Plasma Protein Binding of 109 Per- and Polyfluoroalkyl Substances (PFAS): Using Category-Based New Approach Methods to Inform PFAS Toxicokinetics

Session 4.01: Addressing exposure and risk associated with chemical contaminants in the era of big data

SETAC North America 43rd Annual Meeting

Barbara A. Wetmore, Ph.D.
November 17, 2022

Conflict of Interest Statement; Disclaimer

The authors have no conflicts of interest to disclose.

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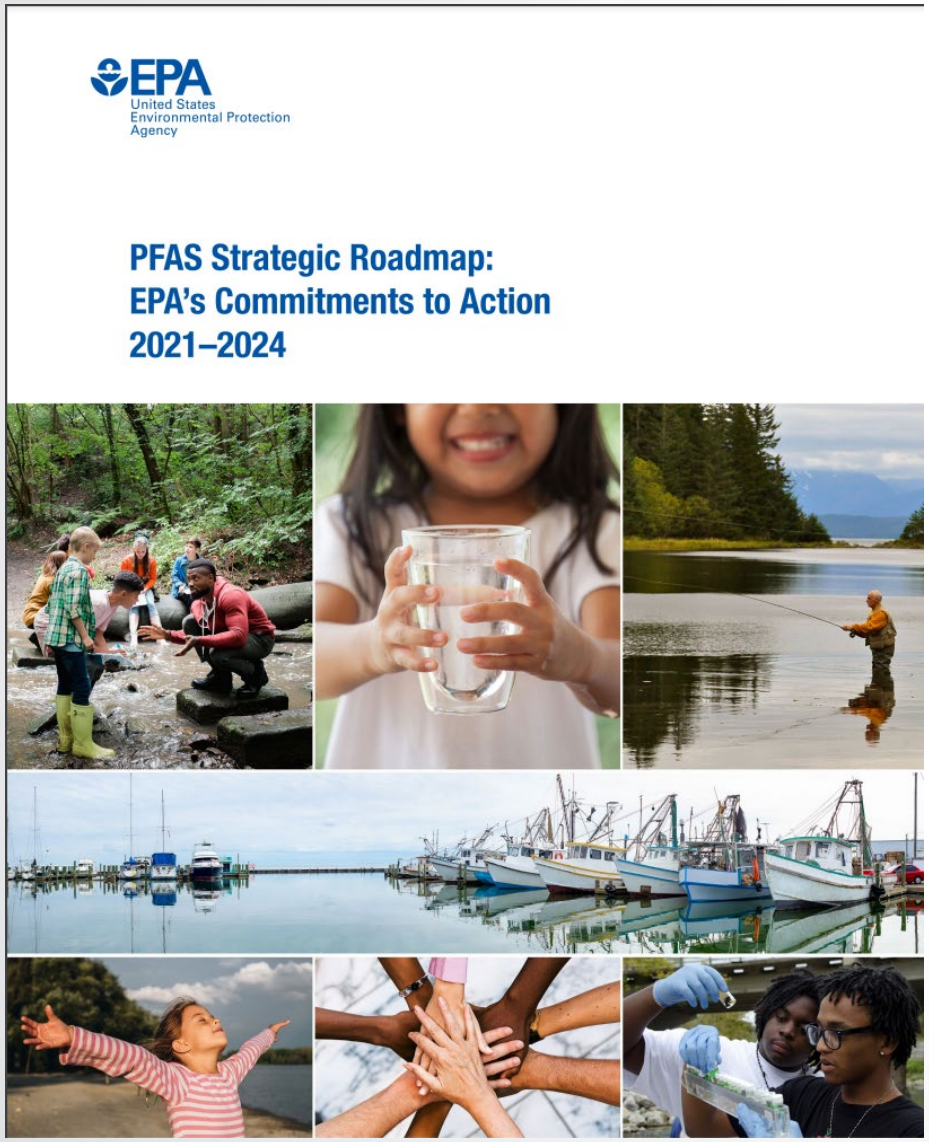
EPA PFAS Strategic Roadmap



RESEARCH

Invest in research, development, and innovation to increase understanding of PFAS exposures and toxicities, human health and ecological effects, and effective interventions that incorporate the best available science.

RESTRICT

Pursue a comprehensive approach to proactively prevent PFAS from entering air, land, and water at levels that can adversely impact human health and the environment.

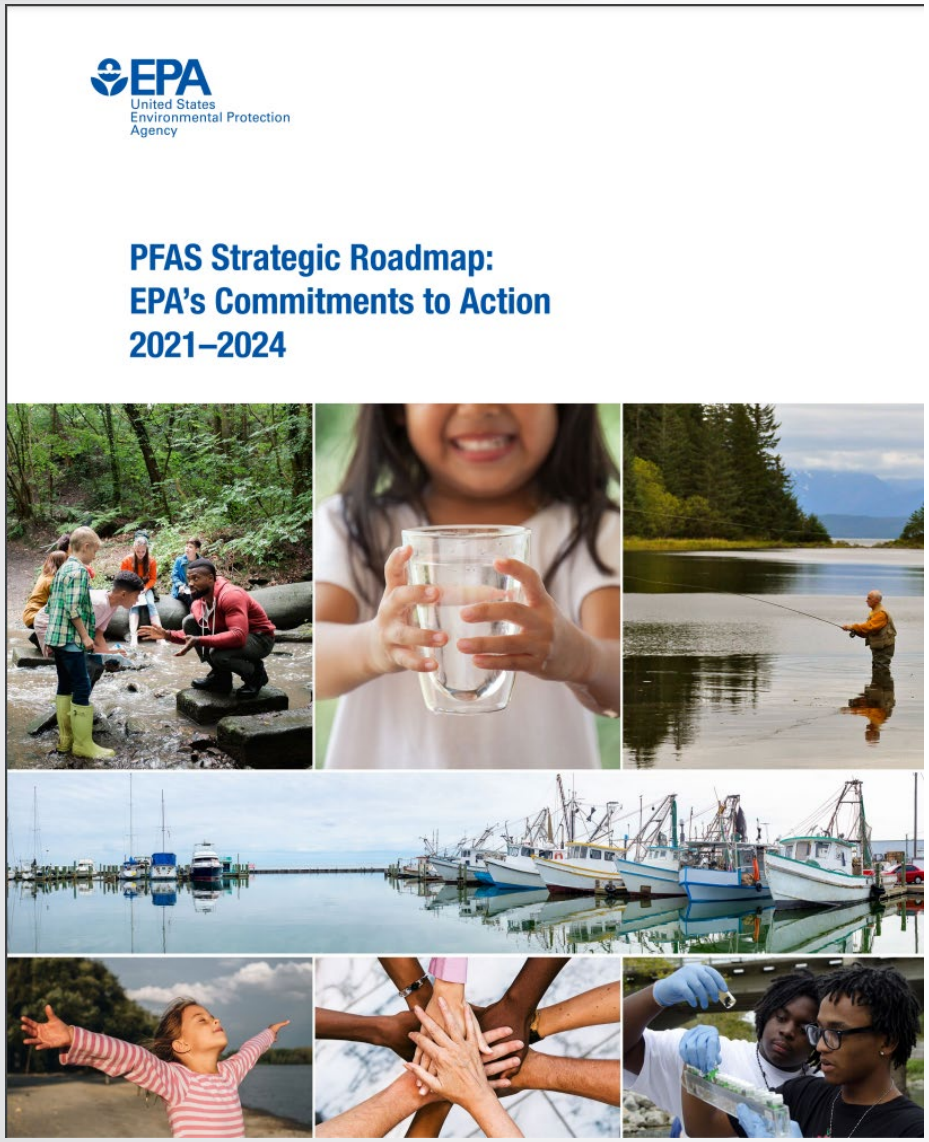
REMEDIATE

Broaden and accelerate the cleanup of PFAS contamination to protect human health and ecological systems.

Employ New Approach Methods to accelerate PFAS hazard evaluation

https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024

EPA PFAS Strategic Roadmap



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Broaden and accelerate the cleanup of PFAS contamination to protect human health and ecological systems.

Employ New Approach Methods to accelerate PFAS hazard evaluation

Use PFAS exposure and toxicity research to inform:

- Environmental release controls
- Remediation strategies

https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024

Using New Approach Methods to Fill PFAS Information Gaps

TOXICOLOGICAL SCIENCES, 169(2), 2019, 317–332
doi: 10.1093/toxsci/kfz058
Advance Access Publication Date: March 5, 2019
Forum

FORUM

The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency

Russell S. Thomas,*,1 Tina Bahadori,† Timothy J. Buckley,‡ John Cowden,* Chad Deisenroth,* Kathie L. Dionisio,‡ Jeffrey B. Frithsen,§ Christopher M. Grulke,* Maureen R. Gwinn,* Joshua A. Harrill,* Mark Higuchi,¶ Keith A. Houck,* Michael F. Hughes,¶ E. Sidney Hunter, III,¶ Kristin K. Isaacs,‡ Richard S. Judson,* Thomas B. Knudsen,* Jason C. Lambert,‖ Monica Linnenbrink,* Todd M. Martin,‖ Seth R. Newton,‡ Stephanie Padilla,¶ Grace Patlewicz,* Katie Paul-Friedman,* Katherine A. Phillips,‡ Ann M. Richard,* Reeder Sams,* Timothy J. Shafer,¶ R. Woodrow Setzer,* Imran Shah,* Jane E. Simmons,¶ Steven O. Simmons,* Amar Singh,* Jon R. Sobus,‡ Mark Strynar,‡ Adam Swank,‡ Rogelio Tornero-Valez,‡ Elin M. Ulrich,‡ Daniel L. Villeneuve,‖ John F. Wambaugh,* Barbara A. Wetmore,‡ and Antony J. Williams*

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Thomas *et al.*, 2019

High-throughput transcriptomics and high-throughput phenotypic profiling

High-throughput targeted screening (ToxCast)

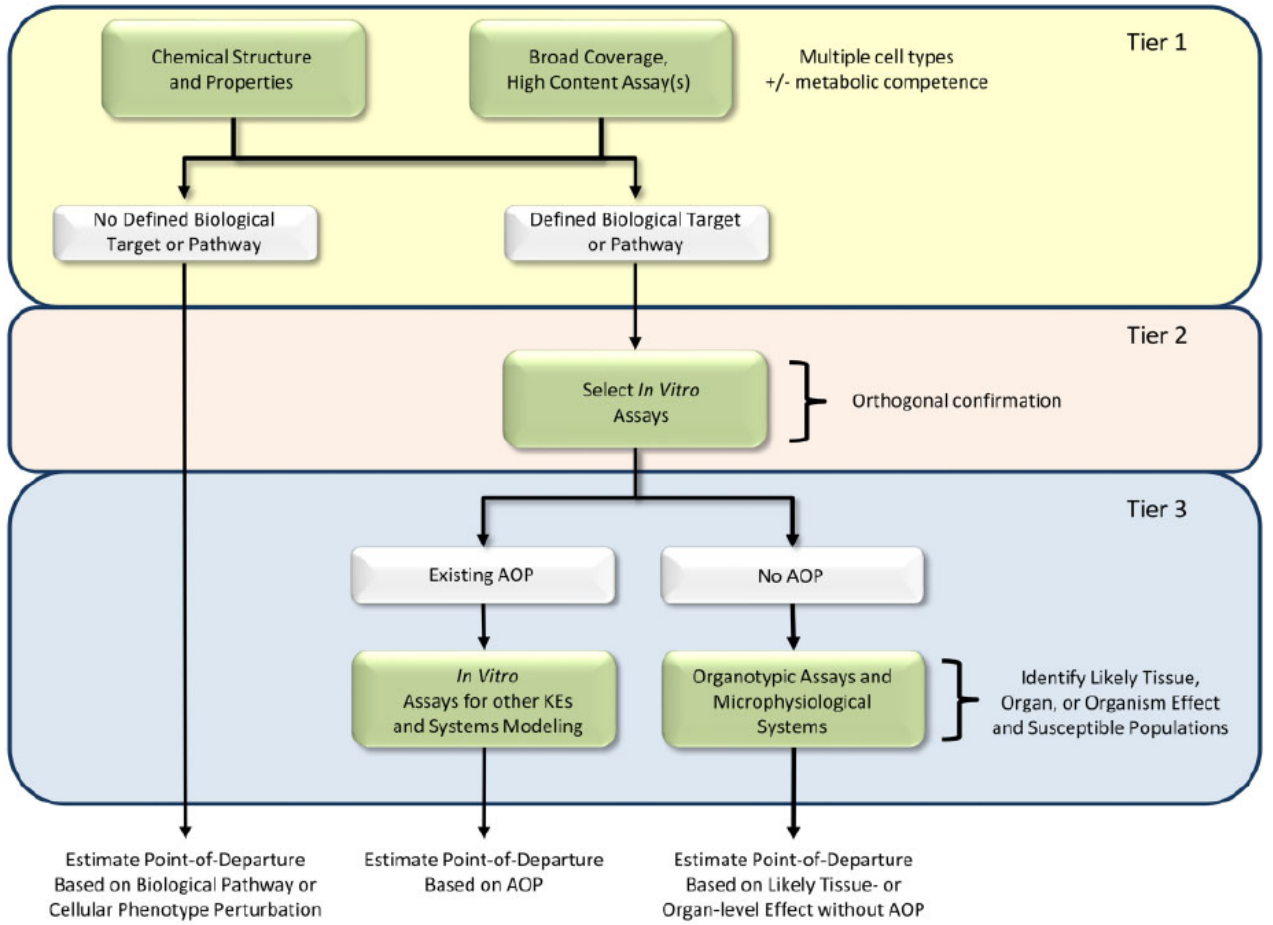
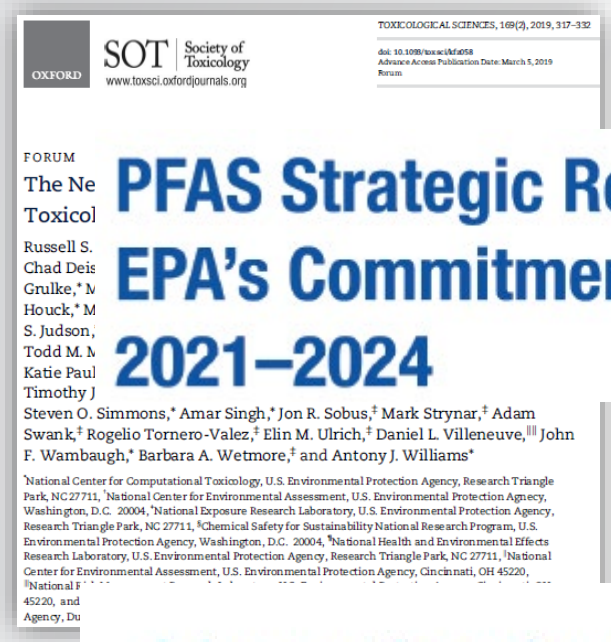


Figure 2. Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals from Tier 1 without a defined biological target / pathway, a quantitative point-of-departure for hazard is estimated based on the absence of biological pathway or cellular phenotype perturbation. Chemicals from Tier 1 with a predicted biological target or pathway are evaluated Tier 2 using targeted follow-up assays. In Tier 3, the likely tissue, organ, or organism-level effects are considered based on either existing adverse outcome pathways (AOP) or more complex culture systems. Quantitative points-of-departure for hazard are estimated based on the AOP or responses in the complex culture system.

Using New Approach Methods to Fill PFAS Information Gaps



PFAS Strategic Roadmap: EPA's Commitments to Action 2021–2024

Advance the science to assess human health and environmental risks from PFAS

High-throughput

and high-

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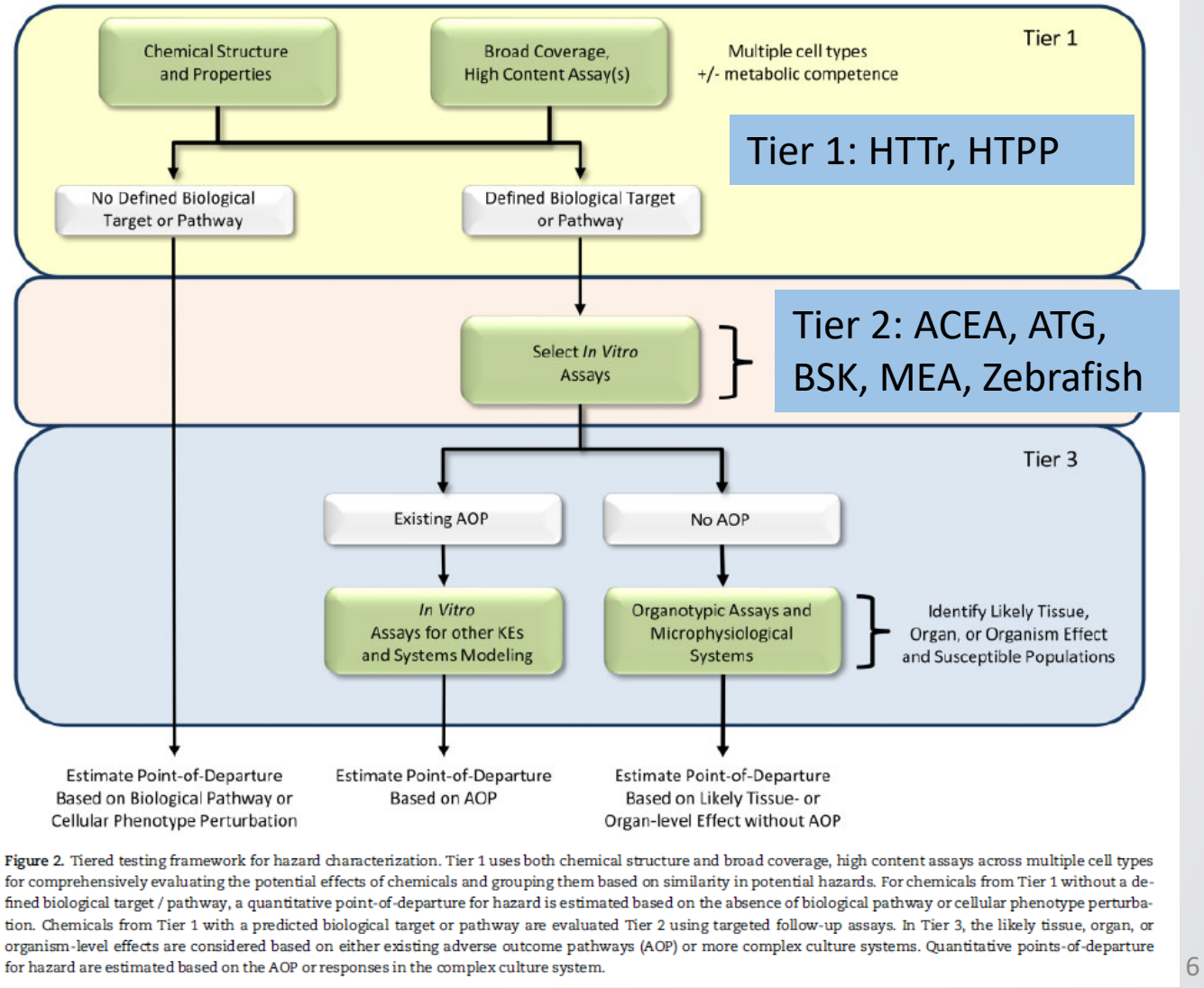
t targeted

screening (ToxCast)



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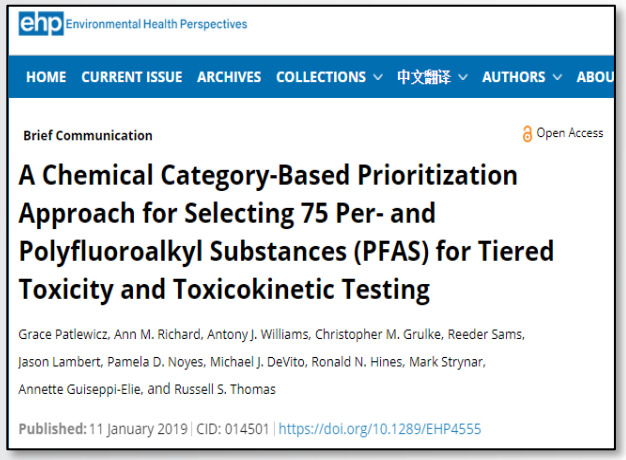
Selecting a Subset of PFAS for Tiered Toxicity and Toxicokinetic Testing

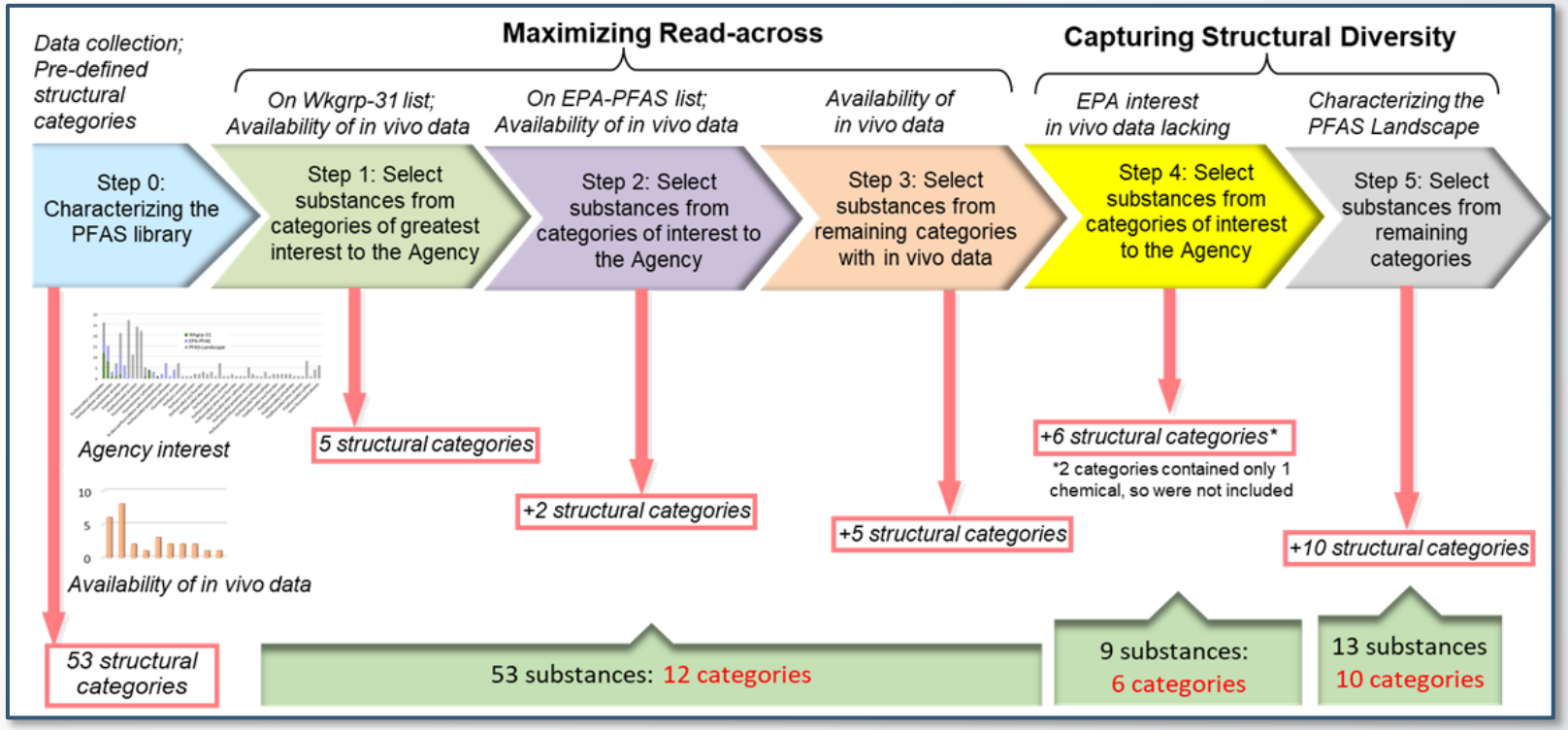


Goals

- Generate data to support development and refinement of categories and read-across evaluation
- Incorporate substances of interest to Agency
- Characterize mechanistic and toxicokinetic properties of the broader PFAS landscape

Selected 150 PFAS in two phases representing 83 different categories

- 9 categories with > 3 members
- Lots of singletons

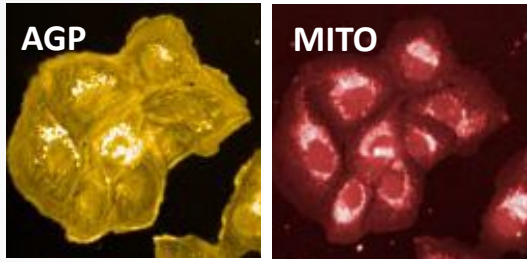


Tony Williams, Ann Richard, Chris Grulke, and Chemical Curation Team

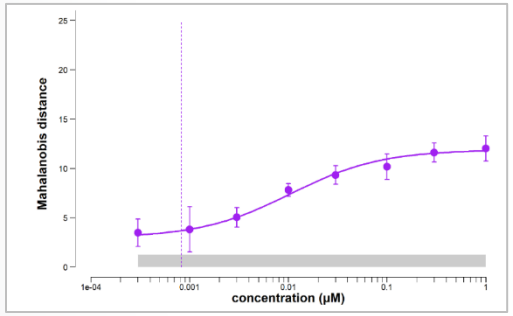
In Vitro-In Vivo Extrapolation (IVIVE) to Inform NAM Dosimetry

NAMs - Advantages

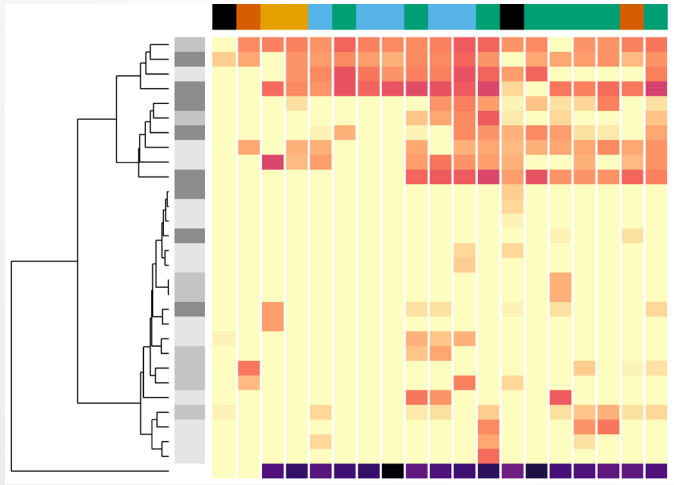
in vitro *assays*
human-specific
relevant pathways



Concentration-response
Point of departure estimation



thousands of endpoints
thousands of chemicals

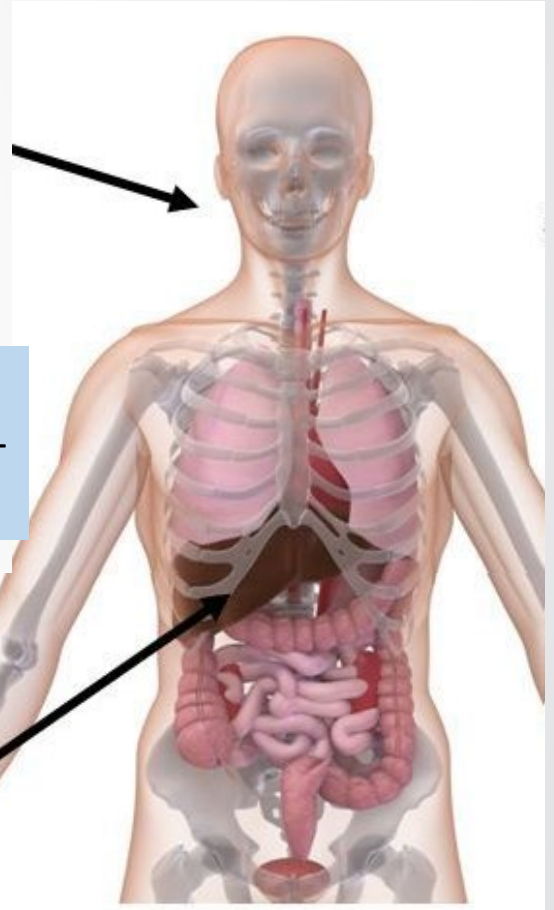


Harrill *et al.*, in preparation
Carstens *et al.*, in preparation

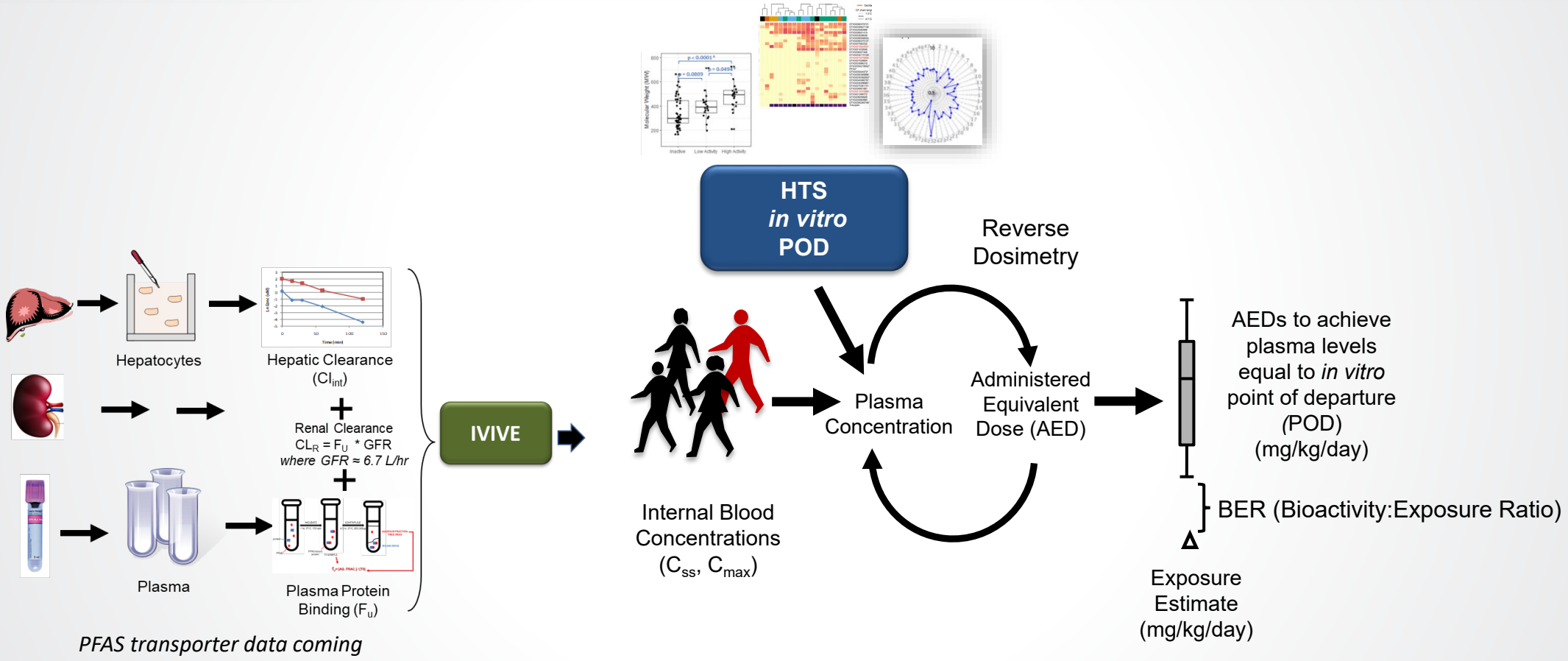
Potential dose:
Ingested, inhaled, applied to skin

Incorporating *in vitro* data with dosimetry key to linking to real-world exposures

Internal dose:
Amount absorbed and available for interaction



Using Toxicokinetics (TK) and IVIVE for Equivalent Dose Estimation



PFAS transporter data coming

***in vitro* toxicokinetic assays**

TK parameters commonly used to evaluate bioaccumulative potential

Highly plasma bound compounds retained in body indefinitely (e.g., dioxin, PFOA, PFOS)

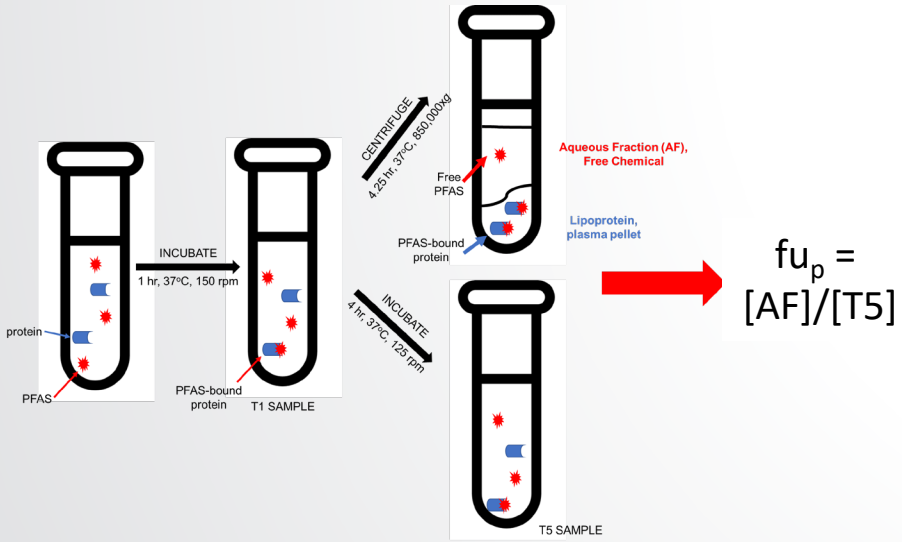
High plasma protein binding + low/no hepatic clearance = highly bioaccumulative

TK Data Generation for PFAS

PFAS Plasma Protein Binding (PPB) Analyses

- Targeted methods successfully developed for ~~109~~ *110* : *in vitro* plasma protein binding
- Method Comparison (Ultracentrifugation (UC) vs. Rapid Equilibrium Dialysis (RED))
- All PFAS analyzed in UC Assay; subset in RED; Stability in plasma monitored

Ultracentrifugation (UC)



fu_p = [AF]/[T5]

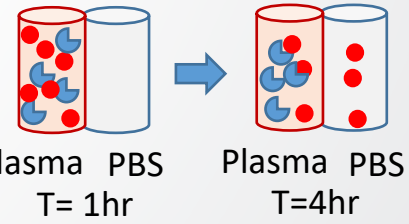
fu_p =fraction unbound in plasma
AF= aqueous fraction

Methodologies

RED assay:
equilibrium required

UC assay:
centrifugation instead of membrane, minimal non-specific binding

Rapid Equilibrium Dialysis



Smeltz et al., submitted: PFAS Stock QC Evaluation
Smeltz *et al.*, submitted: PPB: 73 PFAS; UPLC-MS/MS
Kreutz *et al.*, in preparation: PPB, Hep Cl: 69 PFAS; GC-MS/MS
Crizer *et al.*, in preparation: Hep Cl: ~70 PFAS; LC-MS/MS

PFAS Coverage in PPB Assays

73 Selected for LC-MS/MS:

- *PPB measures successful for 67*
- 67 fell into 25 distinct groups,
- 16 possessing a minimum of two structures
- C chain length and per vs poly analyses possible

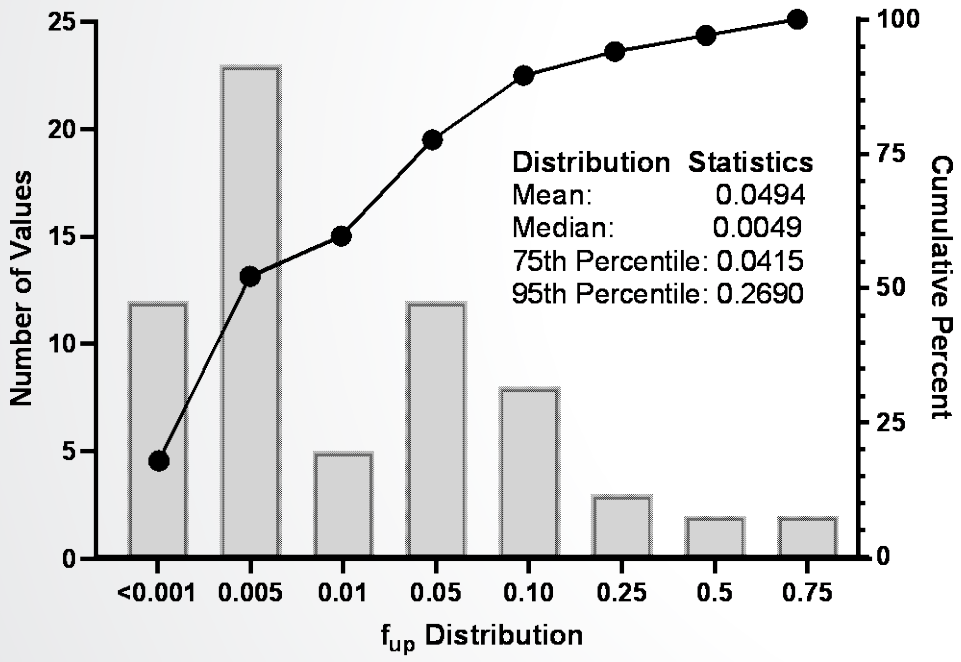
73 Selected for GC-MS/MS:

- *PPB measures successful for 43*
- Coverage across 12 distinct functional classes
- Stability issues noted for several
- Volatility an issue for some groups

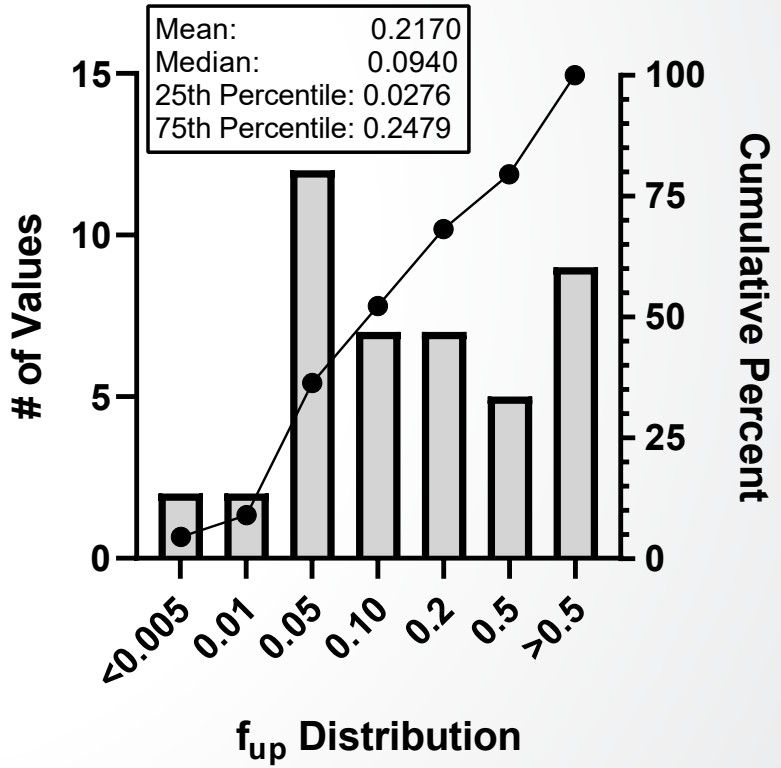
LC-MS/MS PFAS	#	GC-MS/MS PFAS	#
Perfluoroalkyl carboxylates	21	Fluorotelomer Alcohols	10
Perfluoroalkane sulfonates	9	Other Polyfluorinated alcohols	10
Fluorotelomer carboxylates	4	Acrylates	12
Perfluoroalkyl ether carboxylates	4	Ethers esters and ethoxylates	10
Perfluoroalkyl polyether carboxylates	4	Halides	9
Perfluoroalkanoyl chlorides	4	Amides	7
Perfluoroalkane sulfonamides (FASAs)	4	Amines	6
Fluorotelomer sulfonates	3	Sulfur-containing	4
Fluorotelomer phosphonic acids	2	Silanes	2
Two other groups	2	Alkanes	2

High Binding Noted Across Most PFAS

67 PFAS – analyzed by LC-MS/MS



43 PFAS – analyzed by GC-MS/MS



20-fold higher median binding for PFAAs vs PFAS alcohols, amides, etc.

fu_p=fraction unbound in plasma

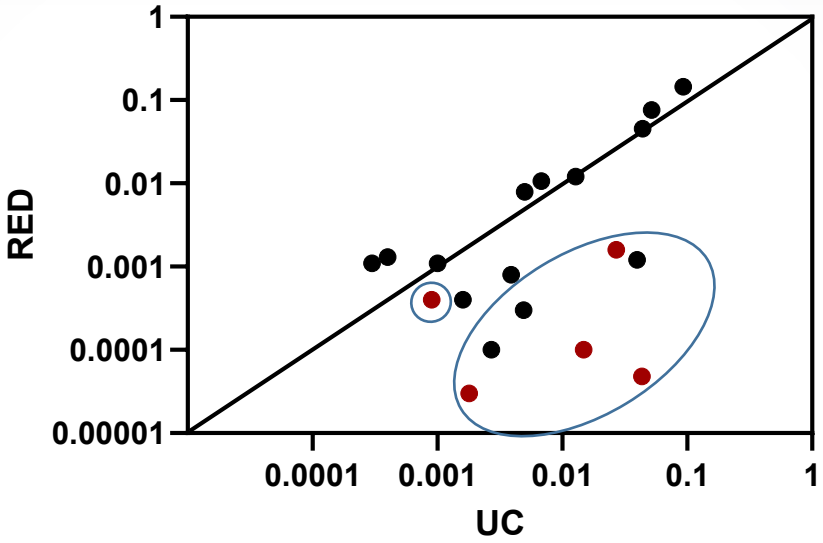
PFAS RED Assay Failures Noted

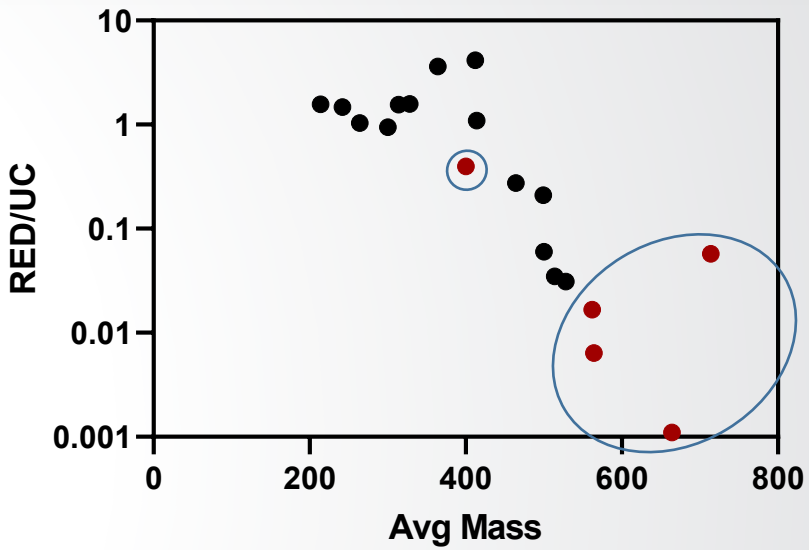
23 PFAS were compared in RED vs UC assay

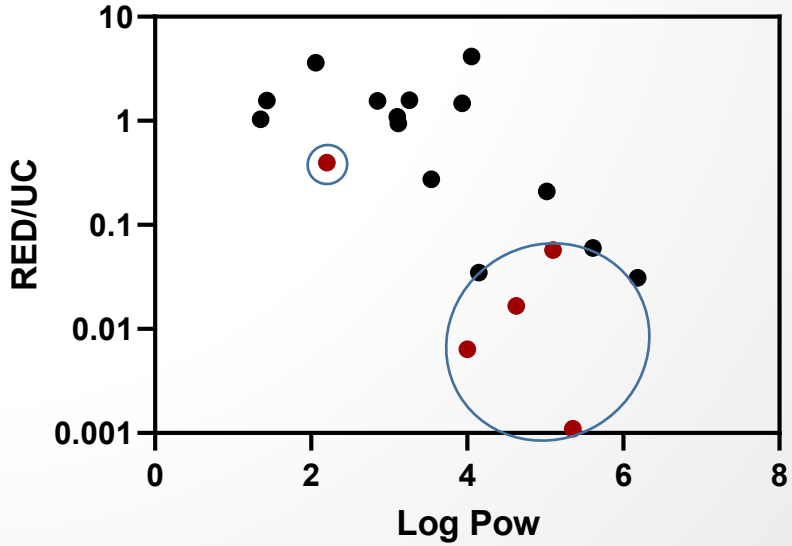
- 4 of 23 - detection issues in RED
- 7 of 23 did not achieve equilibrium in RED
- High M.W. and high Log P_{ow} associated with RED assay failure

Of 14 with acceptable data in both methods:

- 7 showed good agreement (within 3-fold of each other)
- RED assay tended toward higher binding (lower f_{up} measures) than UC assay;
- High M.W. and high Log P_{ow} associated with less agreement

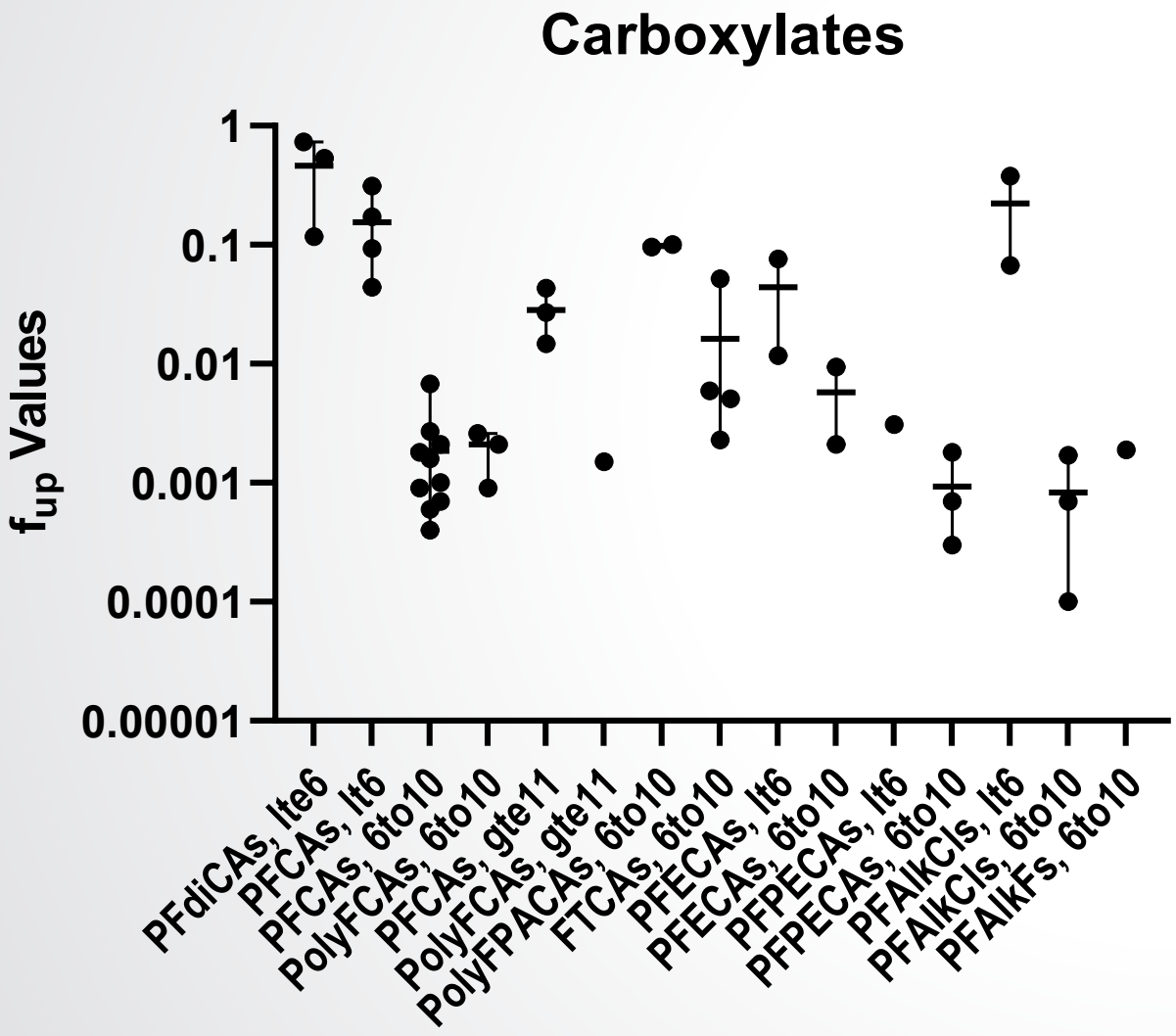


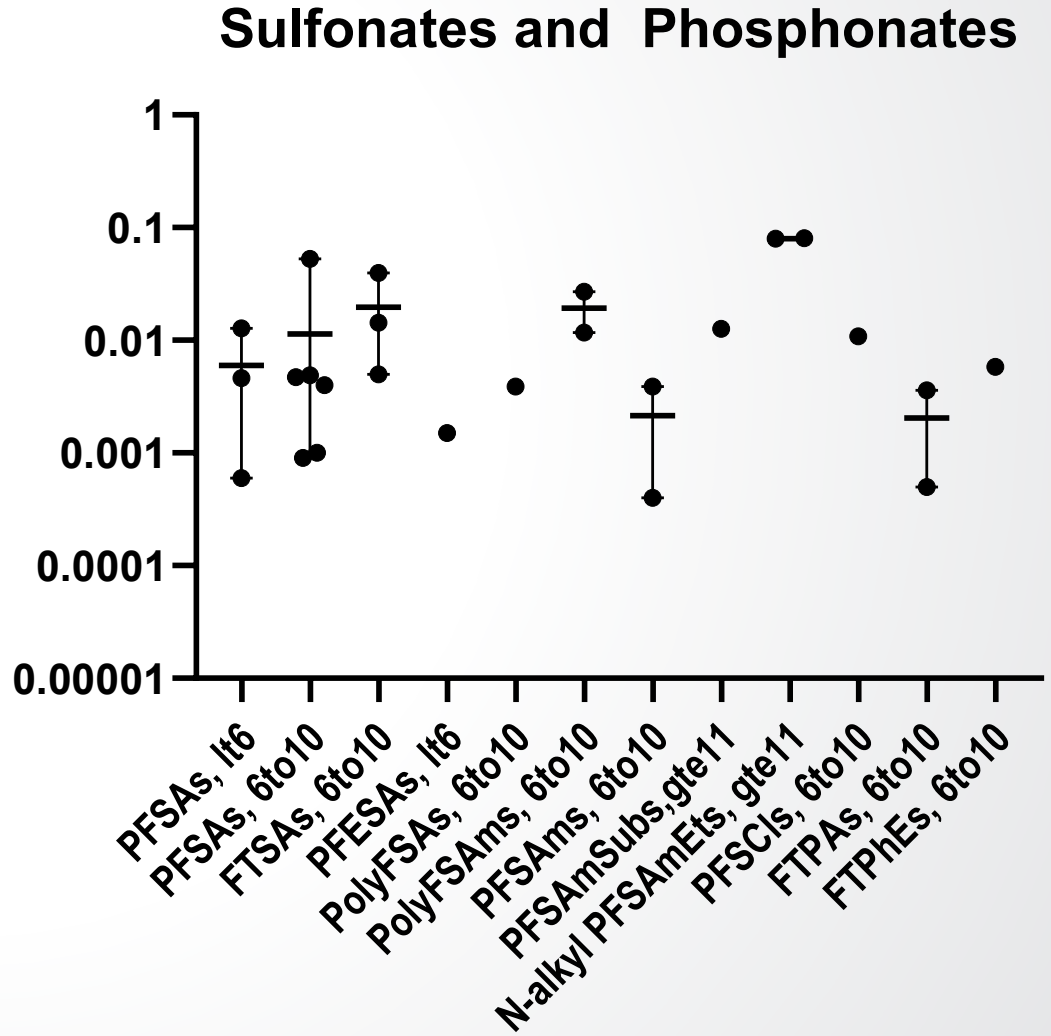




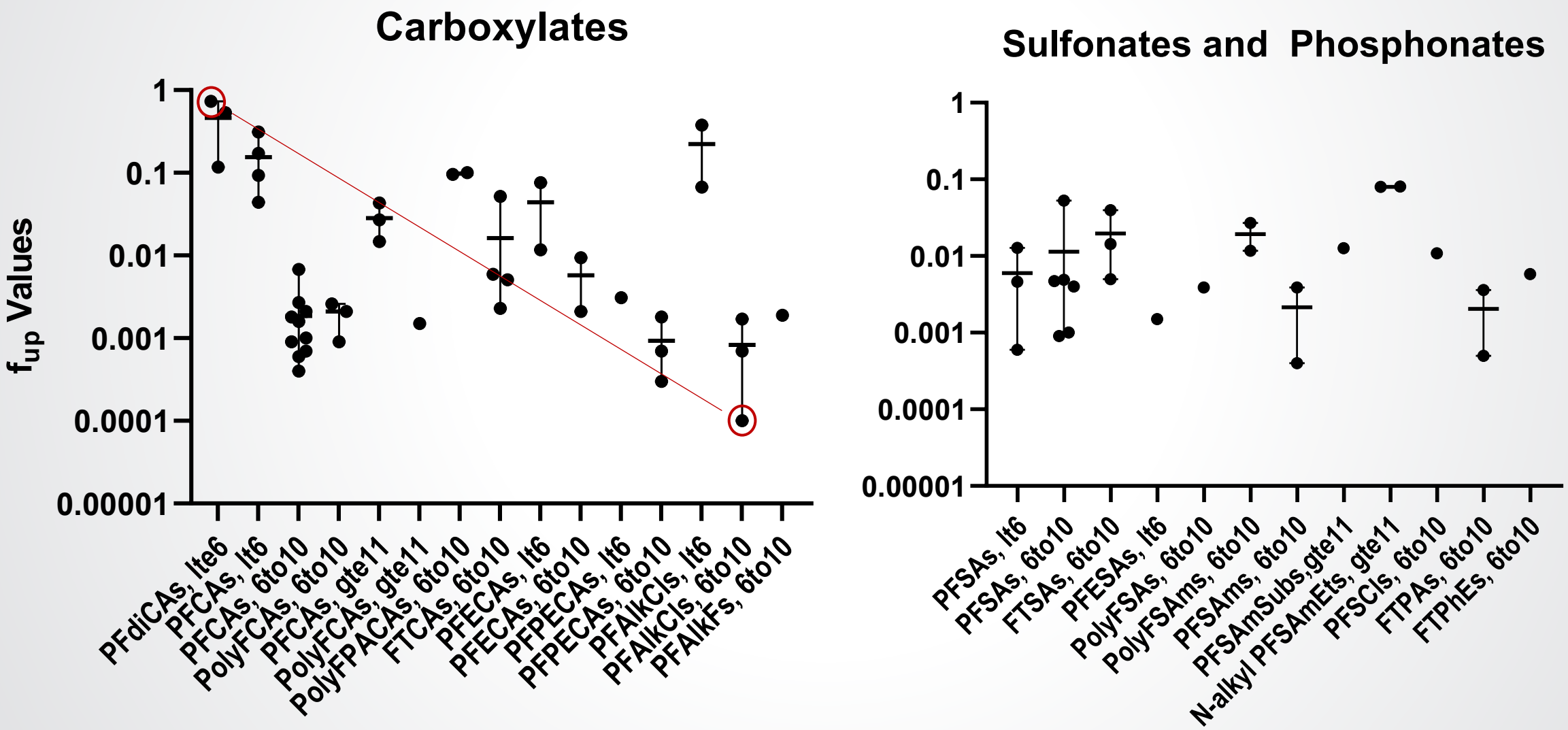
Red circles indicate failure to achieve equilibrium in RED assay

Category-Based Evaluations of PFAS Plasma Protein Binding



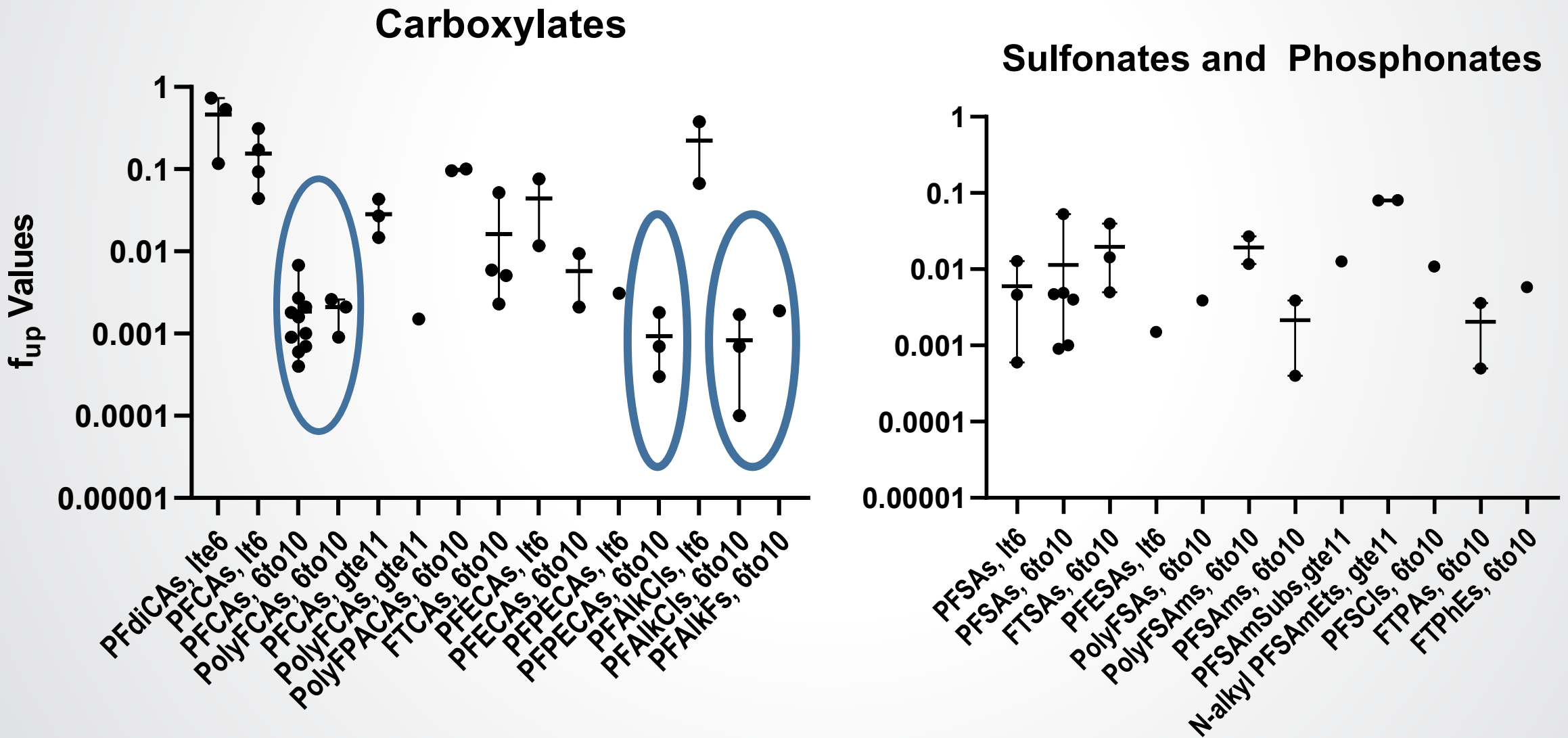


Category-Based Evaluations of PFAS Plasma Protein Binding



7300-fold difference in binding between lowest and highest bound PFAS in this analysis

Trends Noted In Functional Group Presence and Plasma Protein Binding



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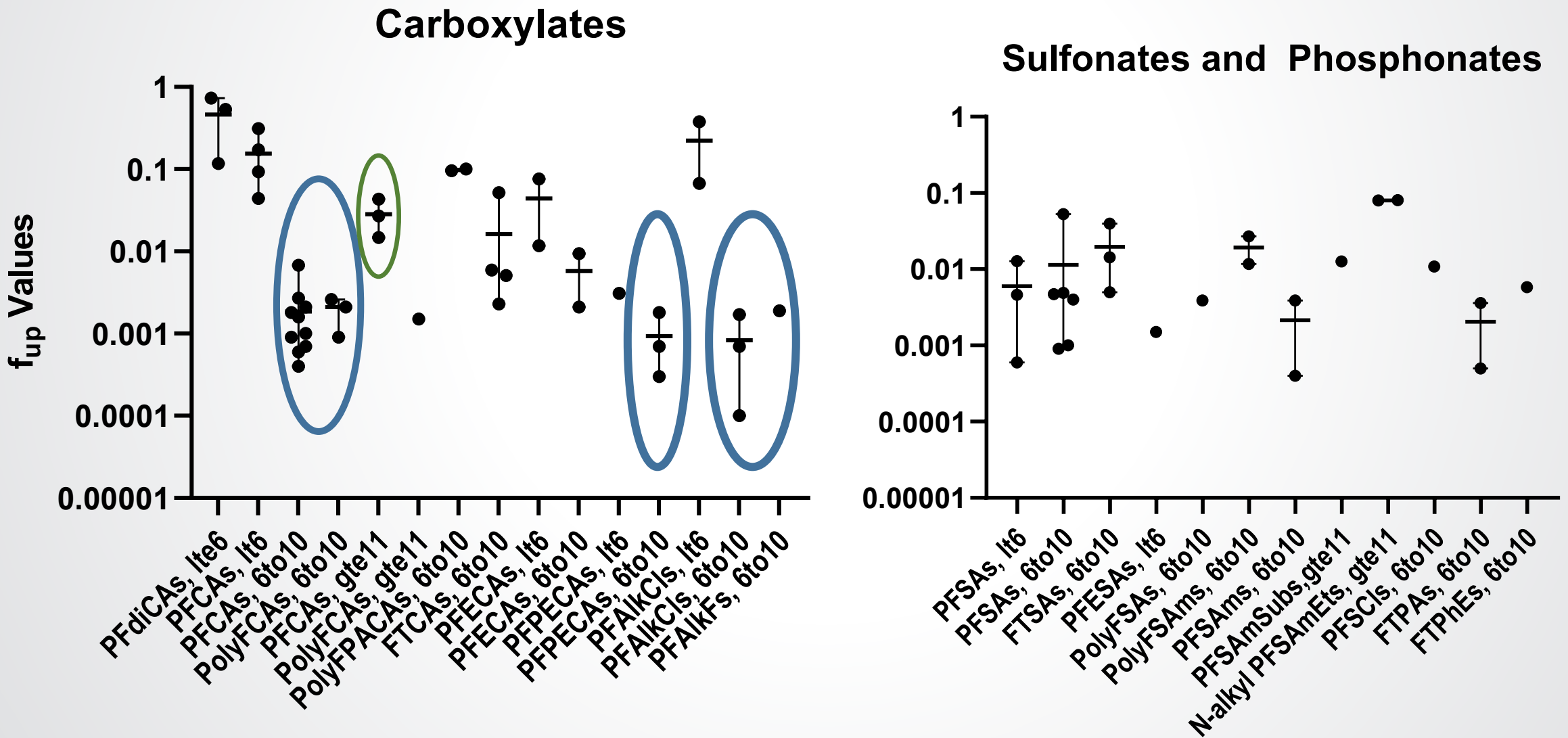

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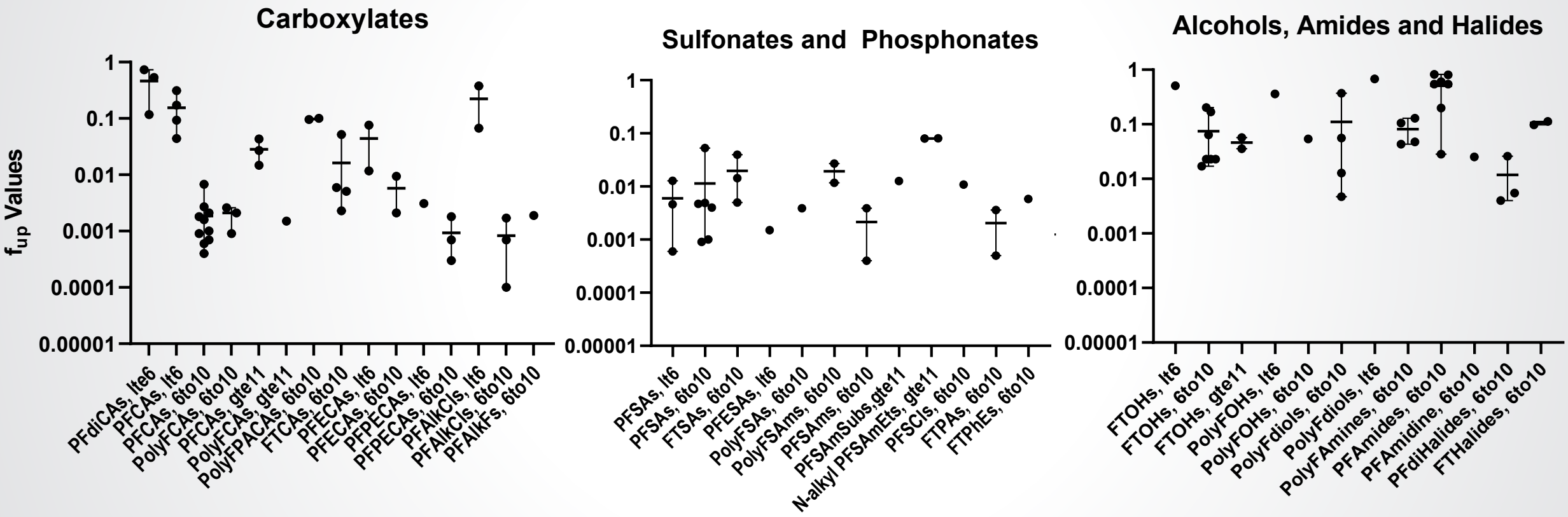
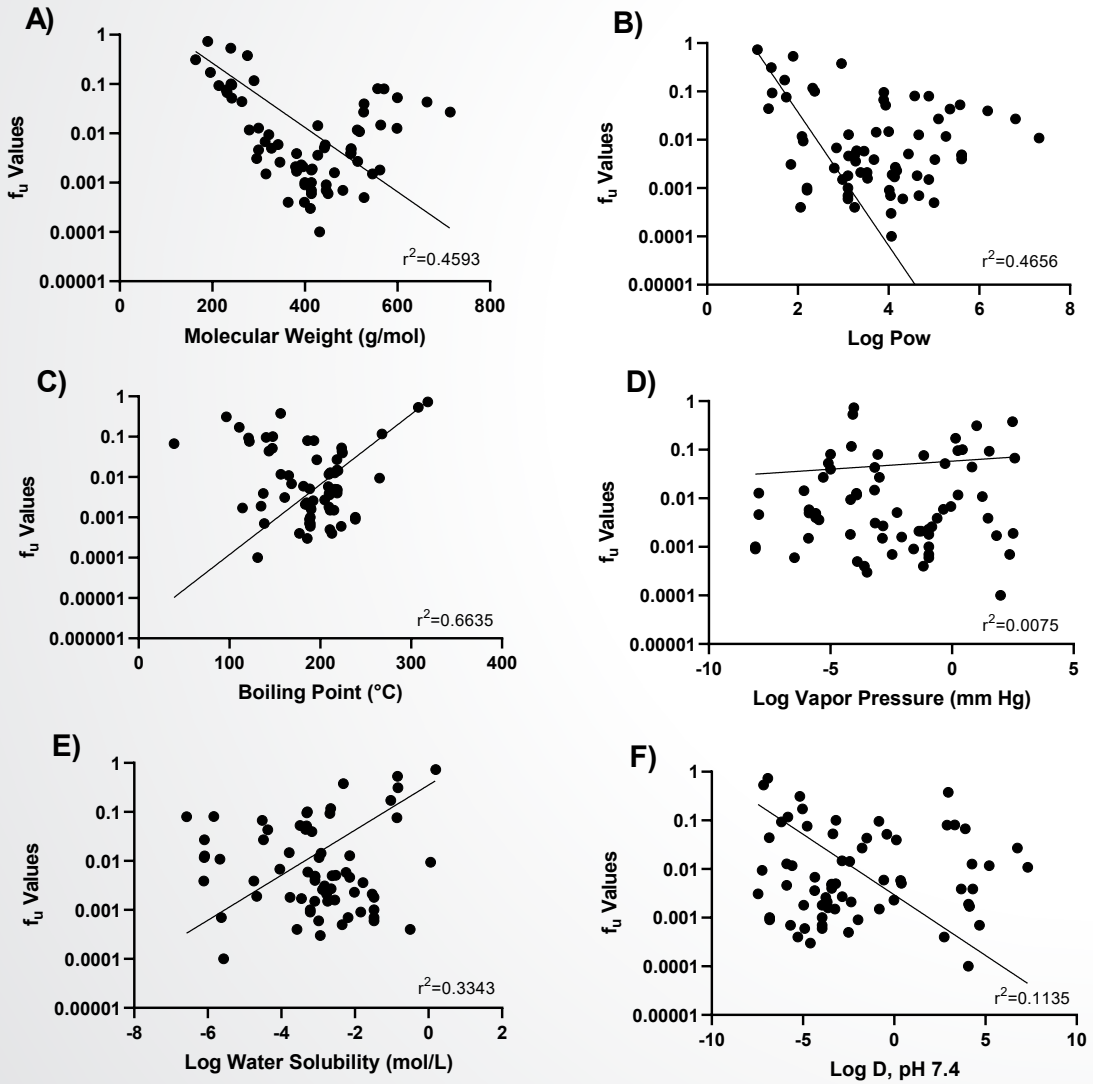


Note lower binding in third panel – PFAS analyzed by GCMS (20-fold lower binding)

Key Observations - PFAS Plasma Protein Binding

- Groups >99.9% binding (i.e., f_{up}≈0.001):
  - Perfluoroalkanoyl chlorides (mean f_{up} = 0.0008)
  - Perfluoropolyether carboxylates (PFPECAs)(mean f_{up} = 0.0009)
  - Perfluoroether carboxylates (PFECAs) (mean f_{up} = 0.0058)
  - Per- and polyfluorocarboxylates with 6-9 carbons (mean f_{up} = 0.0019)
- PFECAs and PFPECAs – “alternative PFAS” purported to be less bioaccumulative. Already discovered in NJ waters (NTA study at levels that exceed the PFOA drinking water standard) and in human follicular fluid samples
- 16-fold lower binding for PFAS with ≥ 11 vs. 6-9 carbons; similar trend observed for sulfonates

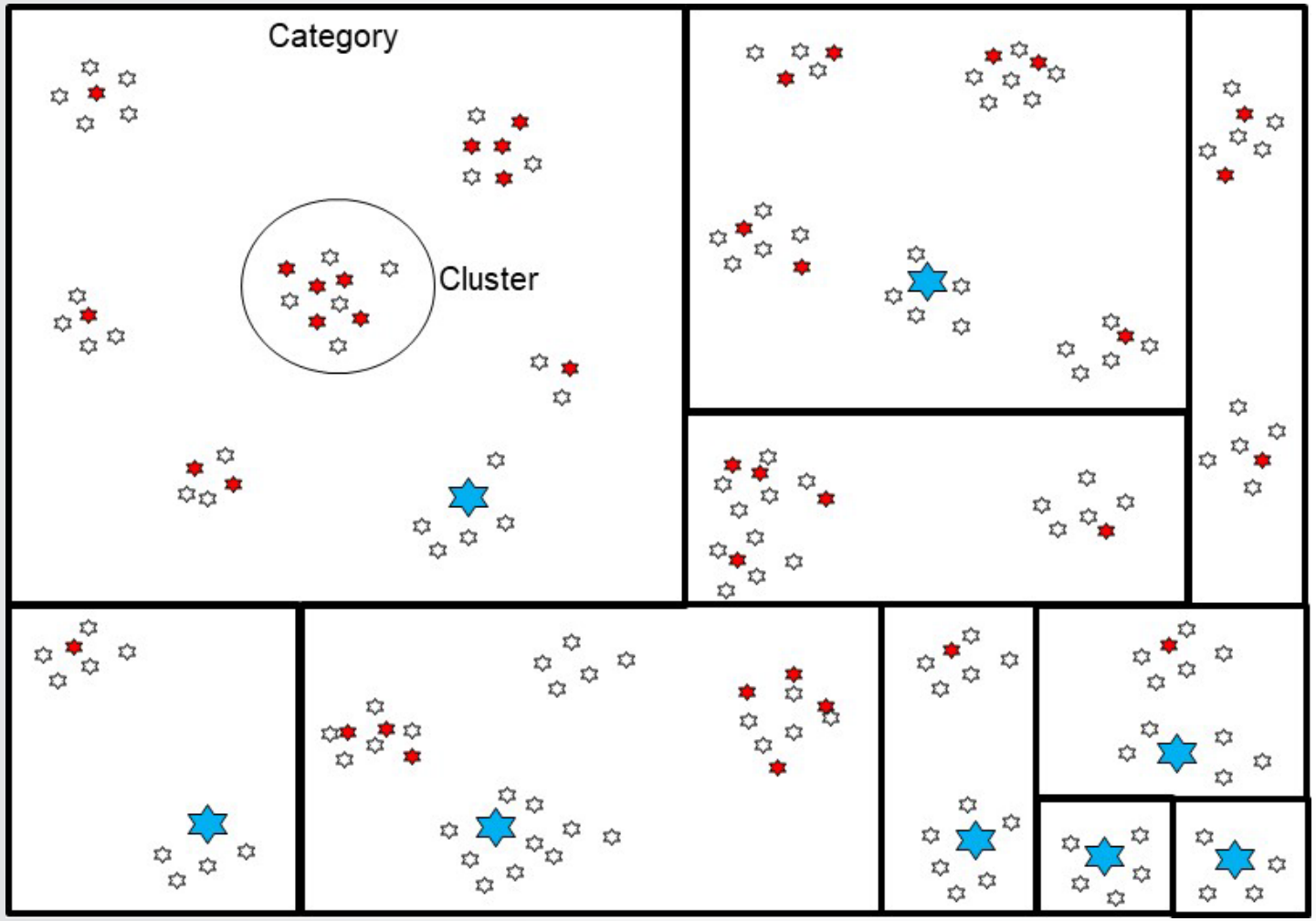
Physicochemical Properties and PFAS Binding



Trends are present…

- Increased binding with:
  - Increase in Log P_{ow}
  - Increase in molecular weight
- But exceptions emerge, indicating importance of functional group presence

Using NAM Data in Category Approach to Inform Testing Decisions



- Chemical substances are placed into high-level categories based on structure
- Sub-categories are derived using NAM, chemical property and existing *in vivo* data
- At least one chemical substance needs *in vivo* data per sub-category for read-across

Approach employed in the National PFAS Testing Strategy (October 2021)

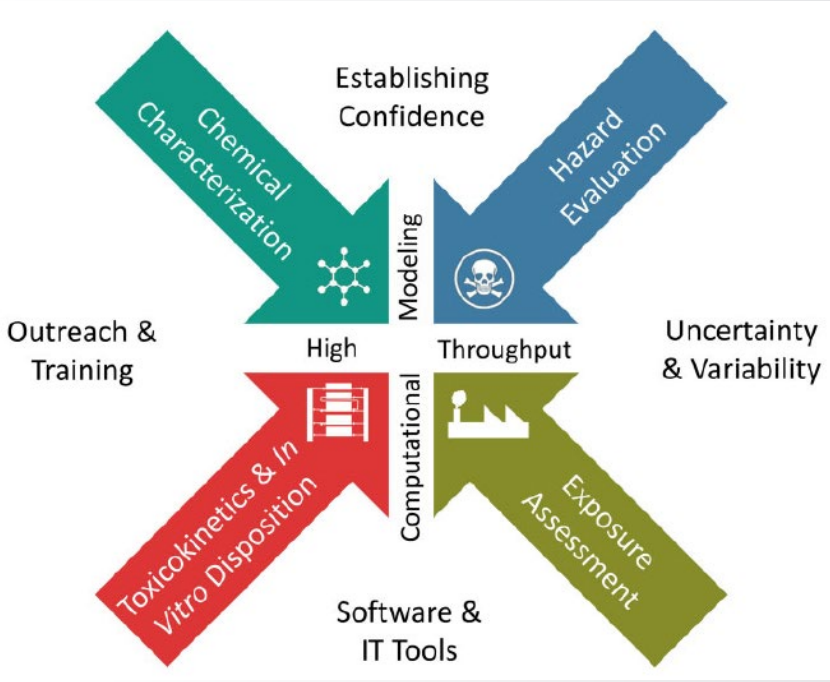
https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/national-pfas-testing-strategy

Chemical Types

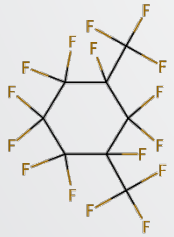
- Untested
- Existing in vivo data
- Proposed for new testing

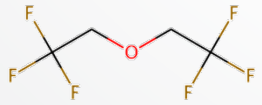
Concluding Thoughts

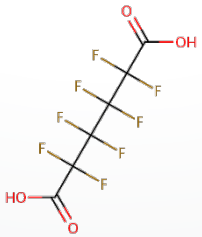
- Subset of ~150 PFAS selected by EPA to support read across evaluations using NAM data streams
- PPB evaluations were successfully conducted on 110 PFAS that span over 40 functional categories.
- Four categories identified with plasma binding exceeding 99.9%:
  - legacy carboxylic acid and sulfonic-acid containing PFAS
  - Perfluoroalkanoyl chlorides (highest bound, at f_{up} = 0.0001)
  - Perfluoro ether and polyether carboxylates
    - perfluoroether carboxylate oligomers found in NJ waters in NTA analyses
- Assay comparisons revealed potential issues with RED, for high mass PFAS and/or highly lipophilic PFAS
- Data will be used in category approach to inform testing decisions

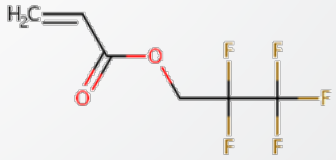


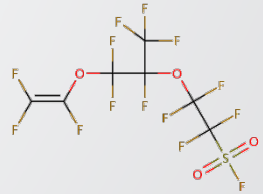
Thomas *et al.*, 2019 *Toxicol Sci*











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Rusty Thomas
John Cowden
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Johanna Nyffeler
Lucas Albrecht
Chris Grulke
Evgenia Korol-Bexell
Anna Kreutz
Jason Lambert
Larry McMillan
James McCord
Myriam Medina-Vera
Mark Strynar
Rogelio Tornero-Velez
Elin Ulrich

National Toxicology Program (NIEHS)
David Crizer
Stephen Ferguson
B. Alex Merrick

With a heart-felt shout out to the PFAS TK Sharktank Team:


Marci Smeltz


M. Scott Clifton


Anna Kreutz


W. Matthew Henderson

In Vitro Hepatic Clearance of PFAS

66 PFAS evaluated for hepatic clearance

0 - <0.9 ul/min*mill cells: 26 PFAS

0.9 – 5 ul/min*mill cells: 21 PFAS

>5 ul/min*mill cells: 19 PFAS

Functional group presence more important than chain length in overall evaluation

For many PFAS, metabolism will result in formation of a subsequent PFAS, which may in turn pose a human and/or ecological hazard. Ongoing metabolic characterization is underway to address these data gaps.