Assessing Generated *In Vitro* Toxicokinetic Data of Per- and Polyfluoroalkyl Substances (PFAS) with *In Vitro-In Vivo* Extrapolation (IVIVE)

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Center for Computational Toxicology and Exposure

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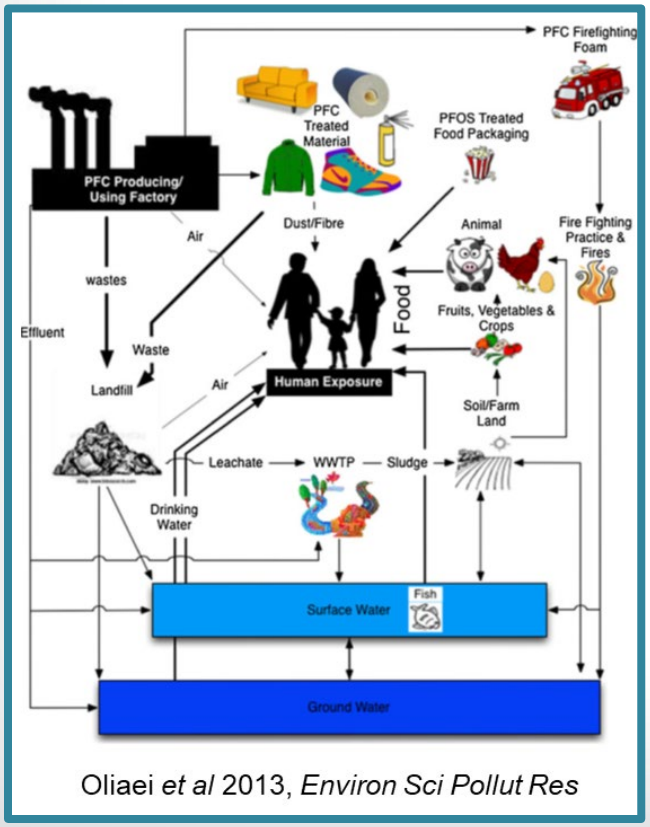
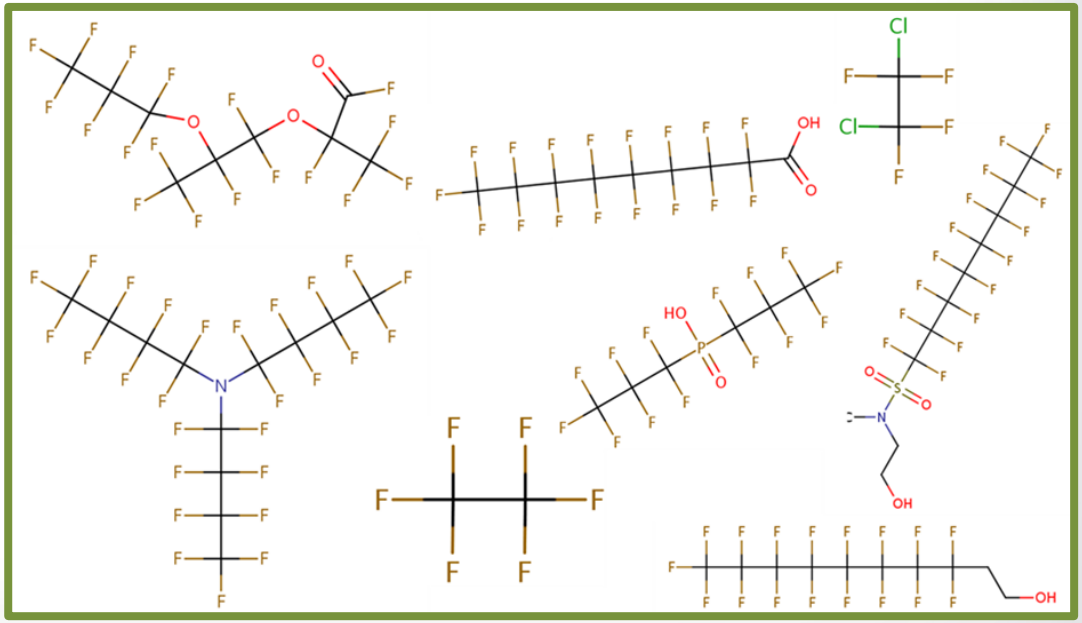
Per- and Polyfluoroalkyl Substances (PFAS)

- 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*



Per- and Polyfluoroalkyl Substances (PFAS)

- Man-made chemicals used in industry and consumer products worldwide since the 1950s
- Repel water, resist heat, and protect surfaces
- Concerns and exposure…
  - **Widespread**: PFAS in 97% of American population, even in arctic polar bears
  - **Persistence**: carbon-fluorine bonds are some of the strongest with little degradation in environment
  - **Bioaccumulative**: absorption > elimination
  - **Abundance**: number will expand with industrial production
    - 6648 PFAS-like structures identified on Dashboard
    - 1223 PFAS on TSCA inventory; 602 currently in use in USA

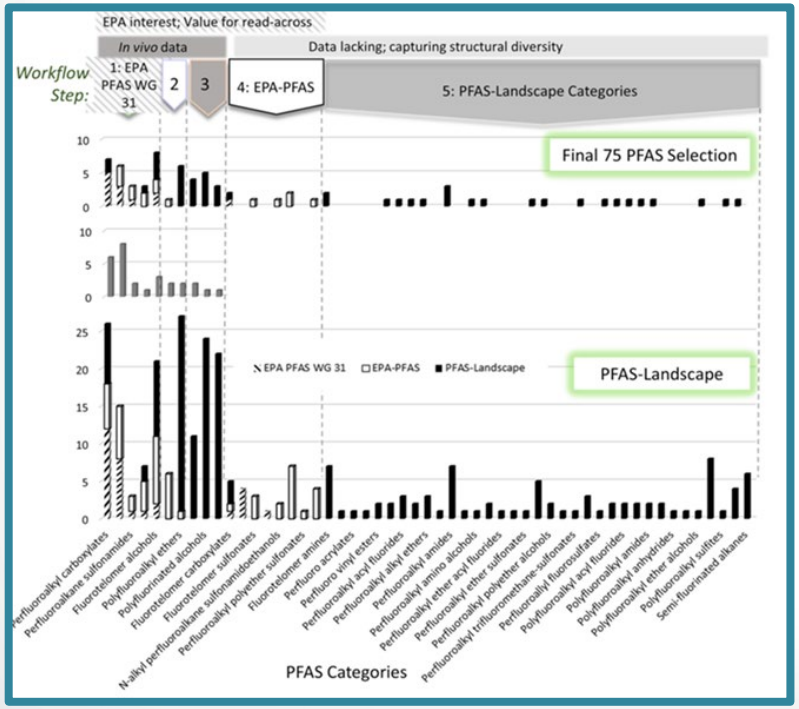
PFAS of Interest for Evaluation

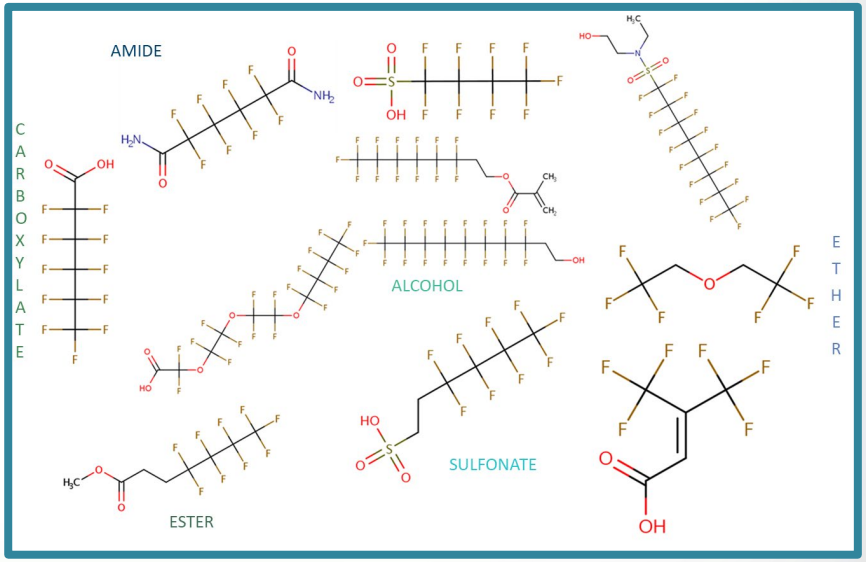
- **PFAS screening library** was established by EPA
  - Maximize read-across
  - Capture structural diversity
  - To date, more than 400 unique compounds included

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]

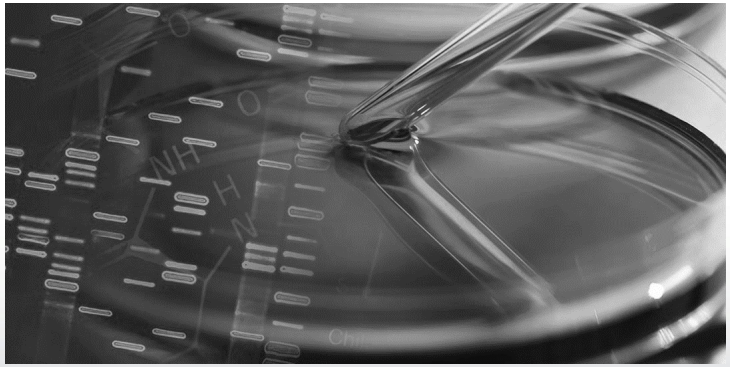
Environmental Health Perspectives 014501-1 127(1) January 2019





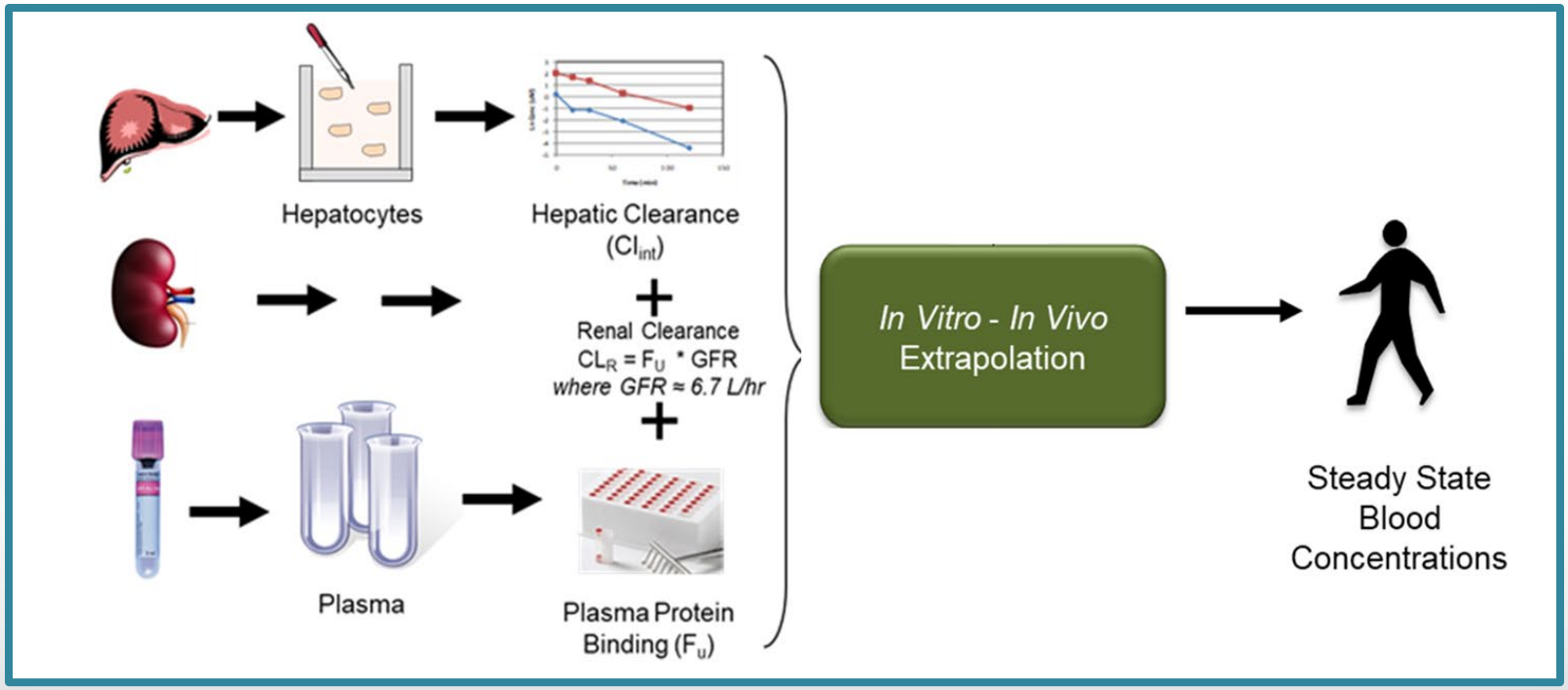
PFAS of Interest for Evaluation

- **PFAS screening library** was established by EPA
  - Maximize read-across
  - Capture structural diversity
  - To date, more than 400 unique compounds included
- A range of targeted and tiered high-throughput toxicity assays employed to serve as a guide for potential human health risk
  - New approach methodologies (NAMs) incorporated – alternative test methods and strategies to reduce, refine, and/or replace mammalian animals
  - Endpoints: hepatotoxicity, immunotoxicity, developmental toxicity, mitochondrial toxicity, ***in vitro*** **toxicokinetics**



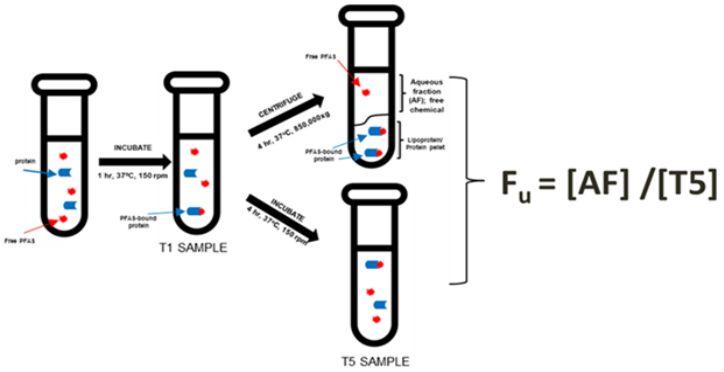
In Vitro Toxicokinetics Assays

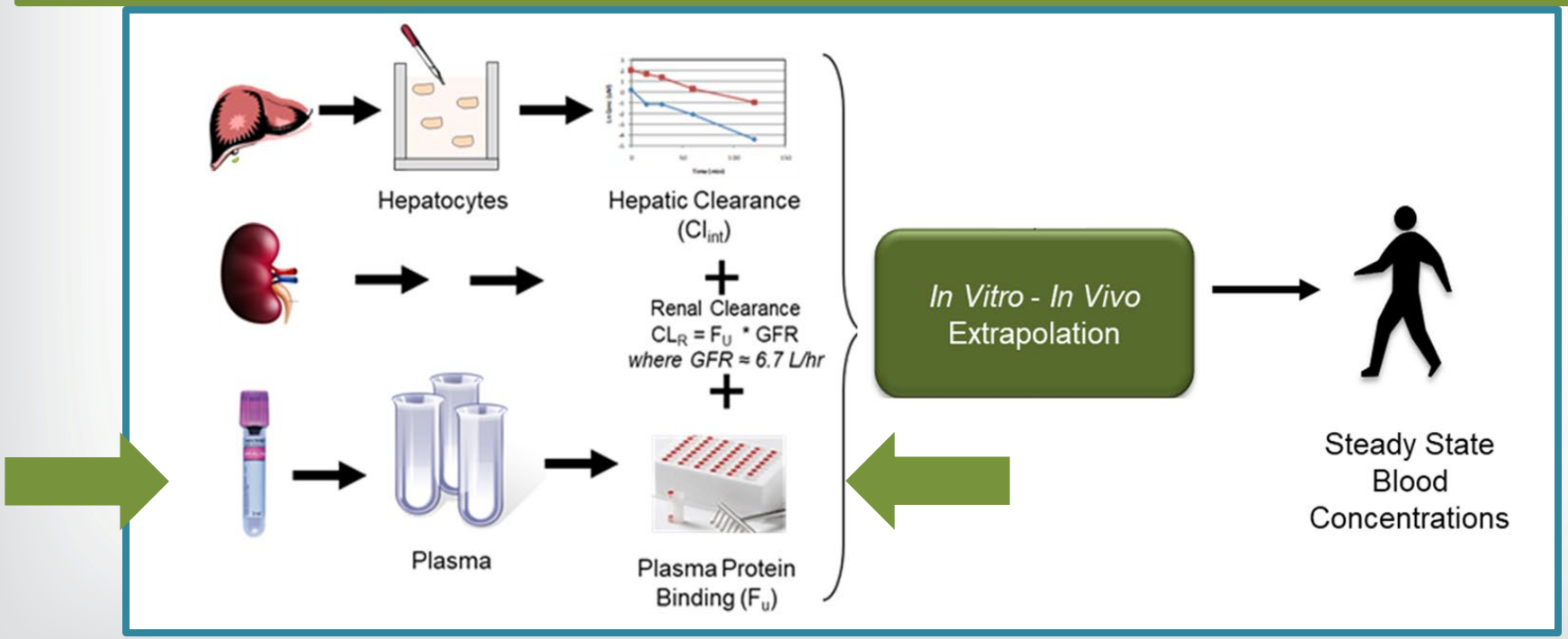
- Toxicokinetics (TK): the study of how a substance gets into the body and what happens to it in the body
  - Can be used to look at how chemicals move throughout the body and lead to harmful effects
  - Often viewed as a function of dose over time



In Vitro Toxicokinetics Assays

- Assay to assess the free (unbound) fraction [F_u] of chemical to proteins within the blood
- Unbound molecules permeate through cell membranes to reach 'target'
- 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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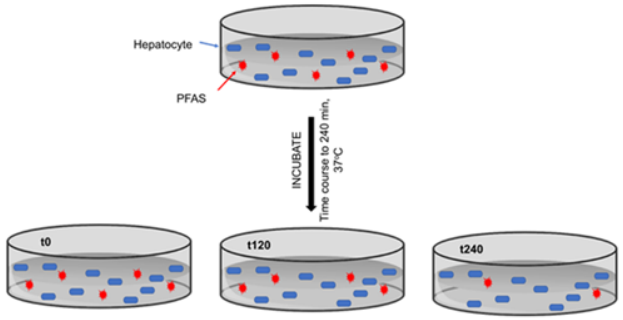
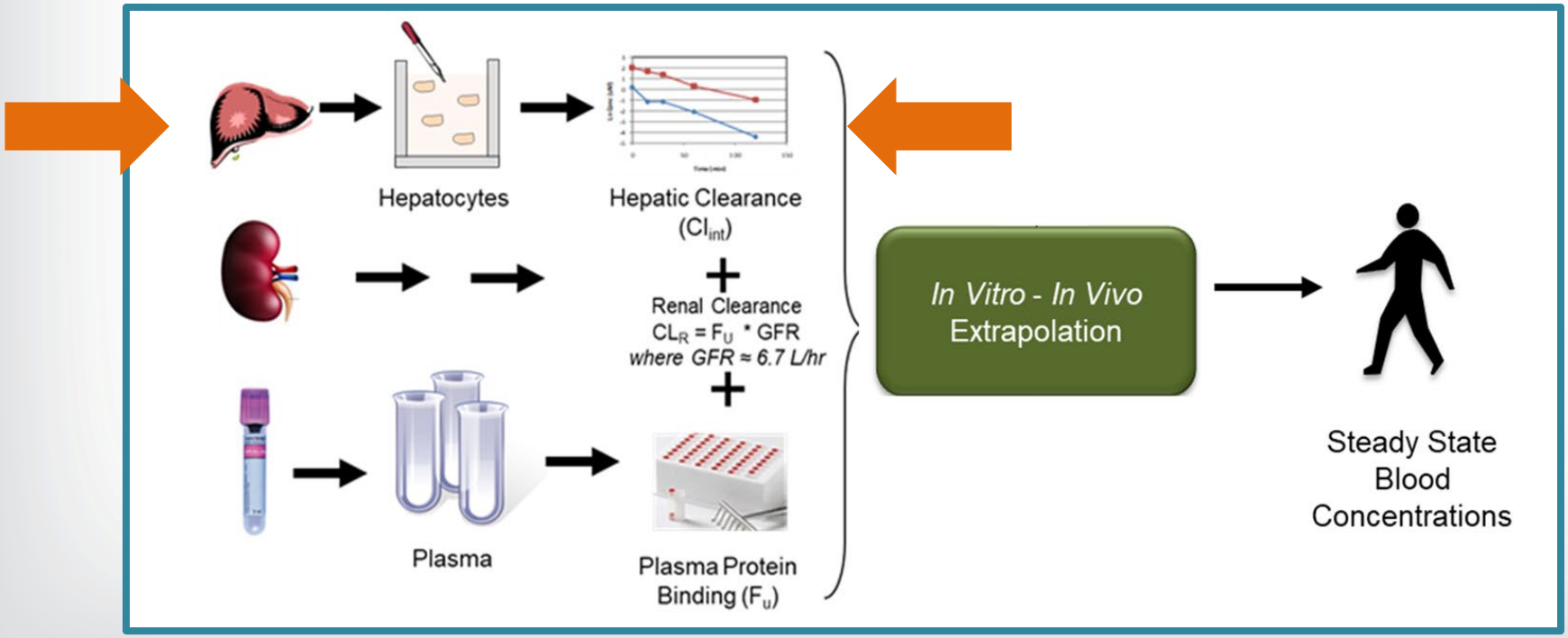
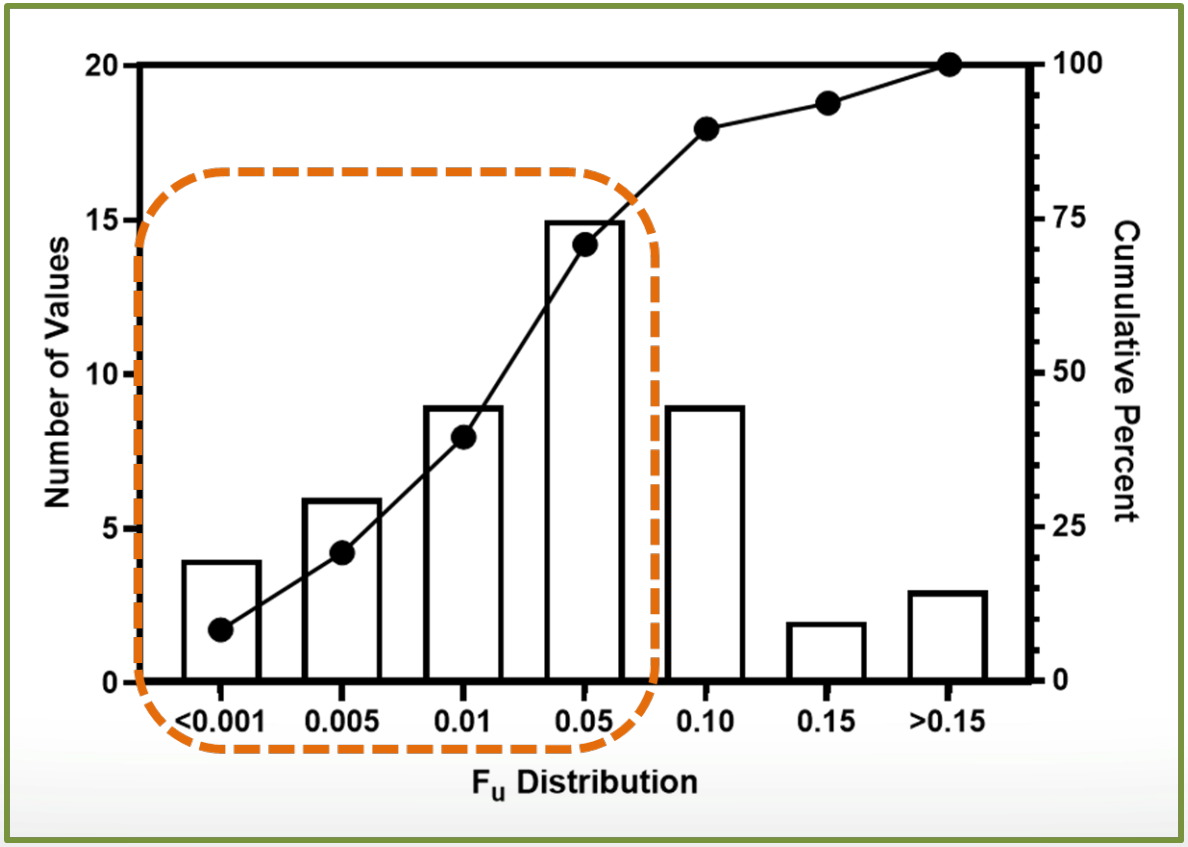


- Hepatic clearance ($CL_{hepatic}$) is measure of the rate of elimination of a chemical from the liver
- Models to study metabolism include human liver microsomes, recombinantly expressed enzymes, and hepatocytes
- Substrate depletion approach using primary human hepatocytes (50-donor pool, mixed sex) at 1 µM PFAS concentration
  - Time course: 0, 15, 30, 60, 90, 120, and 240 min
  - Work completed by collaborator at National Toxicology Program [David Crizer]



PFAS Plasma Protein Binding Results

- 50 LC-able PFAS have determined fraction unbound data
- **F_u ↓ plasma protein binding ↑**



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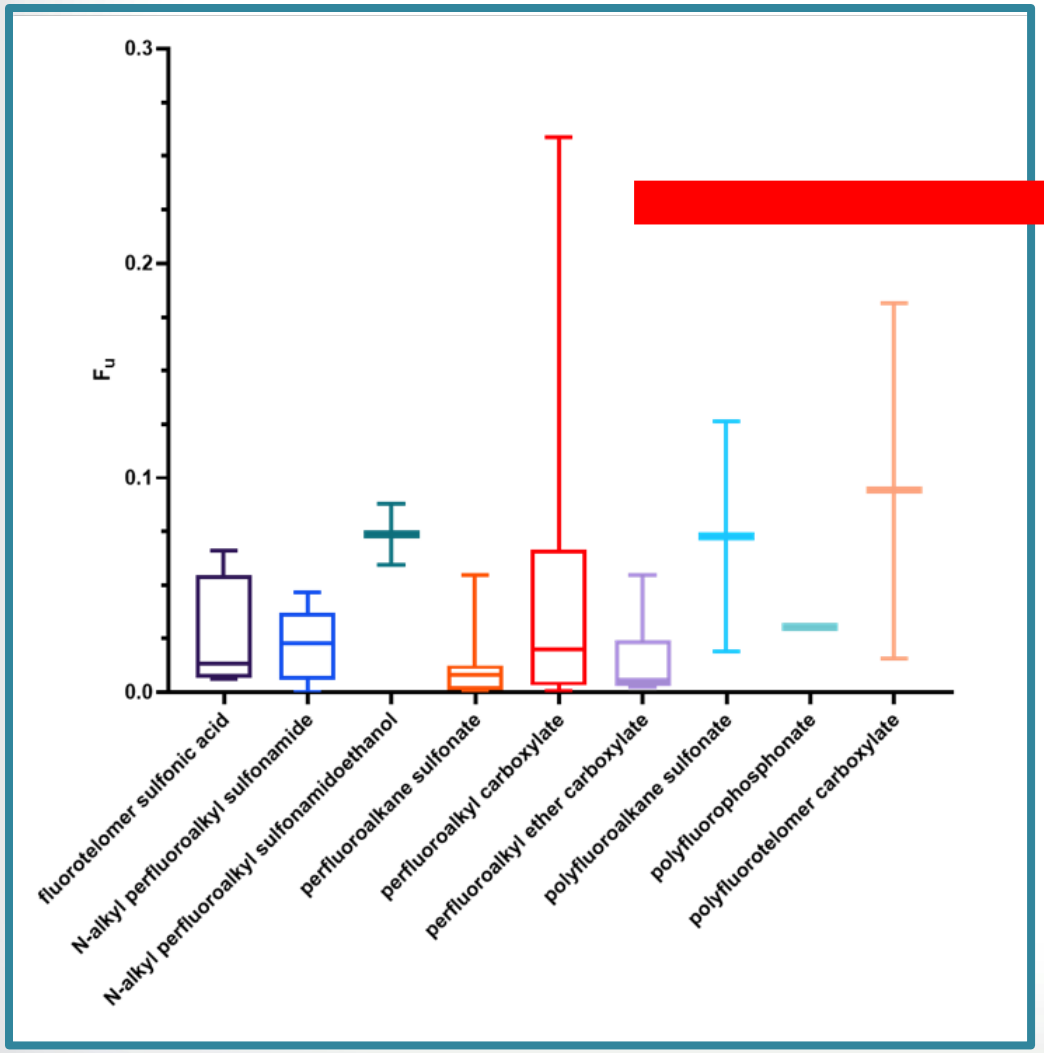
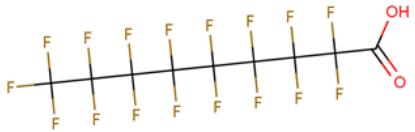


PFAS	Avg Fu
Perfluoropropanoic acid	0.2586
Perfluorobutanoic acid	0.0939
Perfluoropentanoic acid	0.0440
Perfluorohexanoic acid	0.0076
Perfluorooctanoic acid	0.0034
Perfluorononanoic acid	0.0015

↑ chain length ↓ F_u



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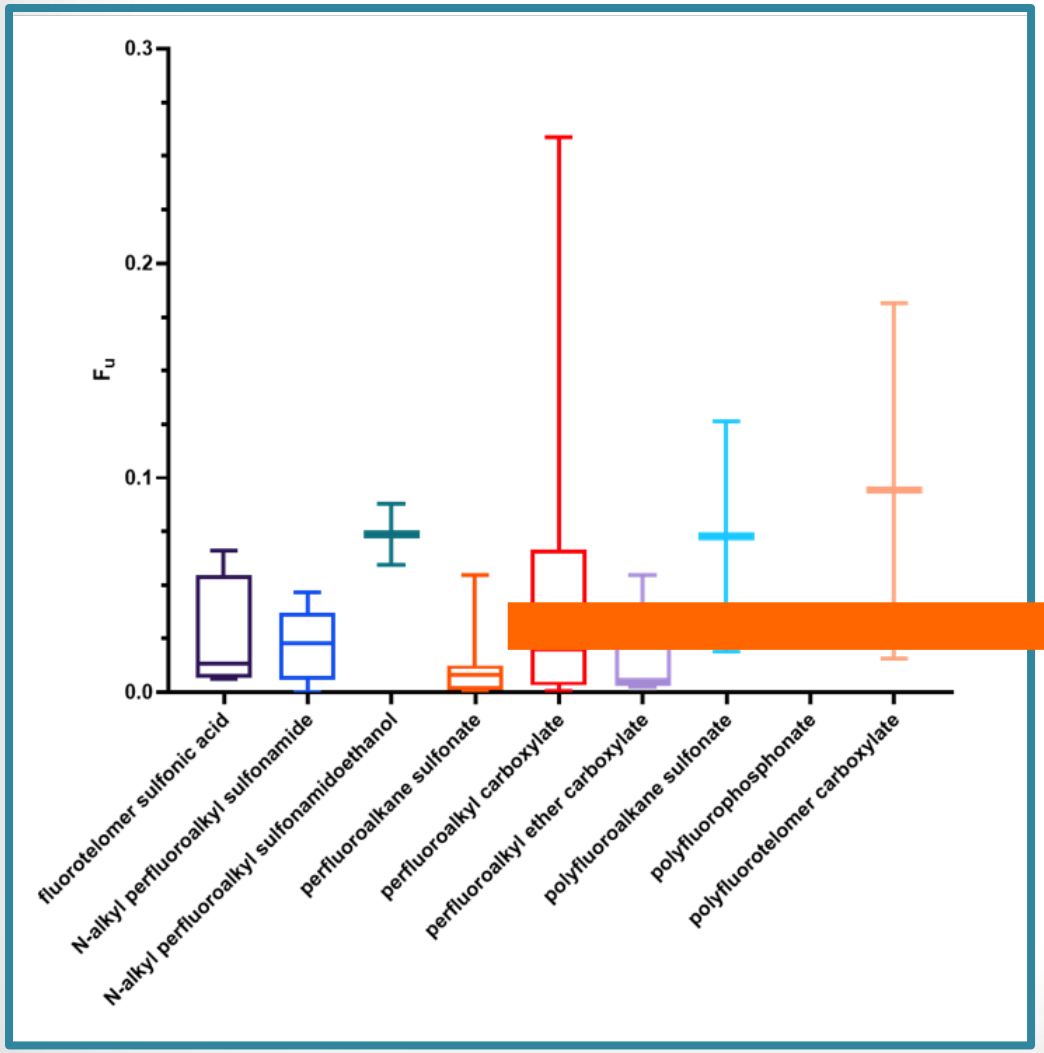
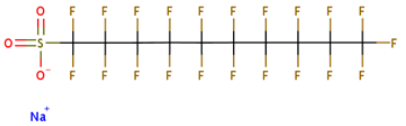
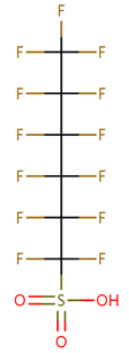




PFAS	Avg Fu	$LogK_{ow}$
Perfluorohexanesulfonic acid	0.0008	3.47
Potassium perfluorooctanesulfonate	0.0040	3.39
Perfluorobutanesulfonic acid	0.0128	2.57
Sodium perfluorodecanesulfonate	0.0546	1.83

$LogK_{ow}$ F_u

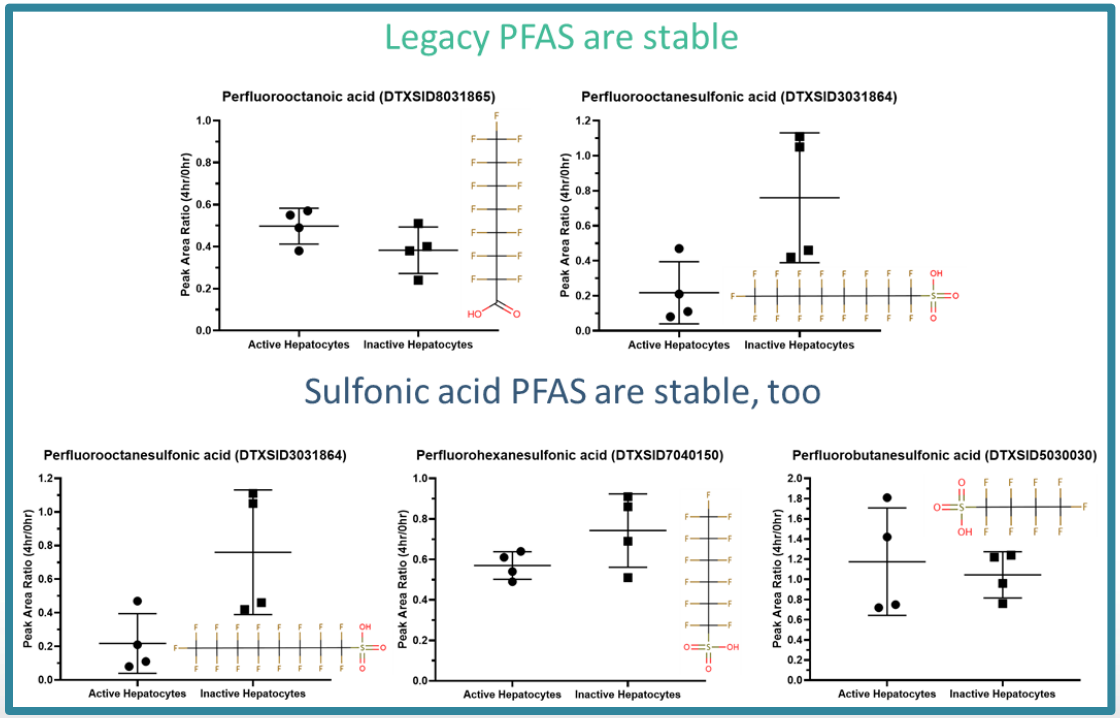


PFAS Hepatic Clearance Data

- **More than 20 LC-able PFAS assessed**

1) *In vitro hepatic clearance screen*

- 0 and 4 hr time points for active and inactive hepatocytes measured
- Ratio of 1 indicates no loss over time (no clearance)



PFAS Hepatic Clearance Data

- **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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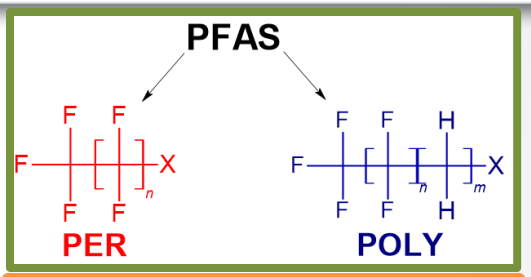
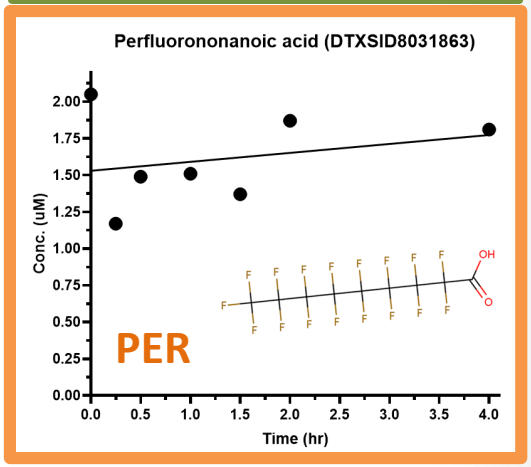
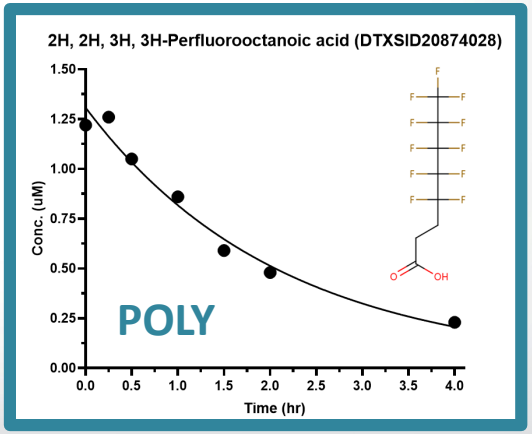
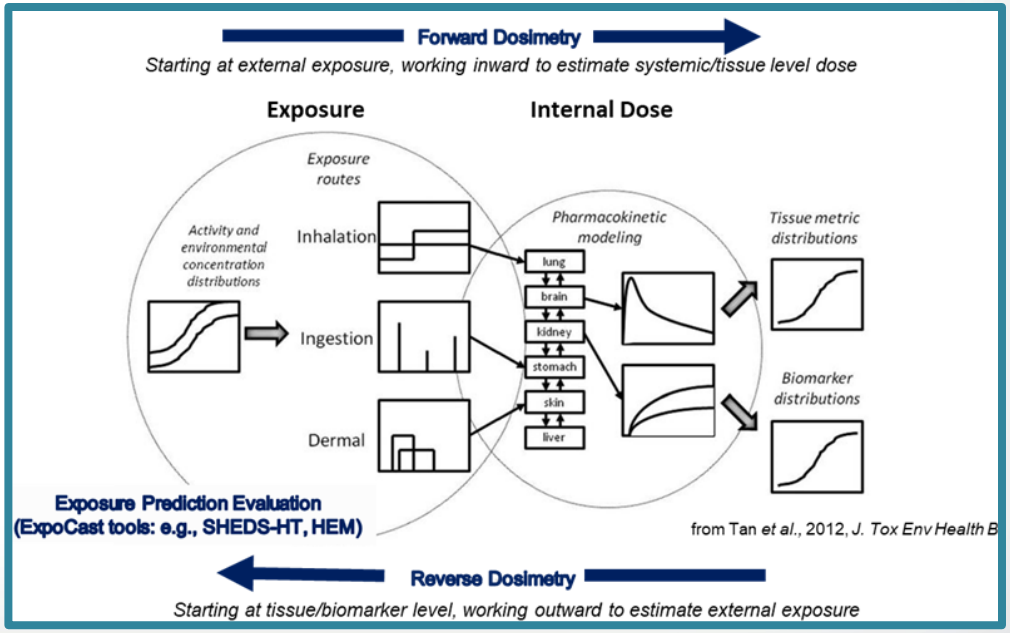






In Vitro-In Vivo Extrapolation (IVIVE)

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



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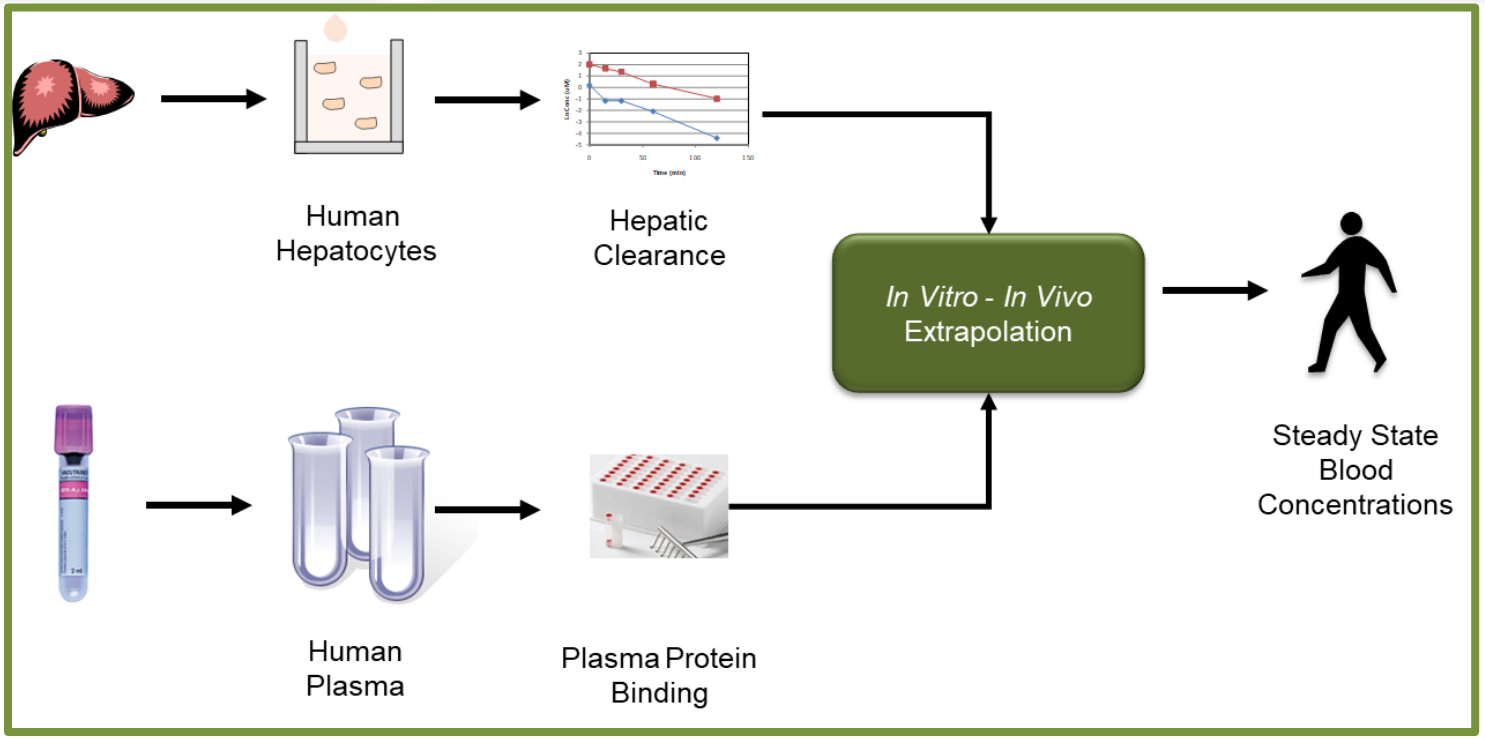
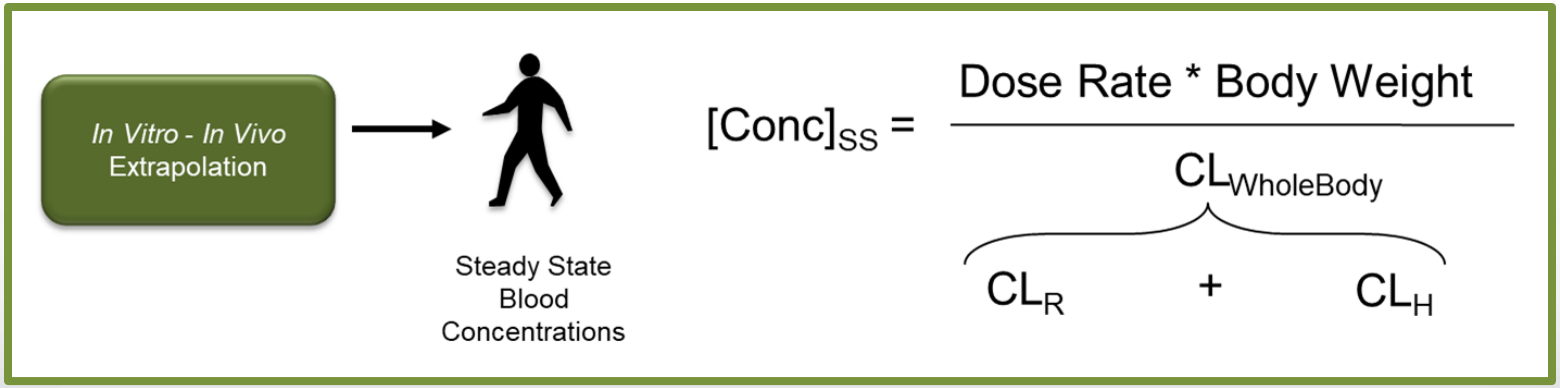




In Vitro-In Vivo Extrapolation (IVIVE)

In Vitro - In Vivo Extrapolation

Steady State Blood Concentrations

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

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

Assumptions

Exposure at 1 µg/kg/day

Linear kinetics

100% oral bioavailability

$$CL_R = F_U * GFR$$

where GFR ≈ 6.7 L/hr

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

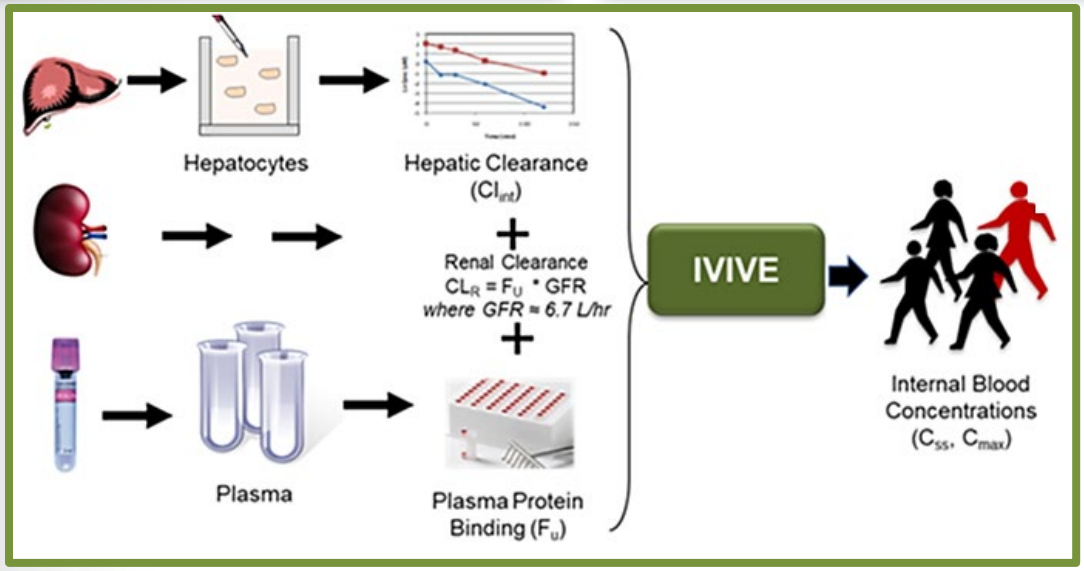
where Q_L ≈ 90 L/hr

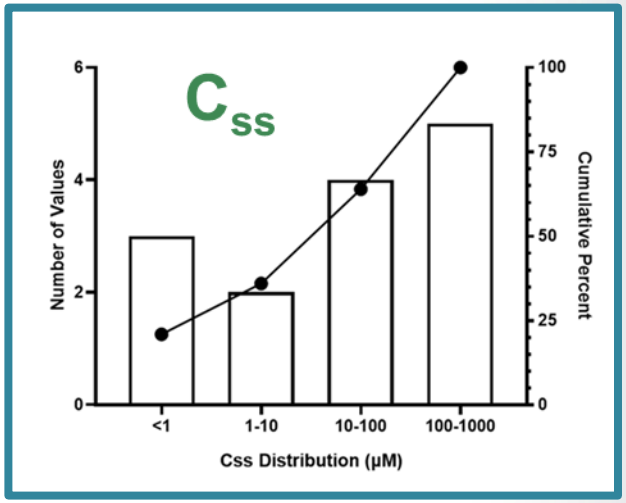
$$CL_{Int} = HPGL * V_L * CL_{invitro}$$

where HPGL ≈ 137 million cells/g

V_L ≈ 1820 g

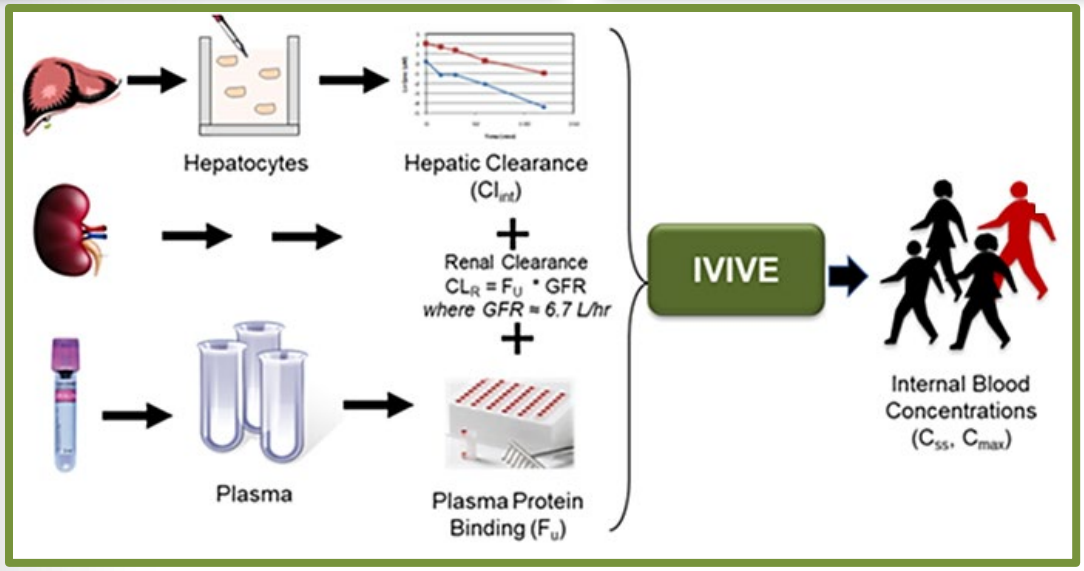
IVIVE Findings on Generated PFAS Toxicokinetic Data

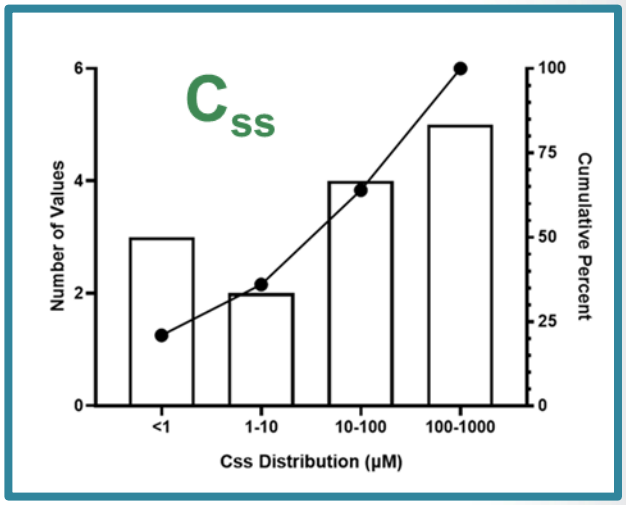




Compound Name	F_u	Cl_{renal} (L/hr)	$Cl_{hepatic}$ (L/hr)	Css (µM)
Potassium perfluorohexanesulfonate	0.0011	0.0075	3.82E-07	894.5132
Ammonium perfluorooctanoate	0.0014	0.0094	1.16E-04	713.7360
Perfluorononanoic acid	0.0013	0.0088	8.33E-03	368.6974
Perfluorohexanoic acid	0.0076	0.0507	2.33E-04	183.6569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

IVIVE Findings on Generated PFAS Toxicokinetic Data





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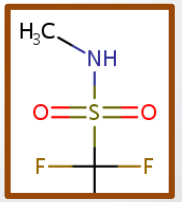
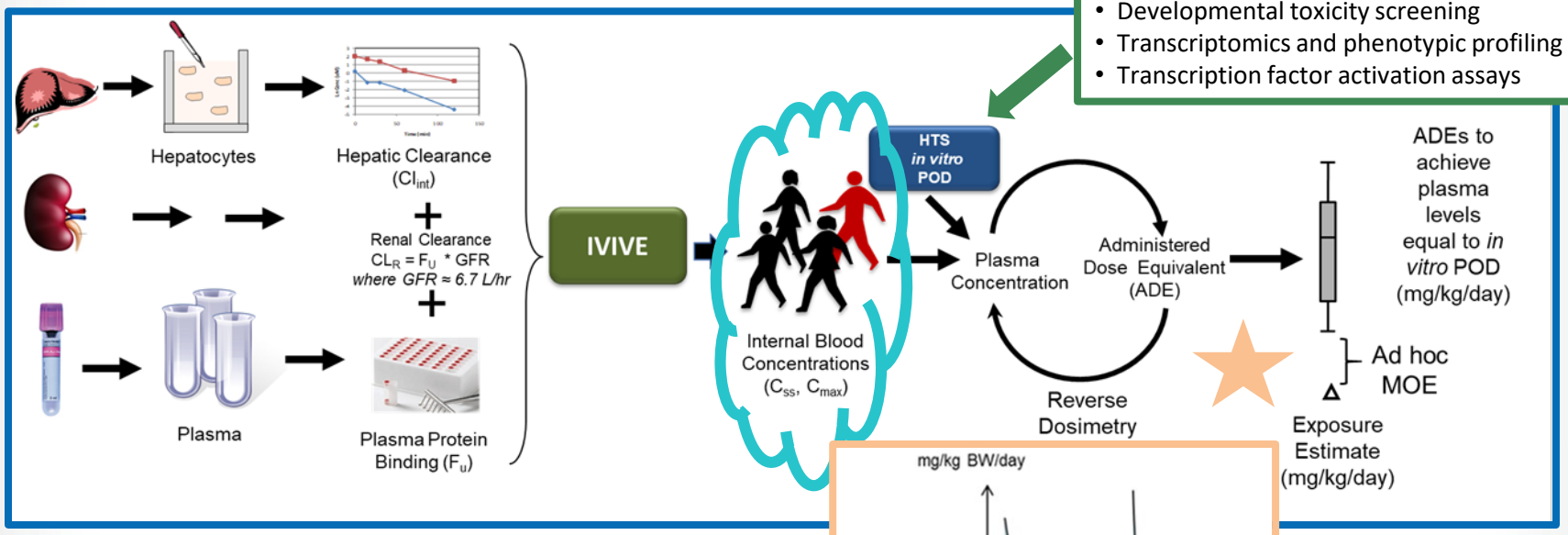
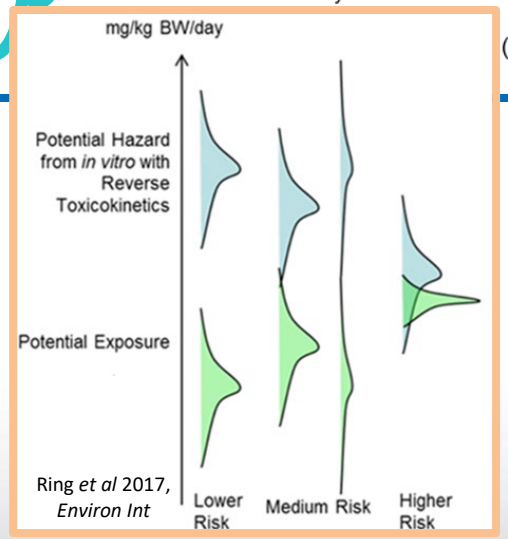


PFAS Finding Application and Summary

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

PFAS Finding Application and Summary

PFAS *In Vitro* Toxicokinetic Data Generation Summary

- 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 mg/kg/day, C_{ss} predictions ranged from 0.16-895 µM, with a median value of 23.29 mM
- Continuing data generation for additional PFAS and toxicokinetic assays for bioavailability, metabolite identification, and renal reuptake

Acknowledgements

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  - *Lucas Albrecht*
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  - Annette Guiseppi-Elie
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  - Keith Houck
  - Michael Hughes
  - Richard Judson
  - *Evgenia Korol-Bexell*
  - *Anna Kreutz*
  - Stephanie Padilla
  - Grace Patlewicz
  - *Matthew Phillips*
  - Ann Richard
  - Timothy Shafer
  - Adam Swank
  - Russell Thomas
  - John Wambaugh
  - *Barbara Wetmore*
  - Antony Williams
- **ORD-CEMM**
  - Scott Clifton
  - Matthew Henderson
  - James McCord
  - Larry McMillan
  - Mark Strynar
- **NTP**
  - David Crizer
- **Waters Corporation**
  - Kari Organtini

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