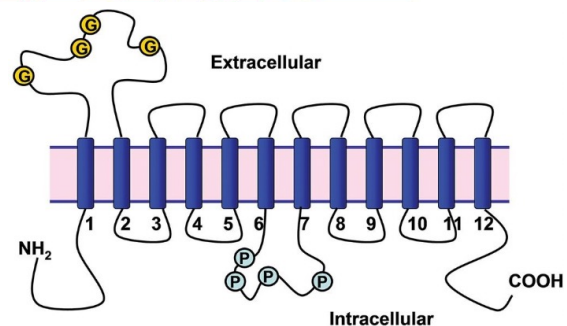


Characterizing Molecular Drivers of PFAS Uptake and Distribution



Carla Ng, University of Pittsburgh
Department of Civil & Environmental Engineering
Department of Environmental and Occupational Health

SRP eRisk Webinar
October 20, 2023

The Ng Lab at Pitt



Graduate Students:

Yuxin Cao
Ruiwen Chen
Meredith Kulak
Melissa Marciesky*
Hajar Smaili
Arundhati Tewari

Postdoctoral Scholars:

Megha Bedi
Zhaokai Dong
Shan Niu*

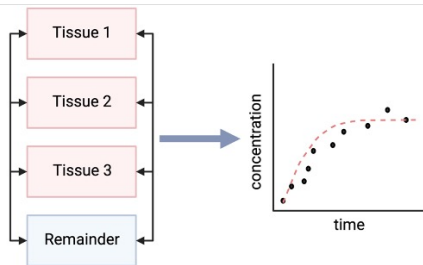
Undergraduate Researchers:

Percy Curtis
Rae Templeton
Aaron Winchell

***SRP-Funded**

The Ng Lab at Pitt

PFAS Toxicokinetics and Toxicodynamics



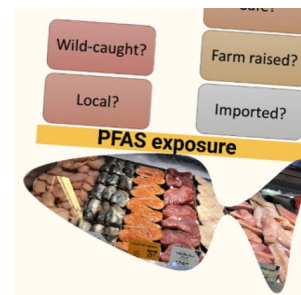
- Physiologically based toxicokinetic models that incorporate protein binding.
- Predict tissue distribution, biological half-life.
- *In silico* and *in vitro* toxicity.

PFAS-Biomolecule Interactions



- Proteins and phospholipids.
- Sorbent development.
- Transporter impacts on toxicokinetics.
- Enzymatic degradation
- PFAS simulations and force fields.

Human Exposure via Food



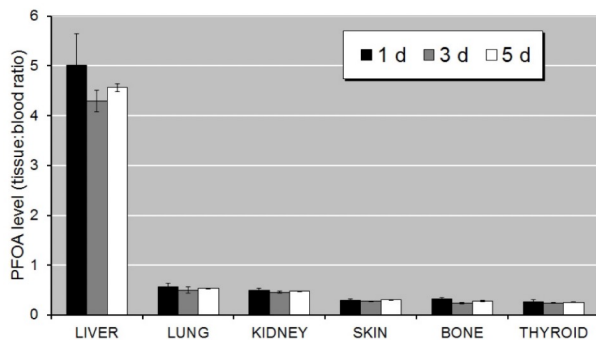
- PFAS in seafood and packaged foods.
- Pesticides, POPs, veterinary drugs in seafood.
- PBDEs in farmed salmon.

Regional PFAS Contamination

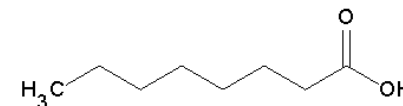
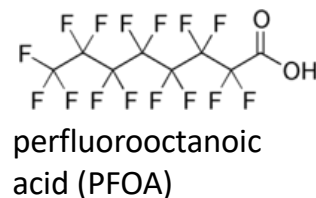


- McKeesport AFFF drinking water spill
- Regional industrial activity (e.g. ethane cracker plant).
- Regional soil-air contamination (e.g. East Palestine derailment).

Observations for PFAS suggest importance of specific interactions



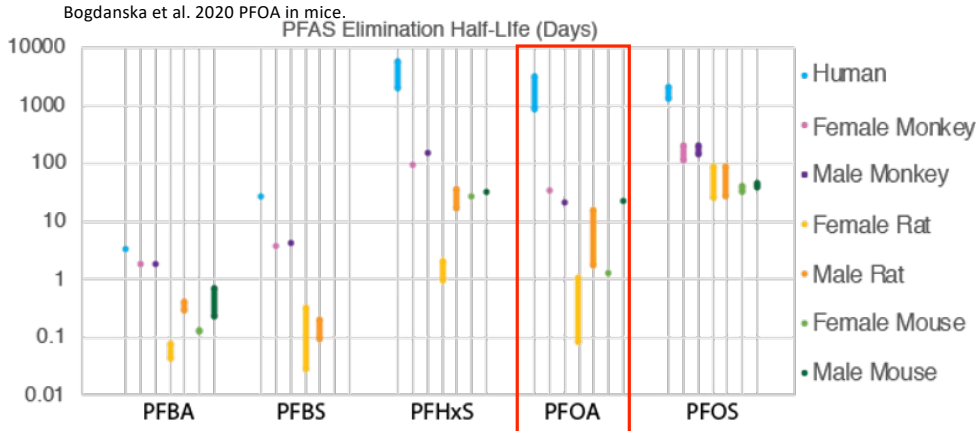
Preferential accumulation in liver and blood (not storage lipid).



octanoic acid

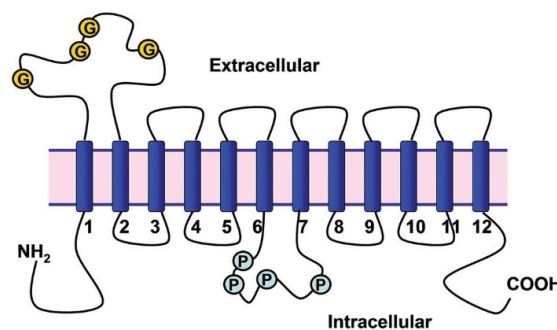


Fatty acid carriers in the body: Serum albumin and liver fatty acid binding protein.



Data extracted from Pizzuro et al. 2019 Reg Toxicol Pharmacol.

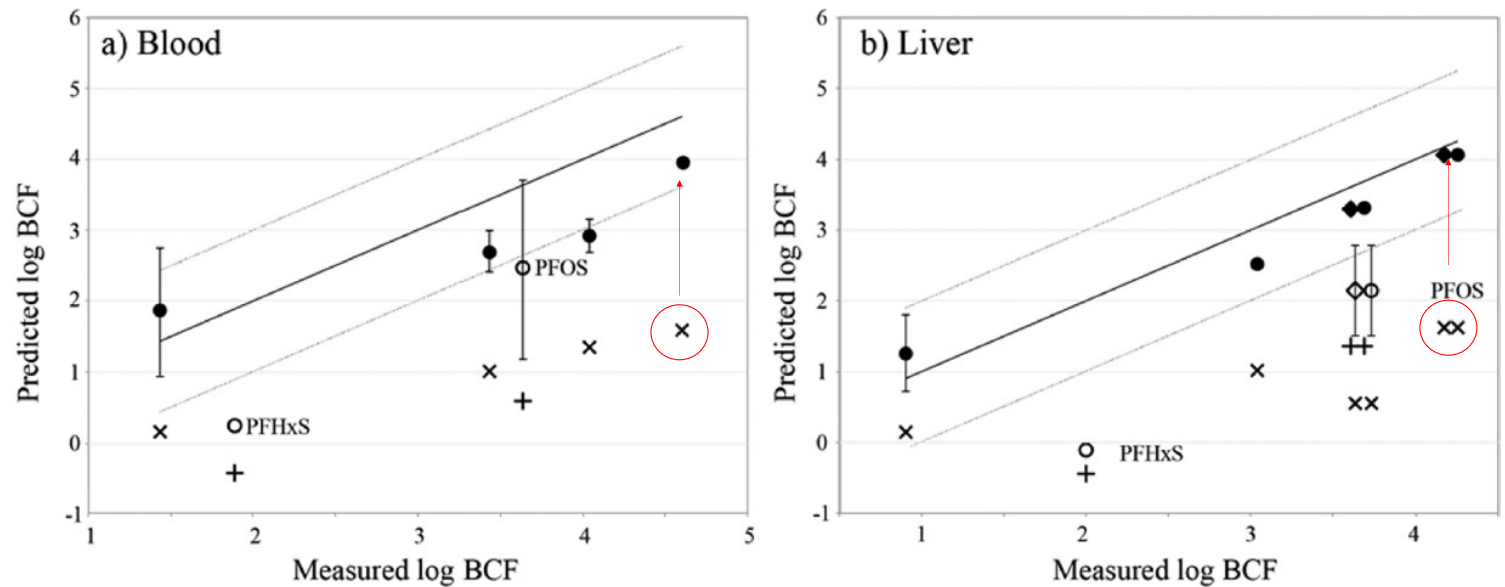
Substantial differences across species and sex.



Nigam et al. 2015 Physiol Rev

Organic anion transport proteins and polypeptides in the liver, kidneys, ... others?

Simple lipid partitioning doesn't predict PFAS behavior

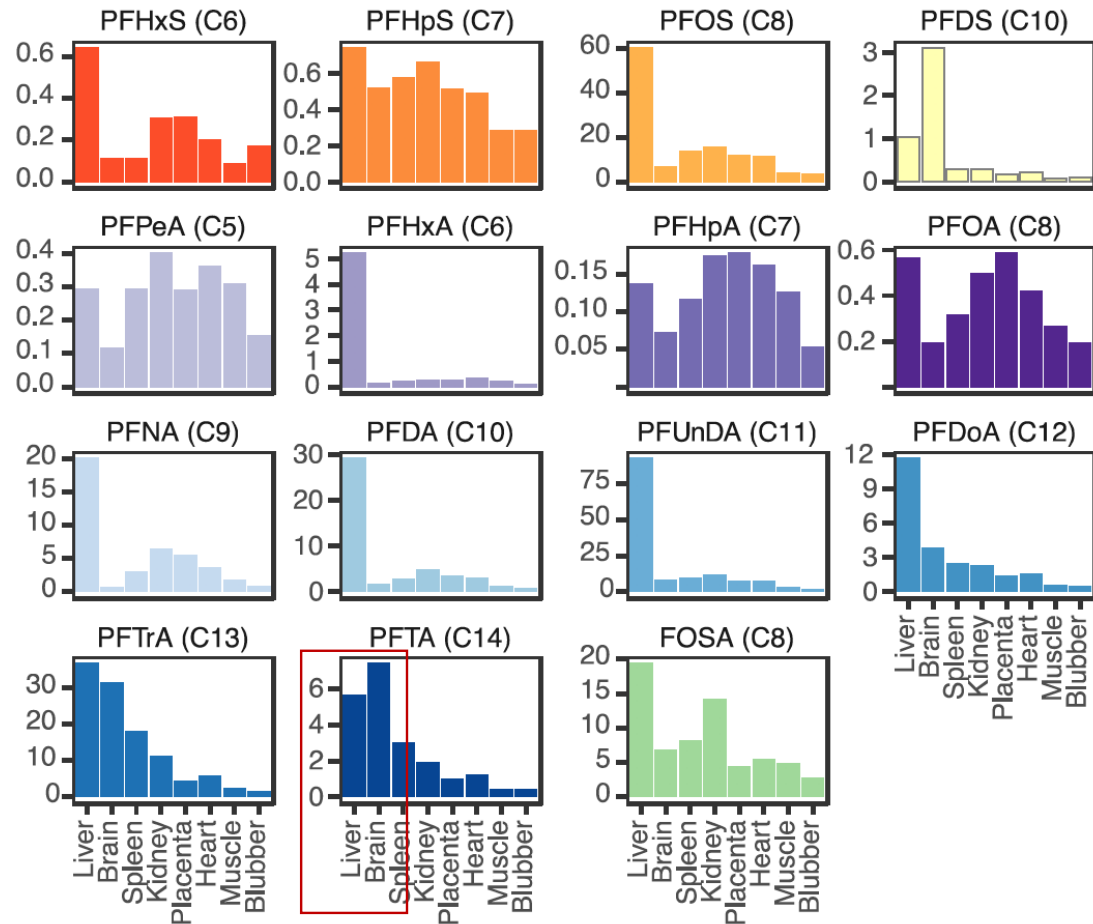


By incorporating key binding proteins (serum albumin, fatty acid binding proteins) models are better able to predict bioconcentration potential.

Tissue-specific patterns suggest further interactions

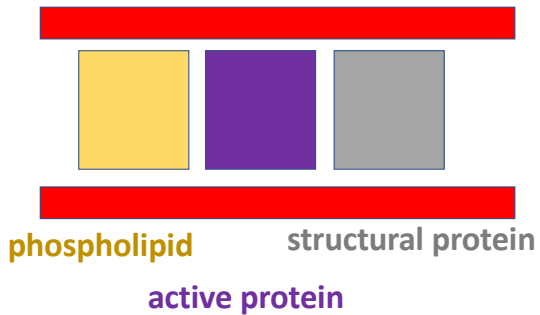
- Not only proteins but phospholipids also shown to contribute.

Need to identify tissue-specific components.



Key needs to advance understanding:

Build tissue-specific descriptions including key phases: proteins, phospholipids.

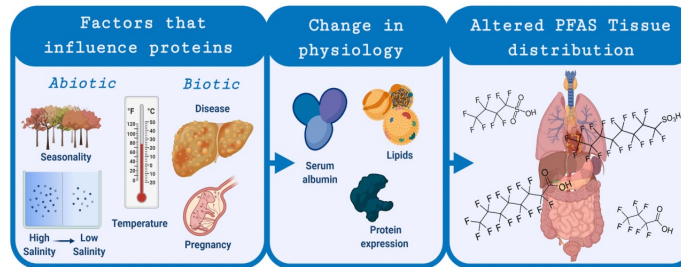


Build in dynamics: proteins, transporters.

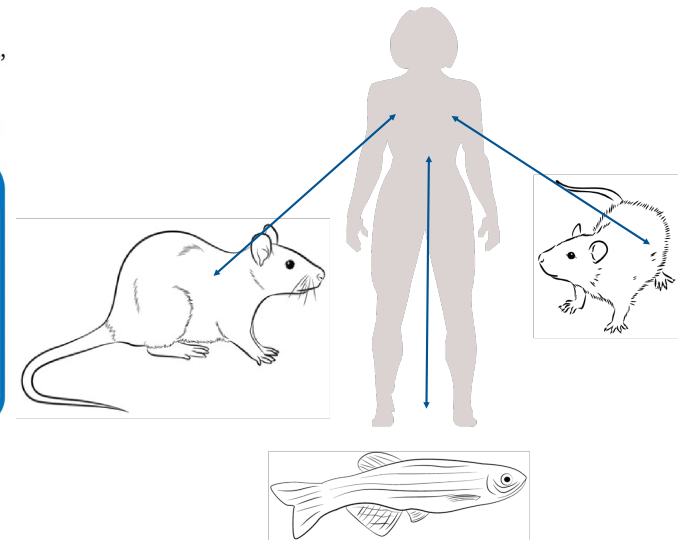
[Environment International 159 \(2022\) 107037](#)

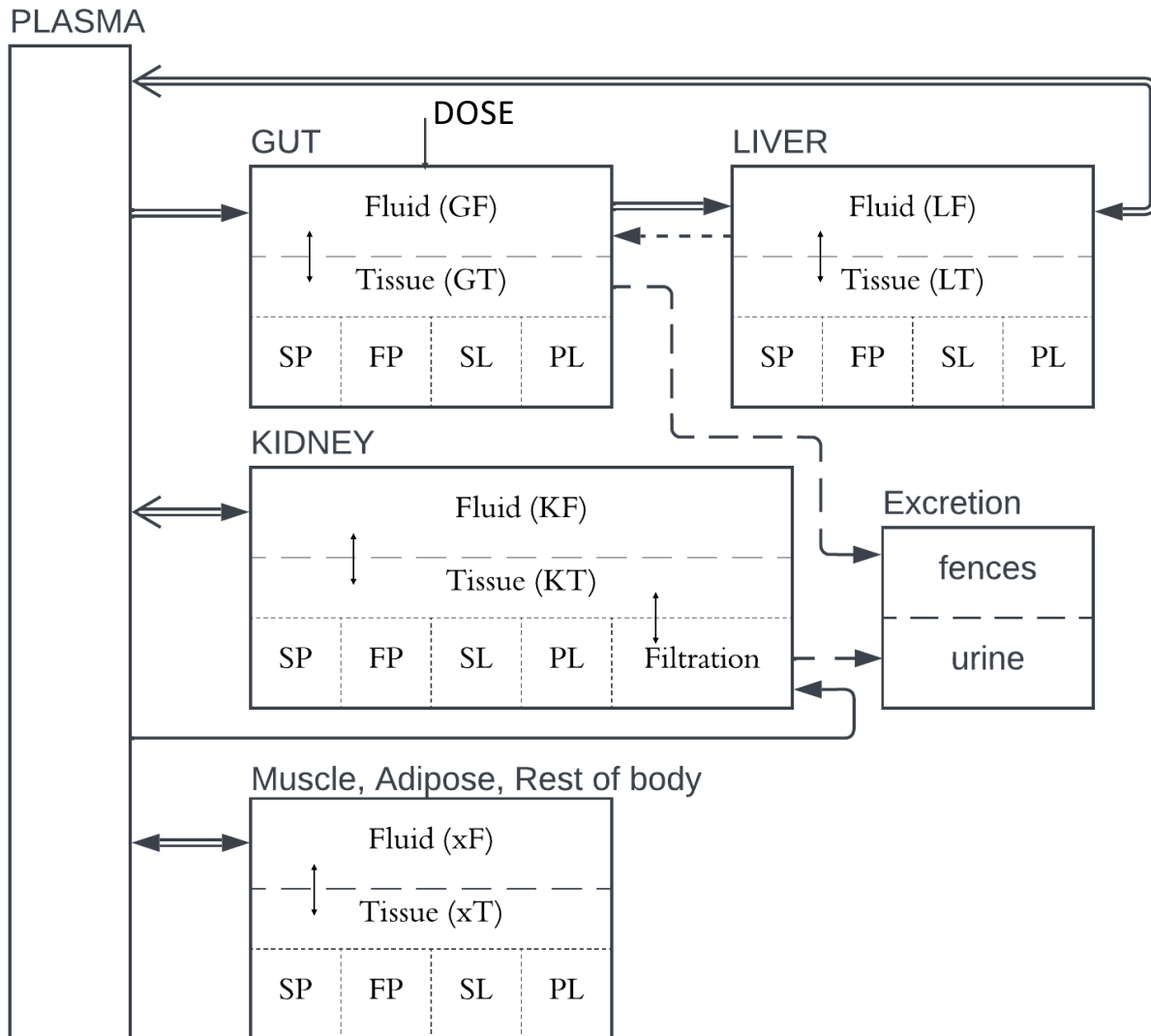
Understanding the dynamics of physiological changes, protein expression, and PFAS in wildlife

Jacqueline Bangma^{a,*}, T.C. Guillette^a, Paige A. Bommarito^b, Carla Ng^{c,d}, Jessica L. Reiner^e, Andrew B. Lindstrom^f, Mark J. Strynar^g

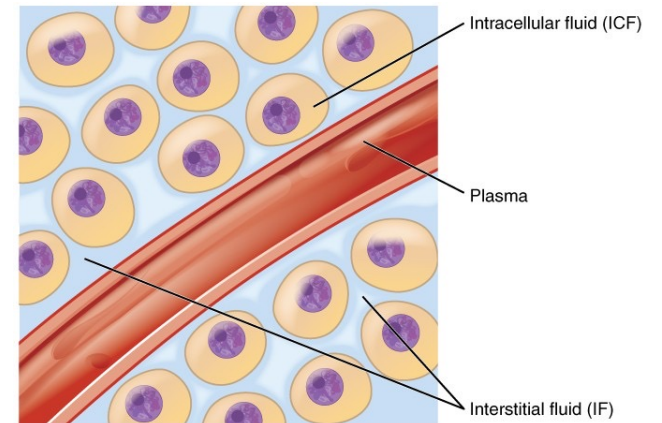


Build multi-species and multi-PFAS frameworks.





In Silico Framework

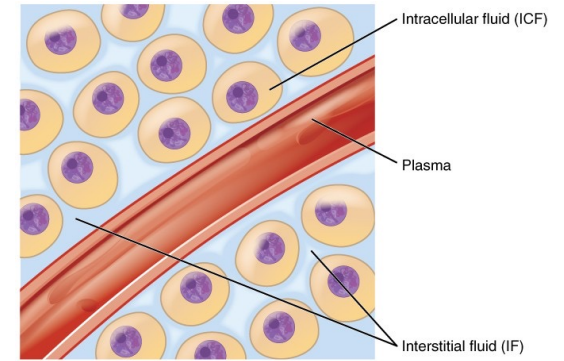


$$\begin{aligned}
 &K_{Tissue-Fluid} \\
 &= K_{PL}f_{PL} + K_{SL}f_{SL} \\
 &+ K_{SP}f_{SP} + K_{FP}f_{FP}
 \end{aligned}$$

In Vitro Evaluation

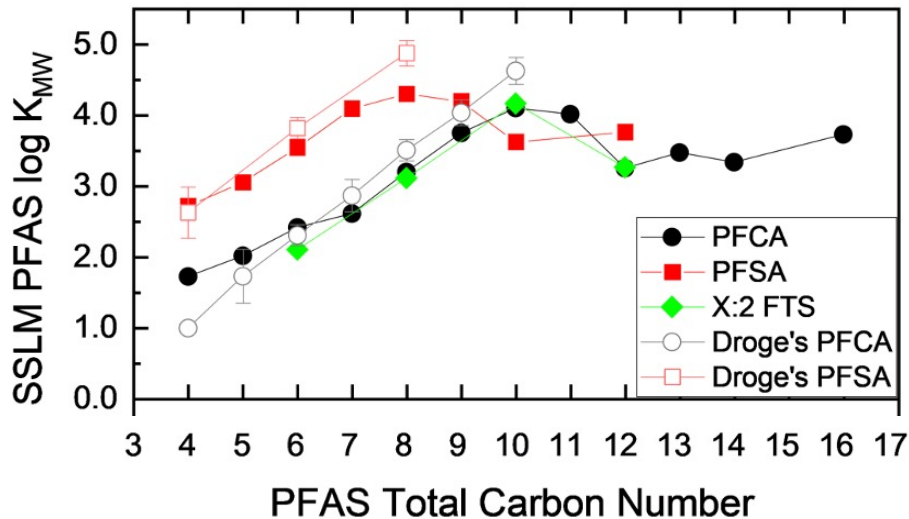


Equilibrium dialysis for protein-PFAS interactions and SSLM assay (Transil assay) for PFAS-phospholipid interactions.

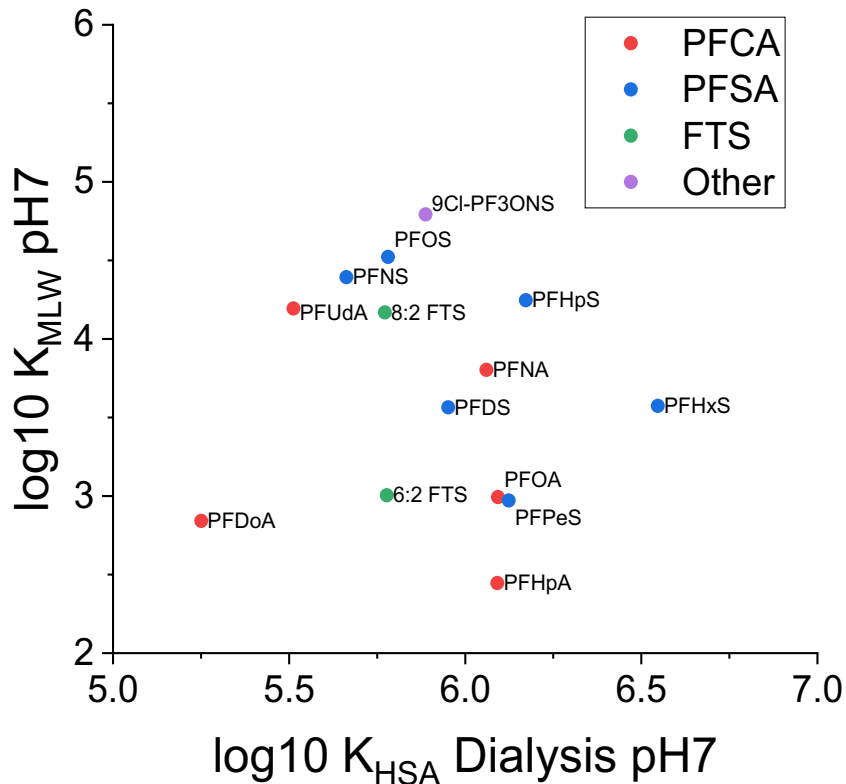


$$K_{Tissue-Fluid} = K_{PL}f_{PL} + K_{SL}f_{SL} + K_{SP}f_{SP} + K_{FP}f_{FP}$$

SP, structural protein
 FP, functional protein
 SL, storage lipid
 PL, phospholipid



Protein and Phospholipid Binding: Strong, Complementary

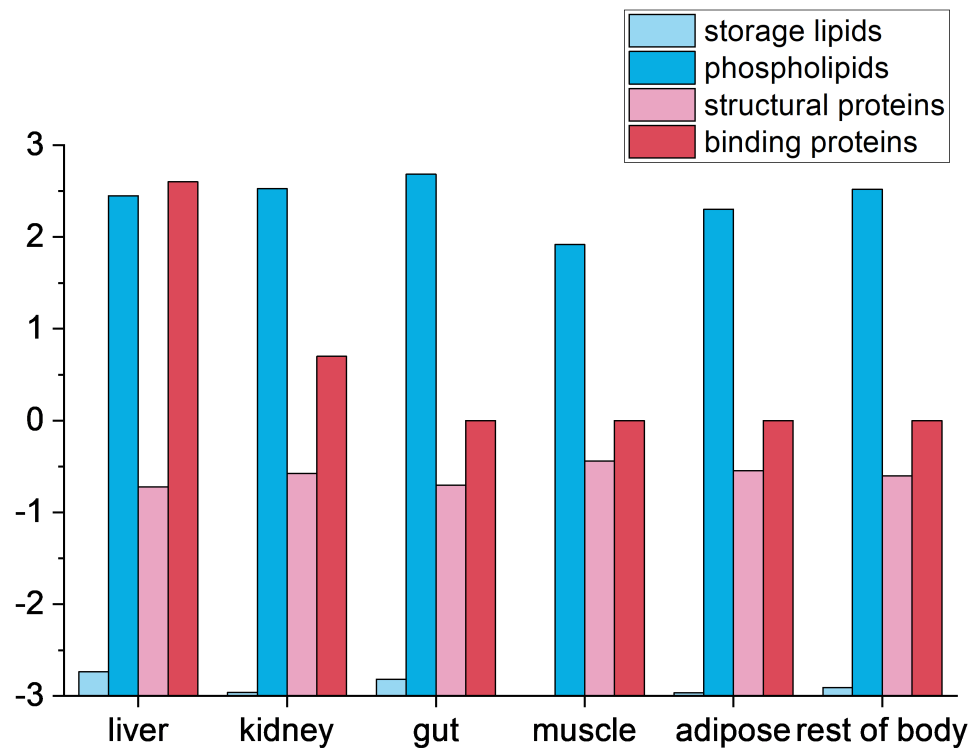


- No strong correlation between membrane (K_{MLW}) and protein (K_A for HSA) binding.
- Suggests different mechanisms and influence of chain length/structural features at play.
- This is good news! These are complementary, not redundant data.

“Other” Lipids and Proteins: Storage and Structural

Phase		Estimation Method	Value for PFOA (log ₁₀ K)
Storage Lipids	D _{OW}	Apparent log K _{ow} , Xiang et al., “Measuring Log K _{ow} Coefficients of Neutral Species of Perfluoroalkyl Carboxylic Acids Using Reversed-Phase High-Performance Liquid Chromatography.”	-2.42
Structural Proteins	D _{PW}	PP-LFERs method from Henneberger, Goss, and Endo, “Partitioning of Organic Ions to Muscle Protein.”	-0.36

Drivers of PFAS-tissue Distribution for PFOA



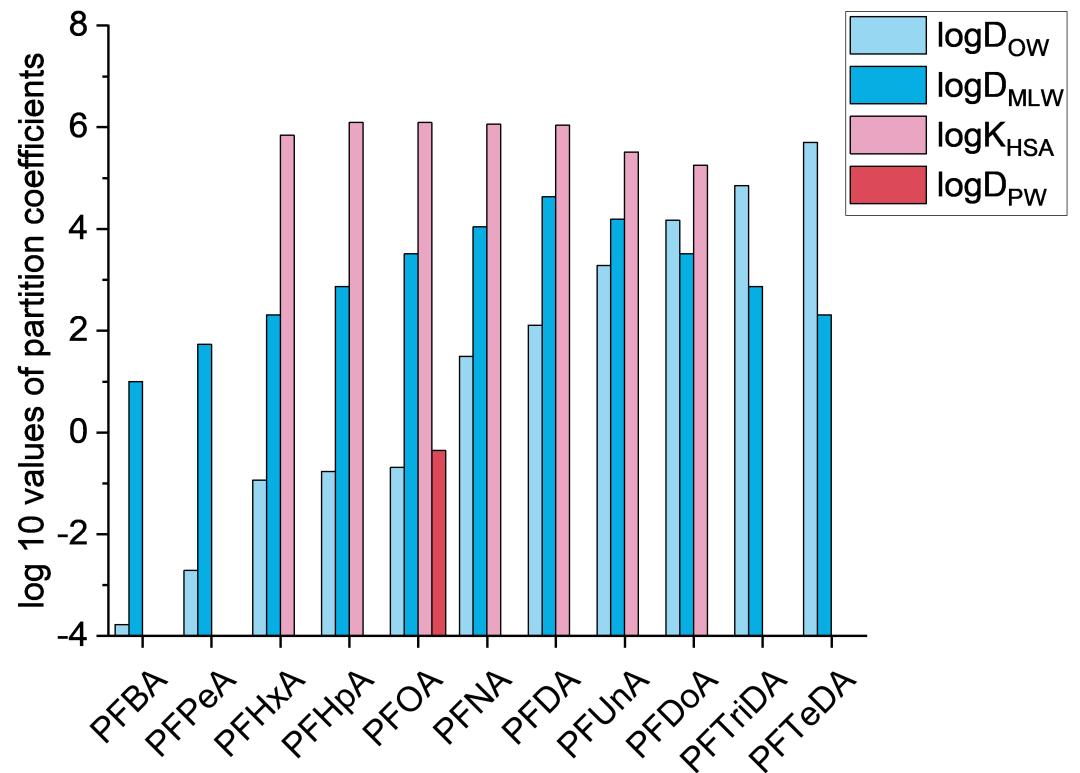
Phospholipids and binding proteins, as expected, contribute most to distribution.

Structural proteins are “neutral”. Storage lipids do not contribute.

Binding proteins in other tissues remains incompletely studied.

Future needs: from PFOA to other PFAS

- Other functional Proteins
 - L-FABP, α 2u-globulin... others?
- Membrane transporters
 - Oat1, Oat3, Oatp1a1, Ntcp, Osta/ β ...
- These additional data can inform tissue partition coefficients:
 - D_{OW} , D_{MLW} , D_{PW} , K_{PW} (specific binding)

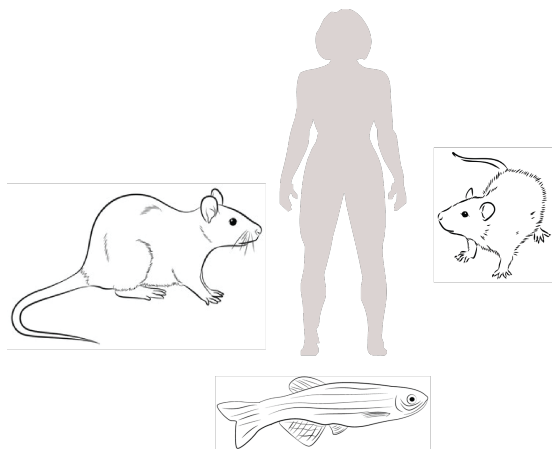


Next: understanding **key differences** across species, ecosystems

Sequence Alignment to Predict Across Species Susceptibility

What is SeqAPASS?

[Sequence Alignment to Predict Across Species Susceptibility \(SeqAPASS\)](#), is a fast, online screening tool that allows researchers and regulators to extrapolate toxicity information across species. For some species, such as humans, mice, rats, and zebrafish, the EPA has a large amount of data regarding their toxicological susceptibility to various chemicals. However, the toxicity data for numerous other plants and animals is very limited.



Which model organism for which purpose?
Which protein? Which PFAS?



Dr. Carlie Lalone, US EPA, Duluth



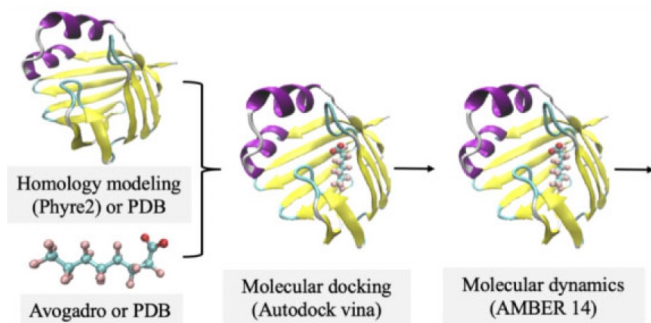
Dr. Jon Doering, now faculty at LSU

Understanding key differences

TOXICOLOGICAL SCIENCES, 2021, 1–12

Integrative Computational Approaches to Inform Relative Bioaccumulation Potential of Per- and Polyfluoroalkyl Substances Across Species

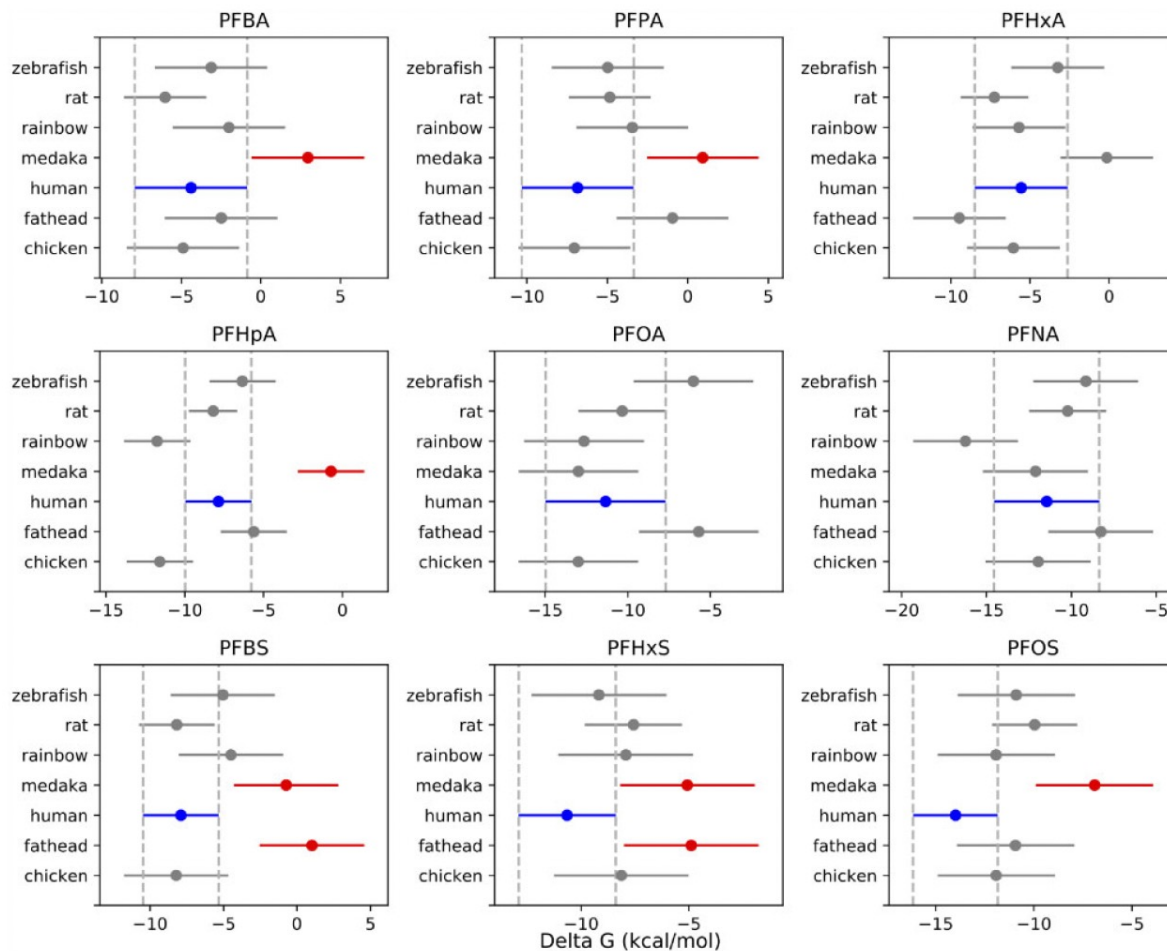
Weixiao Cheng,* Jon A. Doering,[†] Carlie LaLone,^{‡,2} and Carla Ng^{*,§,1,2}



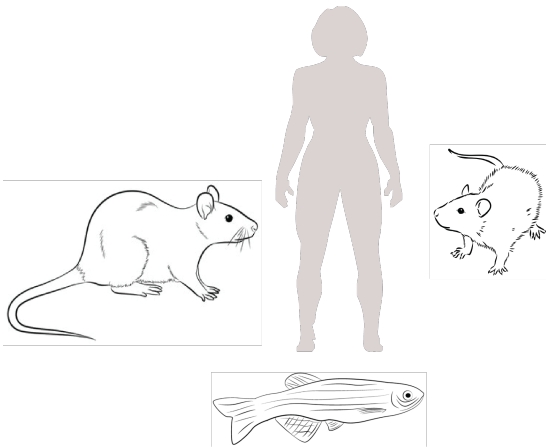
$$\Delta G_{bind} = \frac{1}{N} \sum_{i=1}^N G_i^{Complex} - G_i^{Protein} - G_i^{PFAS}$$

$$G = E_{bond} + E_{electrostatic} + E_{van\ der\ Waals} + G_{polar} + G_{nonpolar} - TS$$

MM-PBSA calculation



Observations on **key differences**



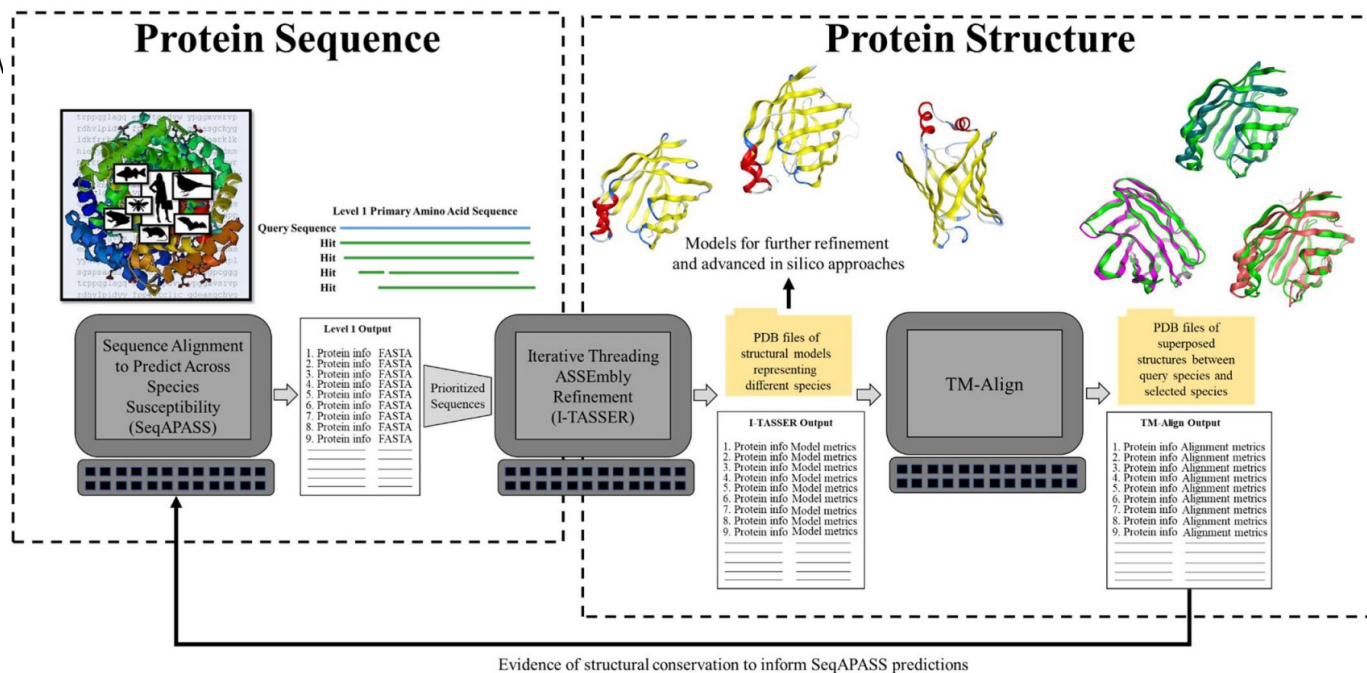
Which model organism for which purpose?

Which protein? Which PFAS?

- Humans **most sensitive species** for LFABP binding for many PFAS.
- Chicken, zebrafish, rainbow trout LFABP show similar affinity.
- Japanese medaka and fathead minnow proteins predicted to bind have **lower affinity for most PFAS**.
- **BUT:** all based on a single protein, and a static picture.

From Protein Sequence to Structure: The Next Frontier in Cross-Species Extrapolation for Chemical Safety Evaluations

Carlie A. LaLone,^{a,*} Donovan J. Blatz,^{a,b} Marissa A. Thomas R. Transue,^{g,h} Wilson Melendez,^h Audrey W

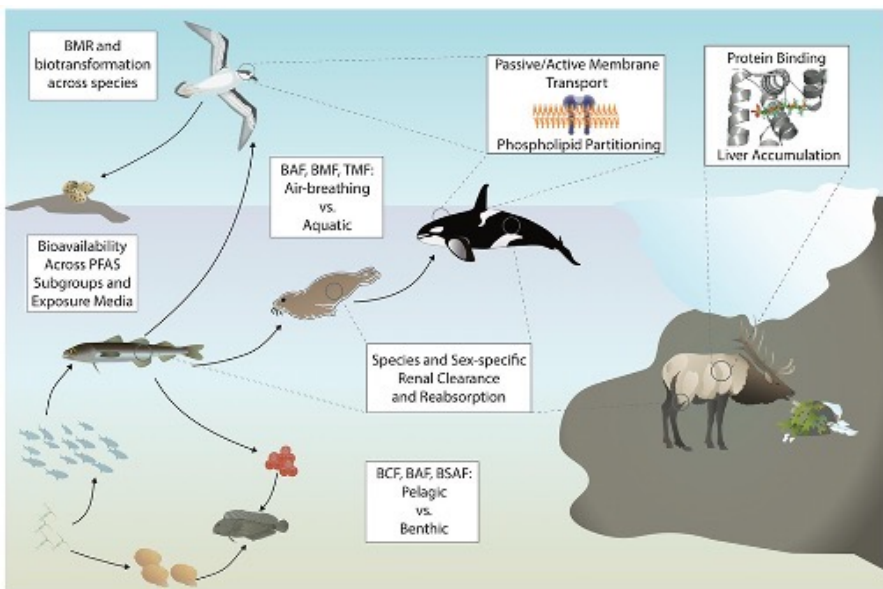


Finally: important to consider *dynamics*

Environmental Toxicology and Chemistry—Volume 40, Number 3—pp. 631–657, 2021

PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding

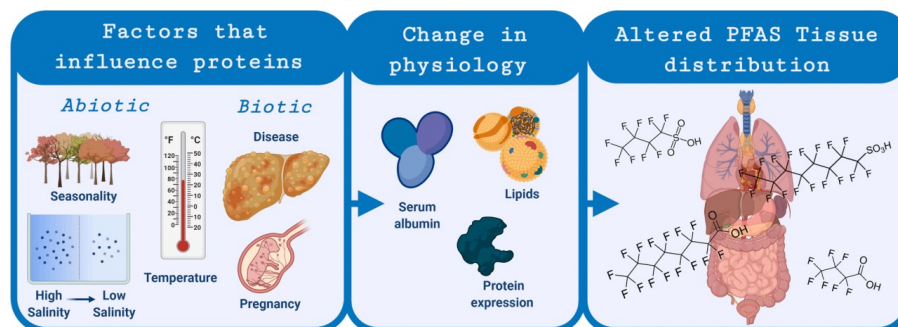
Amila O. De Silva,^a James M. Armitage,^b Thomas A. Bruton,^c Clifton Dassuncao,^d Wendy Heiger-Bernays,^e Xindi C. Hu,^f Anna Kärrman,^g Barry Kelly,^h Carla Ng,ⁱ Anna Robuck,^j Mei Sun,^k Thomas F. Webster,^e and Elsie M. Sunderland^{l,*}



Environment International 159 (2022) 107037

Understanding the dynamics of physiological changes, protein expression, and PFAS in wildlife

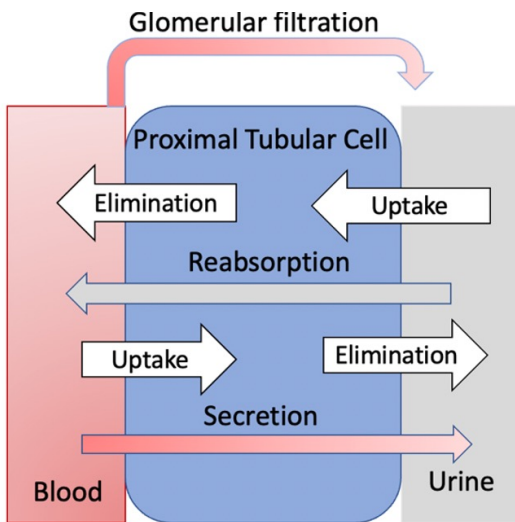
Jacqueline Bangma^{a,*}, T.C. Guillette^a, Paige A. Bommarito^b, Carla Ng^{c,d}, Jessica L. Reiner^e, Andrew B. Lindstrom^f, Mark J. Strynar^g



How do PFAS influence proteins, and how do proteins influence PFAS?

Inter-individual differences

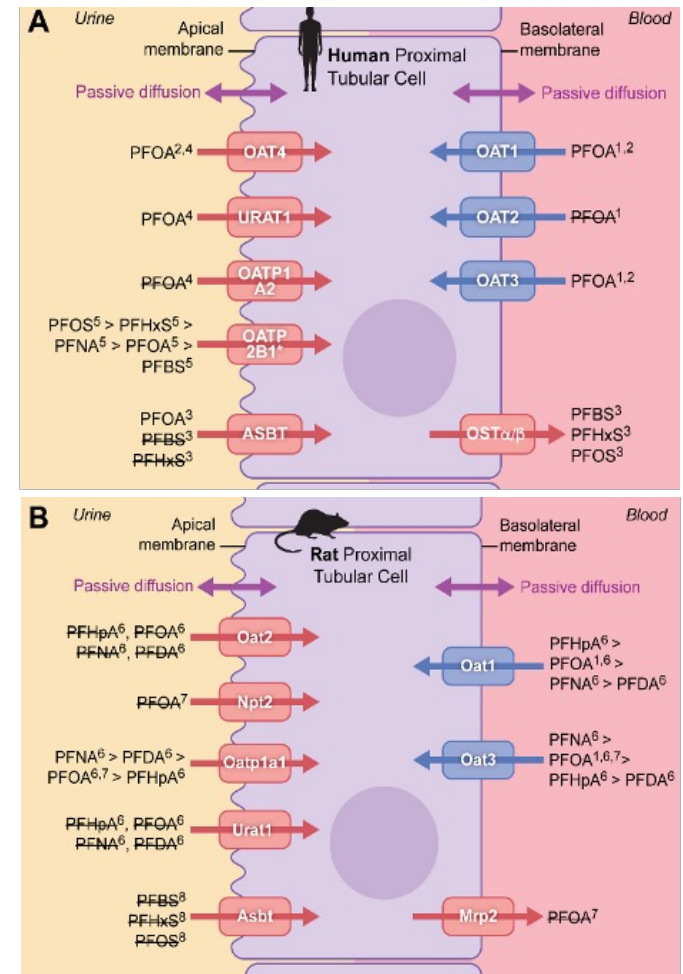
- the role of *dynamic kidney function*



Renal reabsorption is understood to contribute to the long half-life of PFOA in humans and to the sex differences observed between male and female rats.

But many more transporters and many more PFAS exist than have been tested.

And protein expression is dynamic.
 → *Reverse causation vs. causation.*

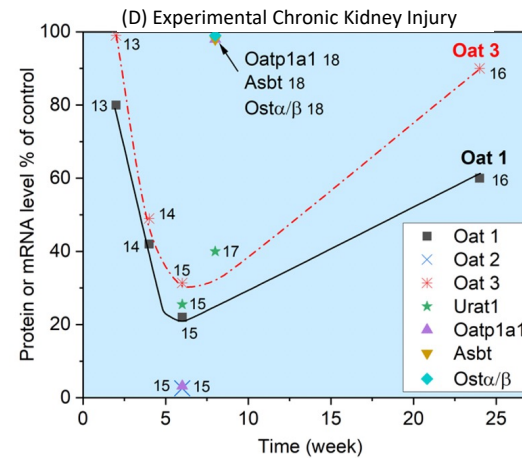
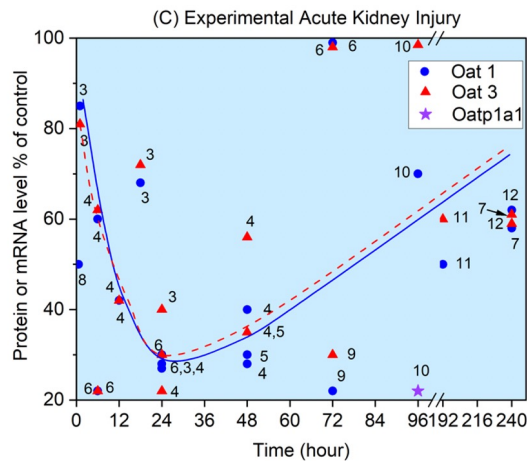
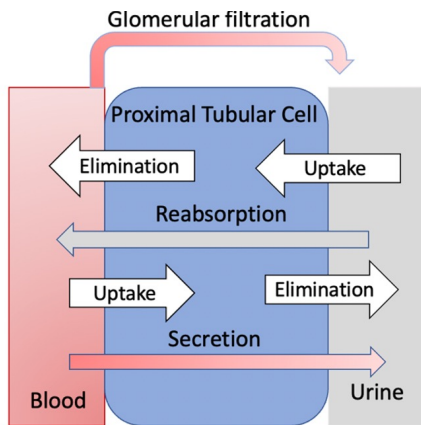


Shan Niu, Ducatman, Sanders and Ng, *in preparation*

Inter-individual differences

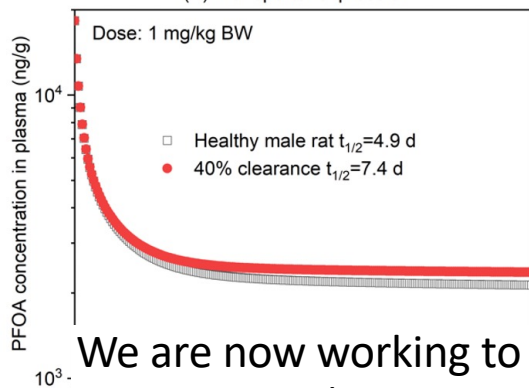
- the role of *dynamic kidney function*

Niu, Ducatman, Sanders and Ng, *in preparation*

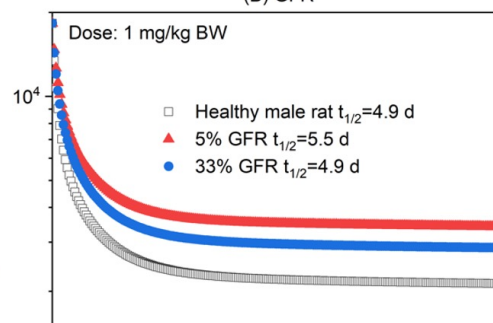


Selected protein level changes for largest difference from normal function (minimum expression level in this case).

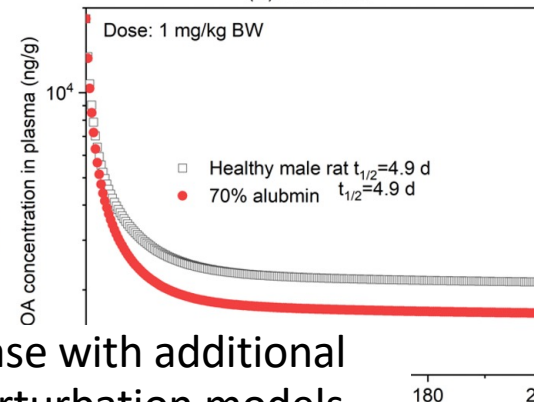
(A) Transporter expression



(B) GFR



(C) Albuminuria



We are now working to build out this knowledge base with additional transporters (OAT1, OAT4, PgP), other PFAS, and perturbation models.

Depending on which part of kidney function is disrupted, PFOA half-life can increase or decrease.

Niu, Ducatman, Sanders and Ng, *in preparation*

Tracking Regional PFAS Contamination

Part of McKeesport under water advisory

July 19, 2021 Mon Valley Independent Latest News



Friday's fire at McKeesport Auto Body has resulted in a water advisory being issued for a portion of the city.

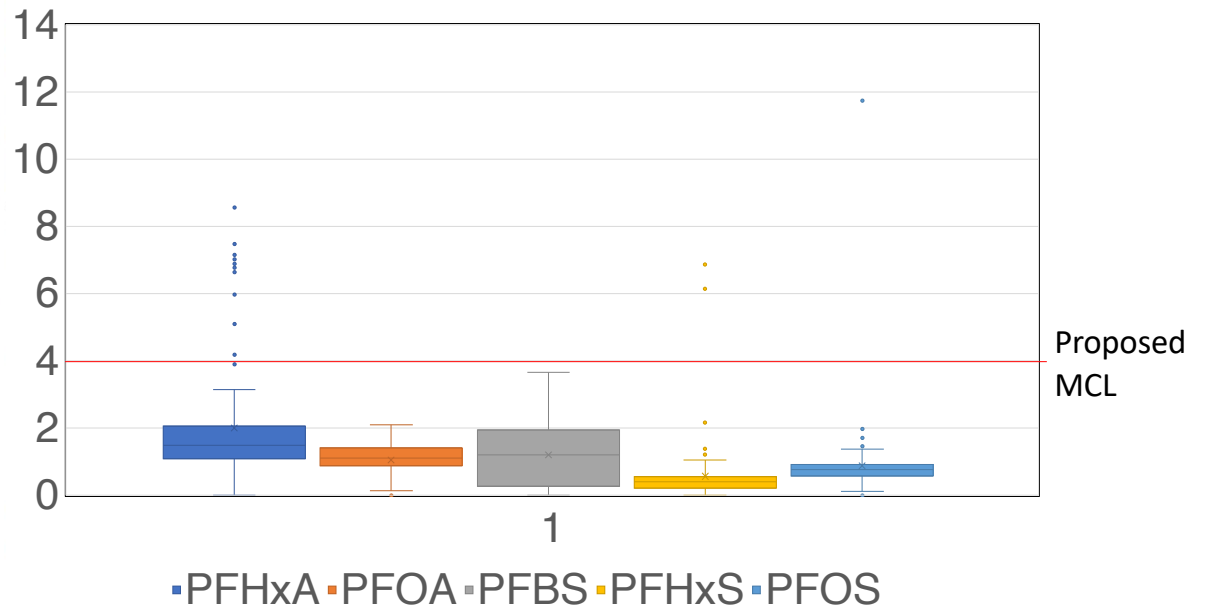
Facebook 0 Tweet 0

By TAYLOR BROWN

tbrown@yourmvi.com

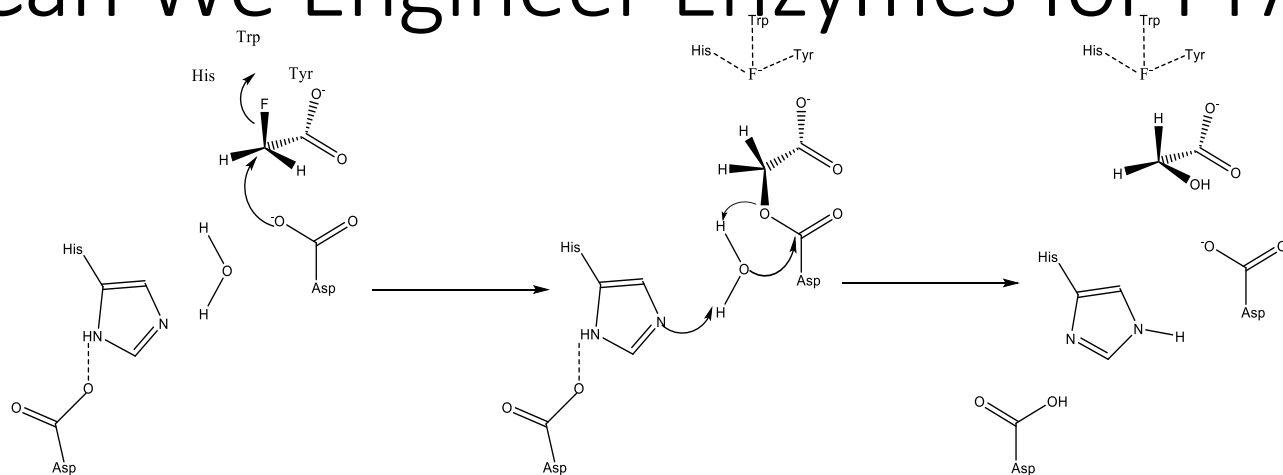
Residents of the Lower 10th Ward in McKeesport are being asked not to use their tap water as a result of a fire in the city Friday.

Drinking Water Results December 2022



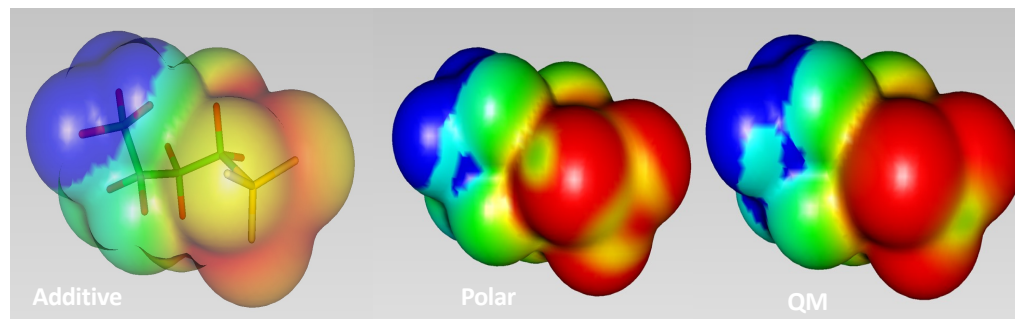
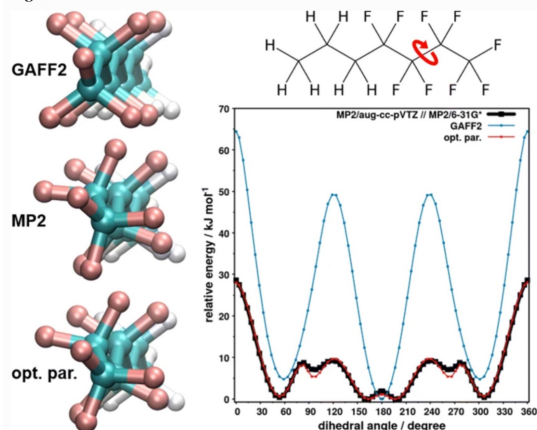
17 Months after the fire drinking water samples were down to background levels. Project has turned to investigating environmental impacts of hydrant flushing.

Can We Engineer Enzymes for PFAS Destruction?



The defluorination pathway of fluoroacetate by FAcD, one of the few known natural defluorination pathways with an identified enzyme.

Fig. 1



1.Träg, J. & Zahn, D. Improved GAFF2 parameters for fluorinated alkanes and mixed hydro- and fluorocarbons. *J Mol Model* **25**, 39 (2019).

Questions?

carla.ng@pitt.edu

[@Ng_lab](#)

