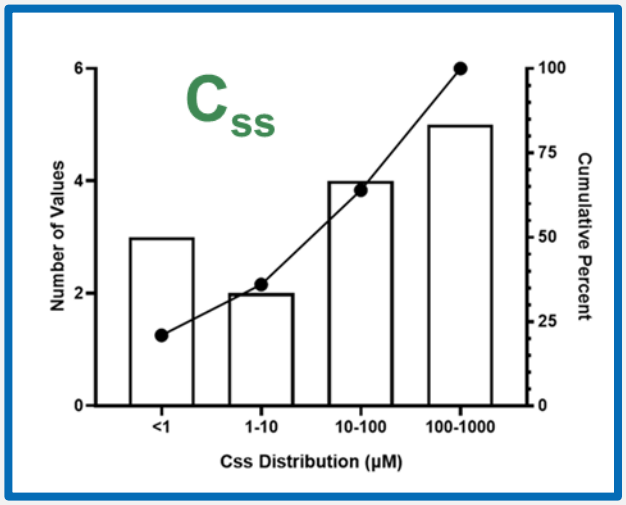
$$CL_H = \frac{F_U * Q_L * CL_{Int}}{Q_L + F_U * CL_{Int}}$$
where Q_L ≈ 90 L/hr

$$CL_{Int} = HPGL * V_L * CL_{invitro}$$
where HPGL ≈ 137 million cells/g

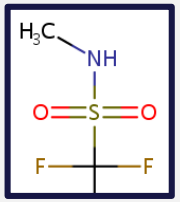
V_L ≈ 1820 g

What did IVIVE show with PFAS data?





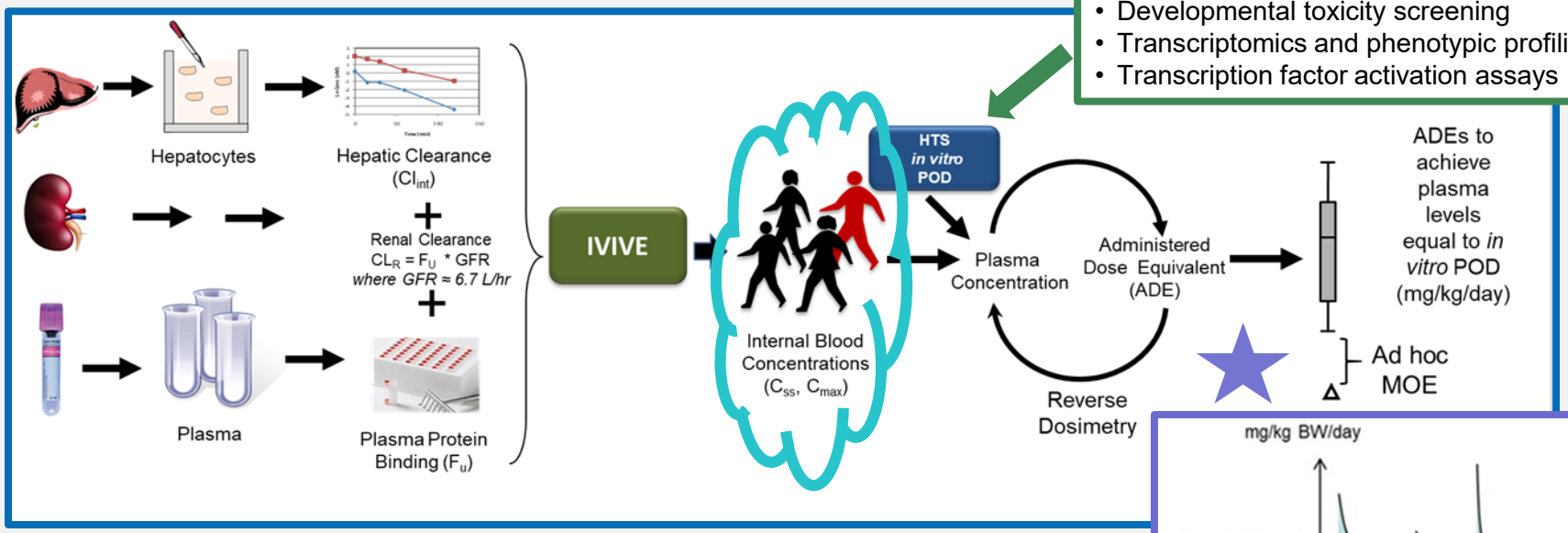
Compound Name	F_u	Cl_{renal} (L/hr)	$Cl_{hepatic}$ (L/hr)	Css (μM)
Potassium perfluorohexanesulfonate	0.0011	0.0075	3.82E-07	894.5132
Ammonium perfluorooctanoate	0.0014	0.0094	1.16E-04	713.7360
Perfluorononanoic acid	0.0013	0.0088	8.33E-03	368.6974
Perfluorohexanoic acid	0.0076	0.0507	2.33E-04	183.6569
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

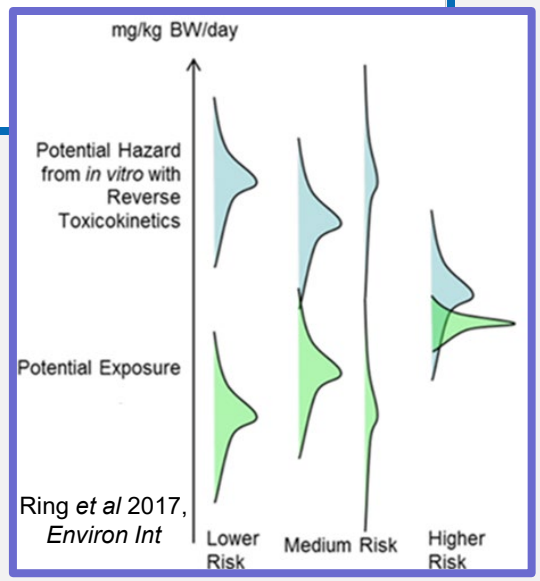


EPA United States Environmental Protection Agency

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



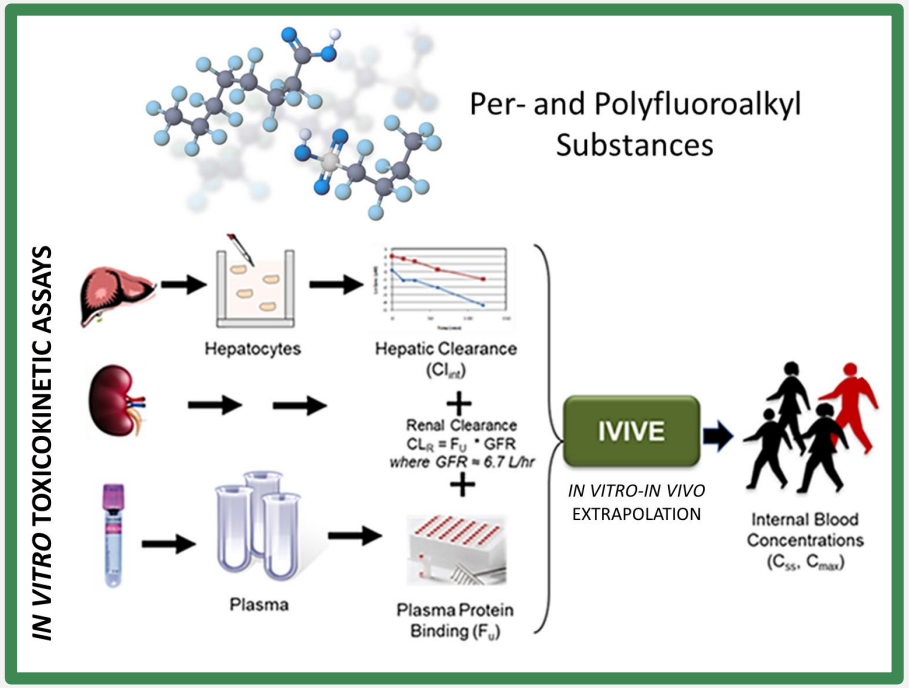


Ring *et al* 2017, *Environ Int*

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

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
  - 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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