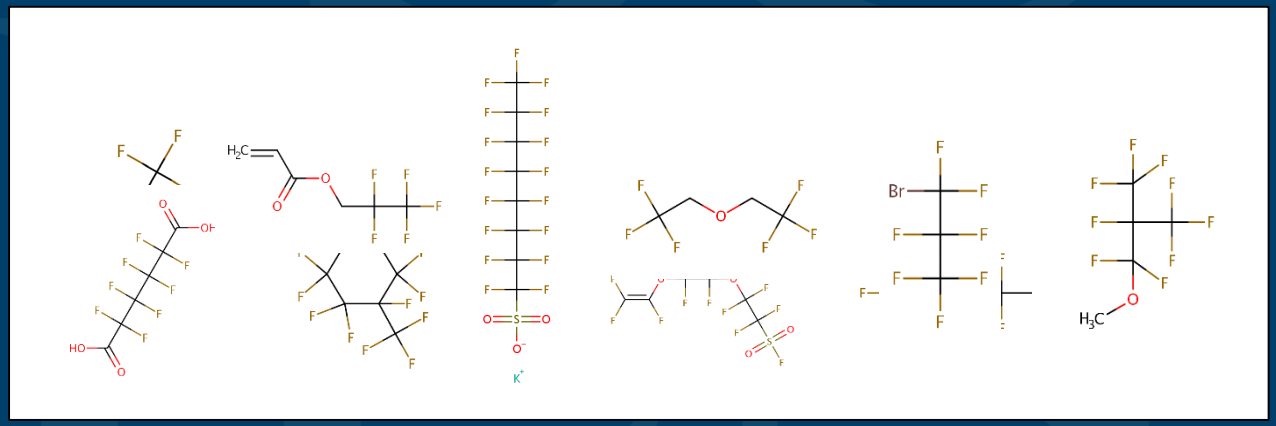
EPA
United States
Environmental Protection
Agency

Application of High-Throughput Toxicokinetics in the Assessment of PFAS



2020 ToxForum Winter Meeting
January 27, 2020

Barbara Wetmore / John Wambaugh
Center for Computational Toxicology and Exposure
Office of Research and Development

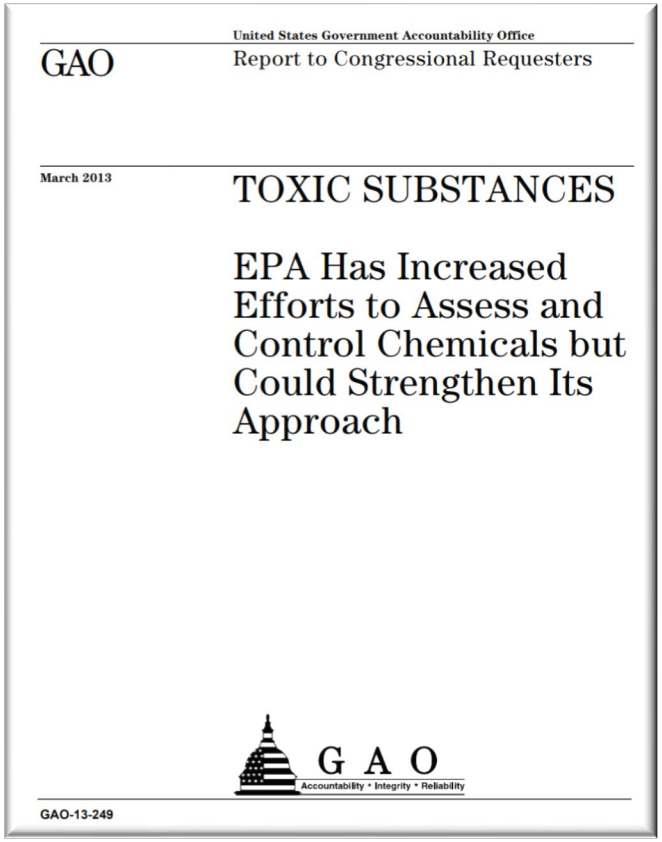
Chemical Regulation in the United States
- Prior to 2016 -

- Chemical safety testing is primarily for food additives, pharmaceuticals, and pesticide active ingredients (NRC, 2007)

- Most other chemicals, ranging from industrial waste to dyes to packing materials, are covered by the Toxic Substances Control Act (TSCA) which is administered by the EPA

"Tens of thousands of chemicals are listed with the Environmental Protection Agency (EPA) for commercial use in the United States, with an average of 600 new chemicals listed each year."
U.S. Government Accountability Office

- Thousands of chemicals on the market were "grandfathered" in without assessment
  Judson et al. (2009), Egeghy et al. (2012), Wetmore et al. (2015)
  Chemical Safety Assessments primarily

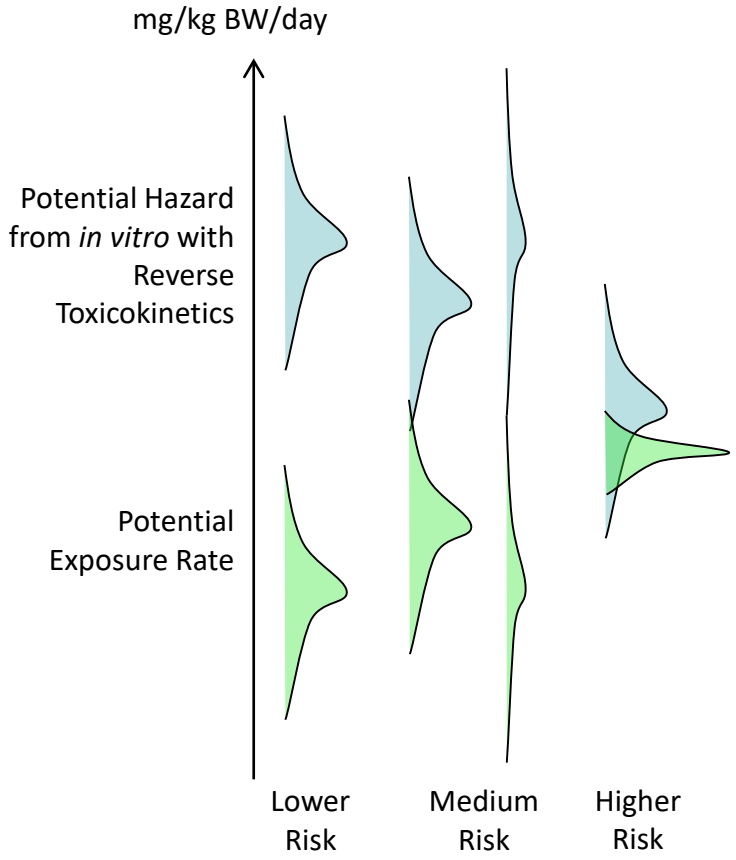


March, 2013

New Approach Methodologies (NAMs) to Inform Risk-based Assessments

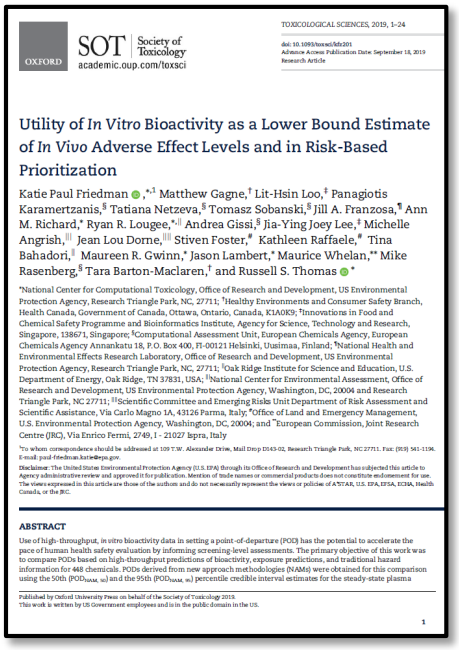
-*Responsive to Lautenberg Chemical Safety Act of 2016* -

- The U.S. National Research Council (1983) identified chemical risk as a function of both inherent hazard and exposure
- Addressing thousands of chemicals requires "new approach methodologies" (NAMs*):
  1. High throughput hazard characterization (Dix et al., 2007, Collins et al., 2008)
  2. High throughput exposure forecasts (Wambaugh et al., 2013, 2014)
  3. High throughput toxicokinetics (i.e., dose-response relationship) linking hazard and exposure (Wetmore et al., 2012, 2015)



*Kavlock et al. (2018)

In Vitro Bioactivity, HTTK, and *In Vivo* Toxic Doses



TOXICOLOGICAL SCIENCES, 2019, 1–24

doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman ,*,1 Matthew Gagne,† Lit-Hsin Loo,‡ Panagiotis Karamertzanis,§ Tatiana Netzeva,§ Tomasz Sobanski,§ Jill A. Franzosa,¶ Ann M. Richard,* Ryan R. Lougee,*,|| Andrea Gissi,§ Jia-Ying Joey Lee,‡ Michelle Angrish,|| Jean Lou Dorne,|| Stiven Foster,# Kathleen Raffaele,# Tina Bahadori,# Maureen R. Gwinn,* Jason Lambert,* Maurice Whelan,** Mike Rasenberg,§ Tara Barton-Maclaren,† and Russell S. Thomas *

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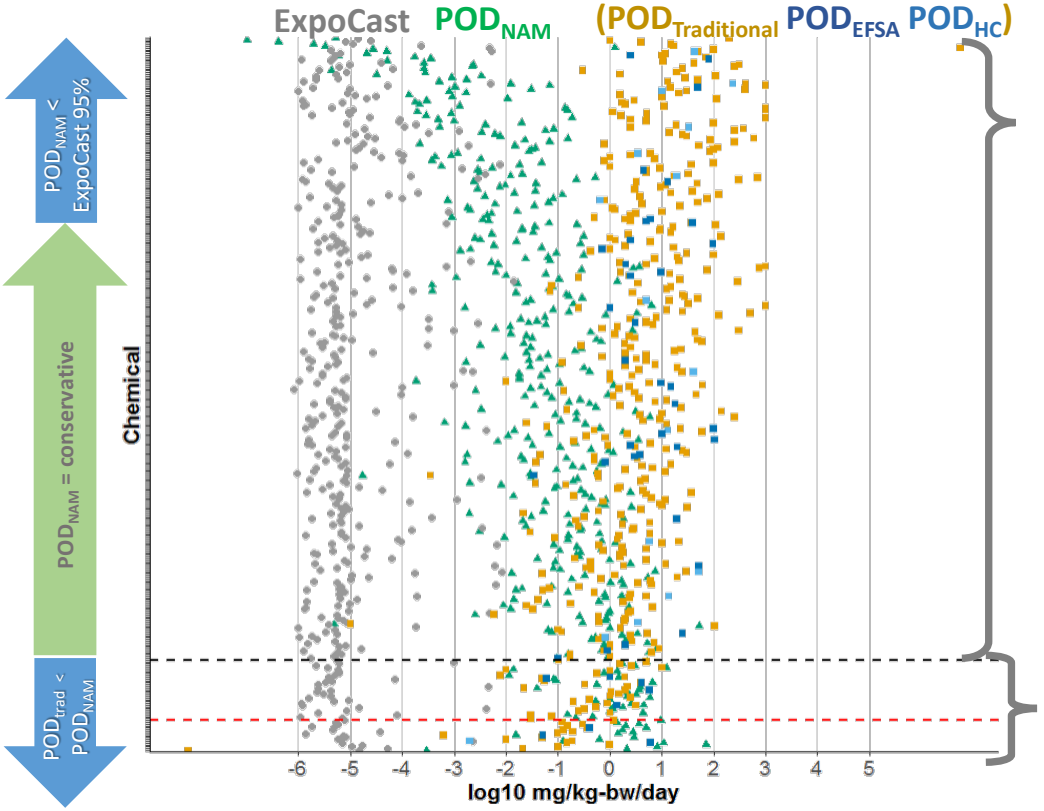
Disclaimer: The United States Environmental Protection Agency (U.S. EPA) through its Office of Research and Development has subjected this article to Agency administrative review and approved it for publication. Mention of trade names or commercial products does not constitute endorsement for use. The views expressed in this article are those of the authors and do not necessarily represent the views or policies of ASTAR, U.S. EPA, EFSA, ECHA, Health Canada, or the JRC.

ABSTRACT

Use of high-throughput, *in vitro* bioactivity data in setting a point-of-departure (POD) has the potential to accelerate the pace of human health safety evaluation by informing screening-level assessments. The primary objective of this work was to compare PODs based on high-throughput predictions of bioactivity, exposure predictions, and traditional hazard information for 448 chemicals. PODs derived from new approach methodologies (NAMs) were obtained for this comparison using the 50th ($POD_{NAM,\ 50}$) and the 95th ($POD_{NAM,\ 95}$) percentile credible interval estimates for the steady-state plasma

International case study with EPA, ASTAR, ECHA, Health Canada, and EFSA

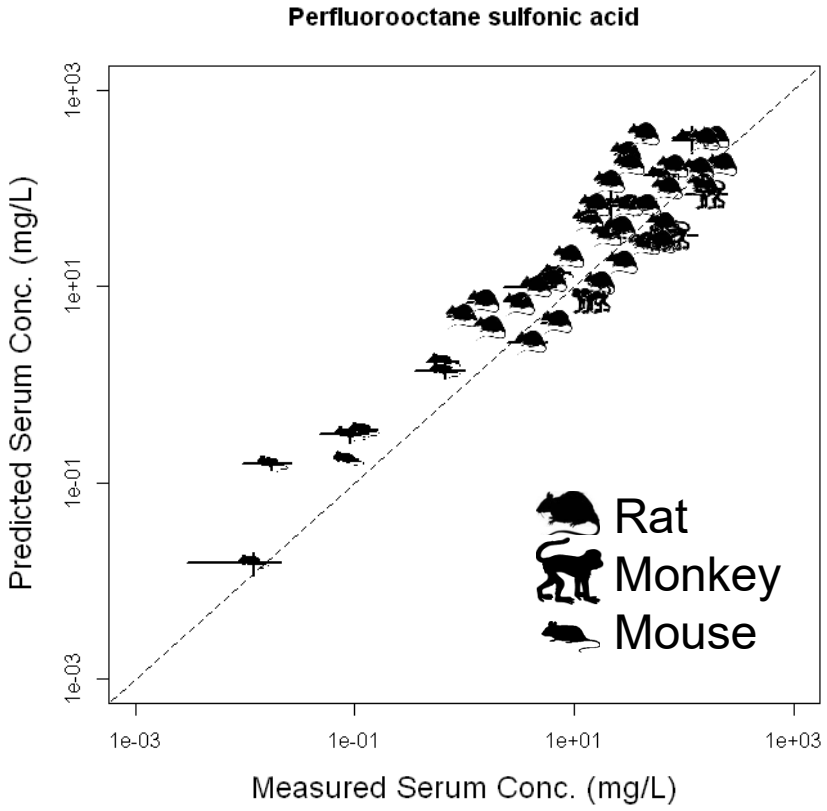


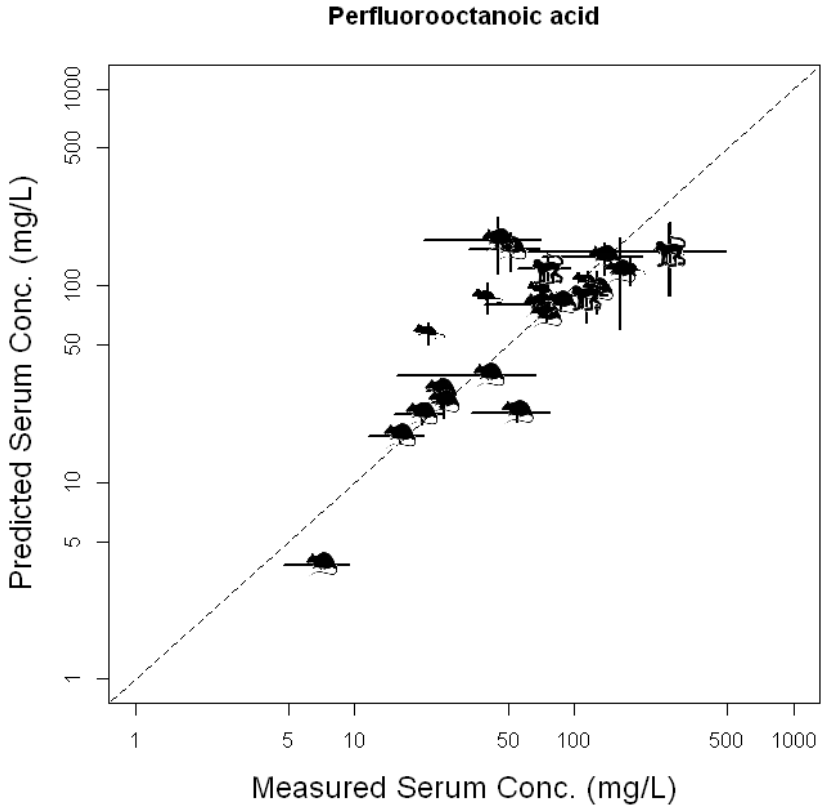
For ~89% of the chemicals, POD_{NAM} was conservative.

(~100-fold on average), but less conservative than a TTC

Chemicals where POD_{NAM} was not conservative enriched in OPs/carbamates

Toxicokinetic Modeling - PFAS

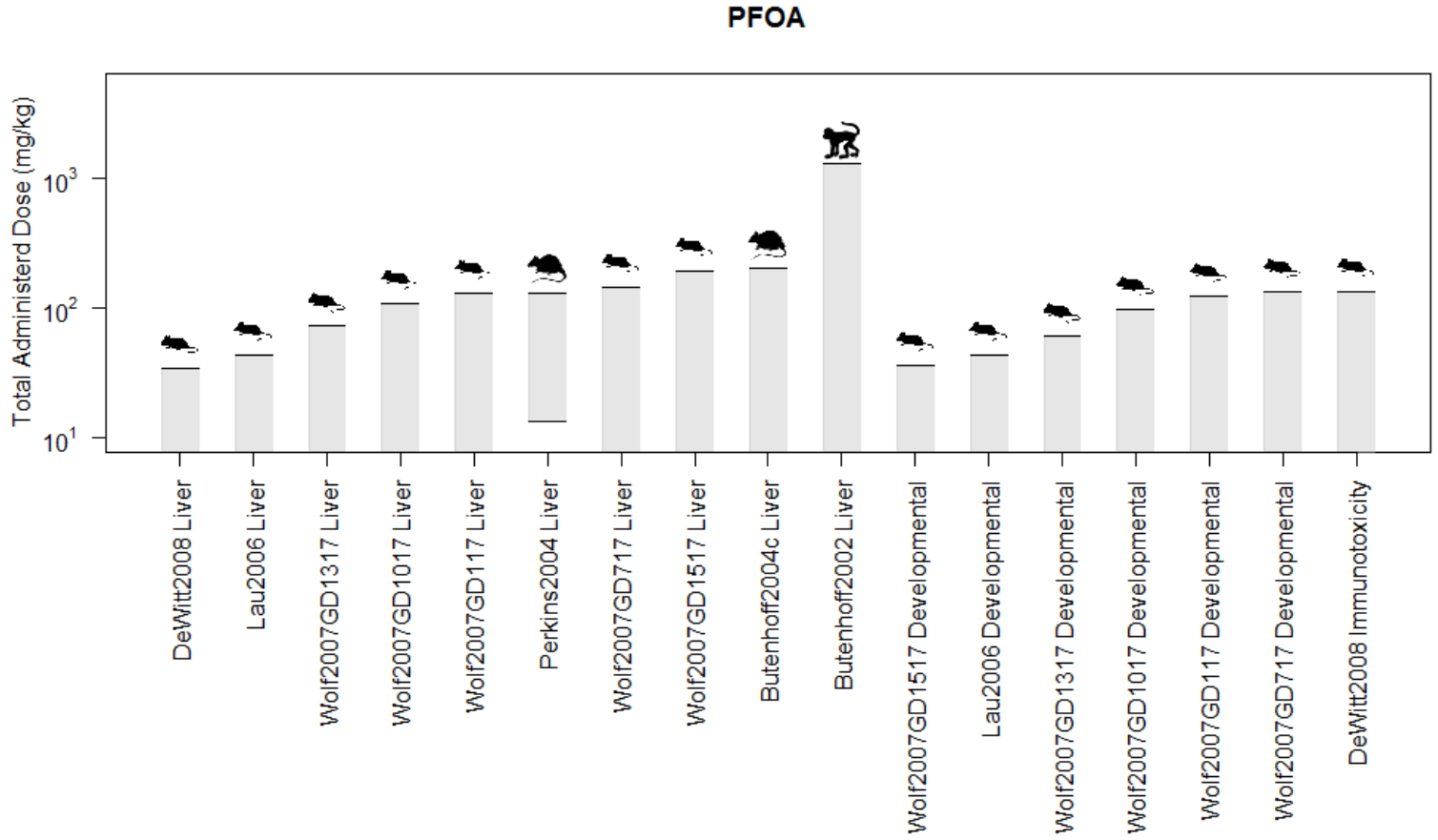




If we have sufficient data, TK modeling is informative

Wambaugh *et al.* (2013)

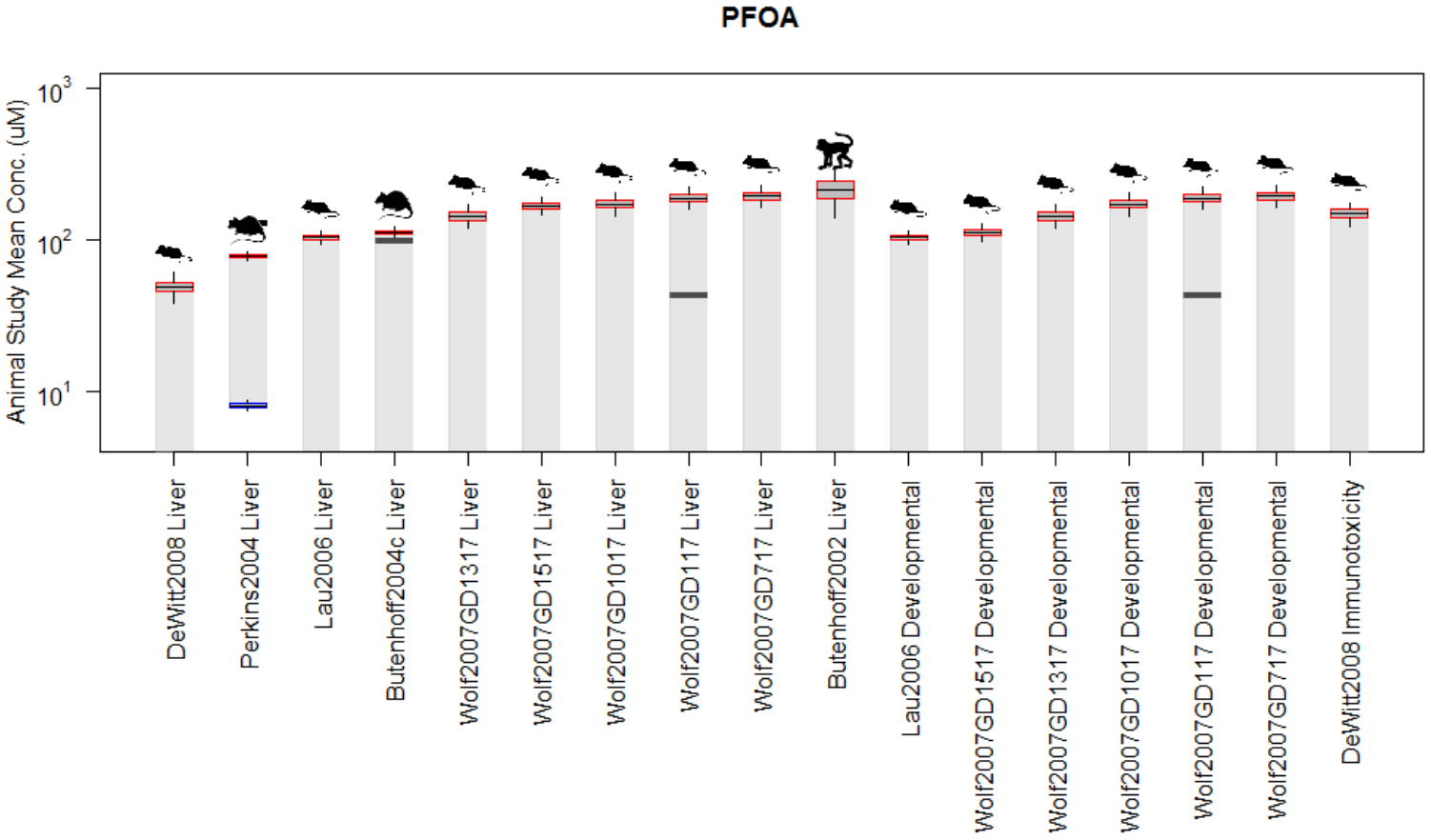
Contribution of Toxicokinetics
- *Data Interpretation* -



Differences in species and dosing regimen can create apparent differences in doses needed to produce adverse effects across in vivo studies

Wambaugh *et al.* (2013)

Contribution of Toxicokinetics
- *Data Interpretation* -



PK modeling of tissue concentrations can reconcile study-specific differences and better align findings across multiple in vivo studies

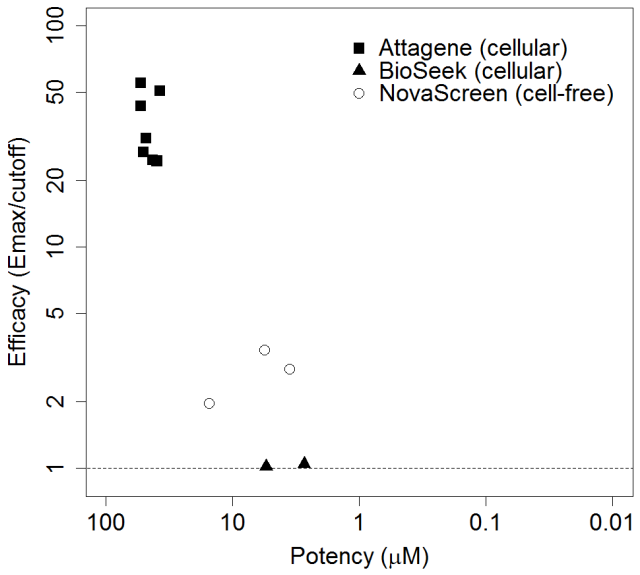
Wambaugh *et al.* (2013)

In Vitro – In Vivo Concordance

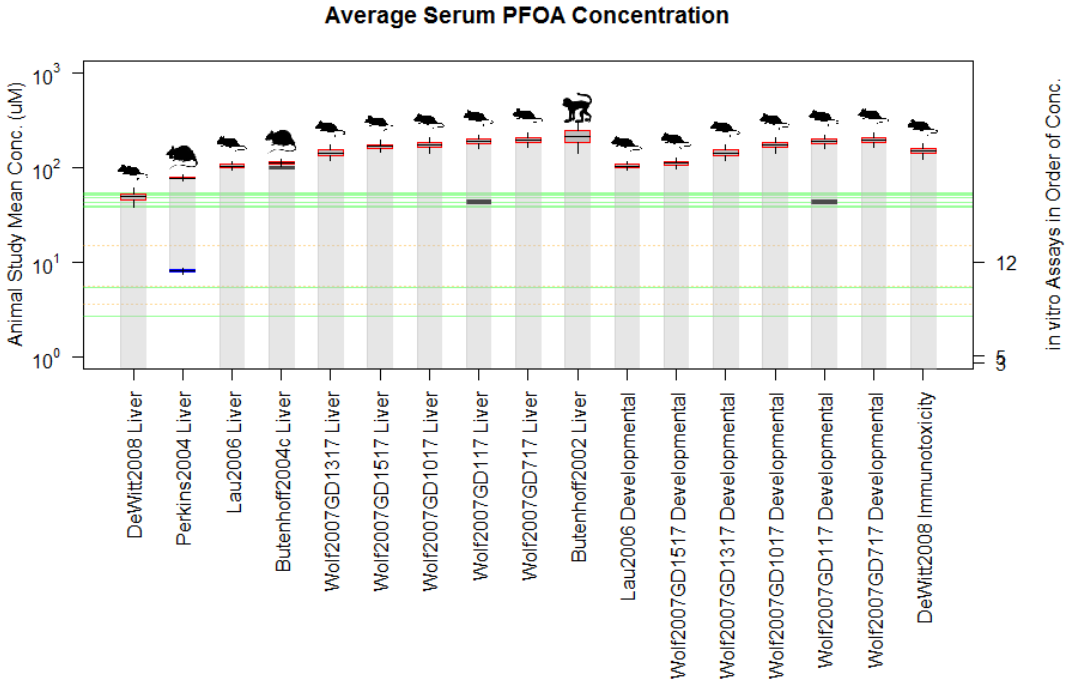
United States Environmental Protection Agency

Overlay of I*n Vivo* Serum PFOA Levels with *in Vitro Bioactivity*

ToxCast HTS



Peer-reviewed *In Vivo* Studies



LOEL/NOEL (credible interval) = red/blue box and whiskers
Green and yellow lines – *in vitro* bioactivity
Black boxes = benchmark doses (3 studies only)

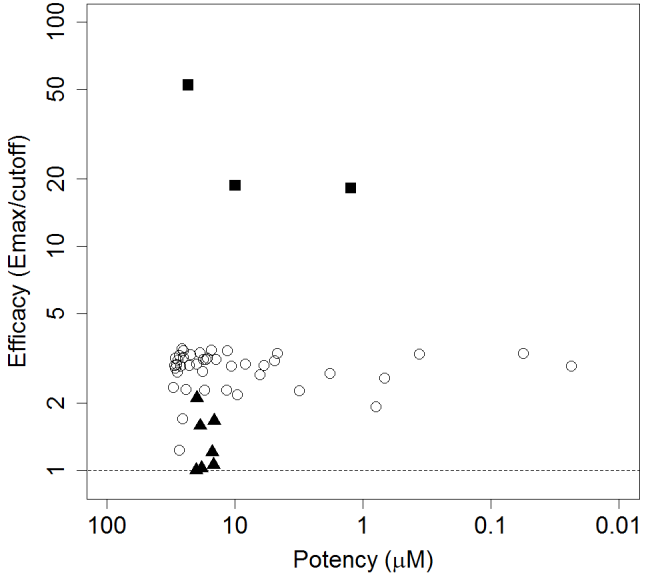
Wambaugh *et al.* (2013)

In Vitro – In Vivo Concordance

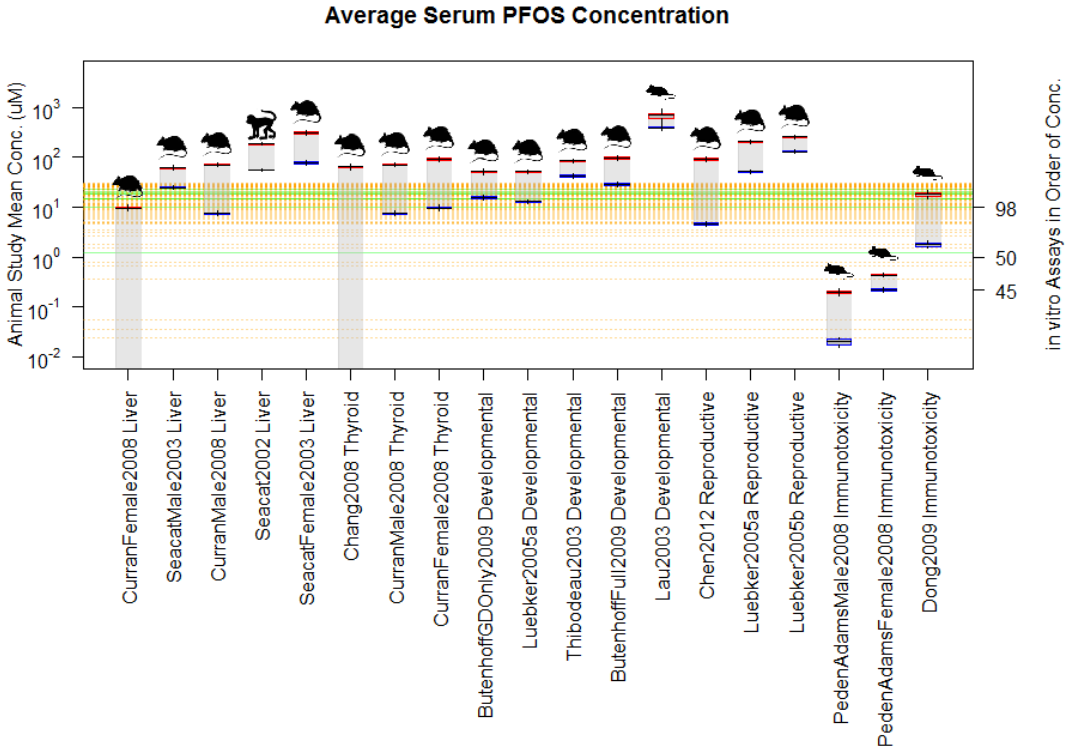
United States Environmental Protection Agency

Overlay of *In Vivo* Serum PFOS Levels with *in Vitro Bioactivity*

ToxCast HTS



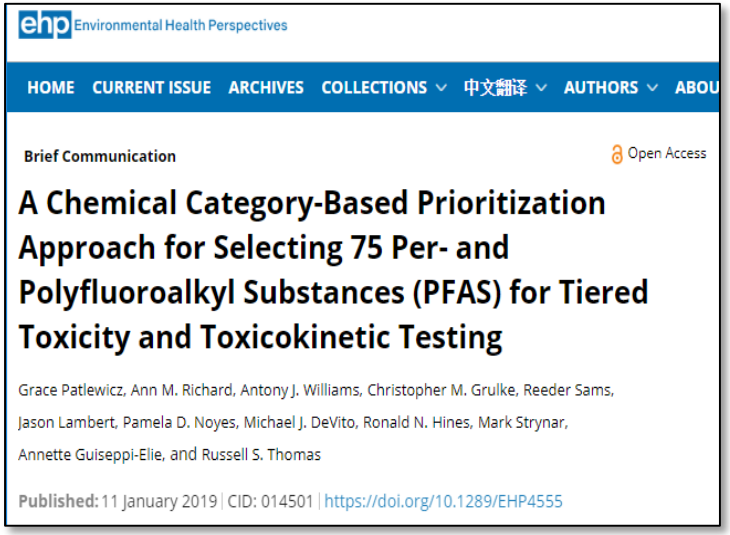
Peer-reviewed *In Vivo* Studies



LOEL/NOEL (credible interval) = red/blue box and whiskers
Green/yellow lines – cell based/cell-free *in vitro* bioactivity

Wambaugh *et al.* (2013)

Selecting a Subset of PFAS for Tiered Toxicity and Toxicokinetic Testing



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Brief Communication

Open Access

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

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

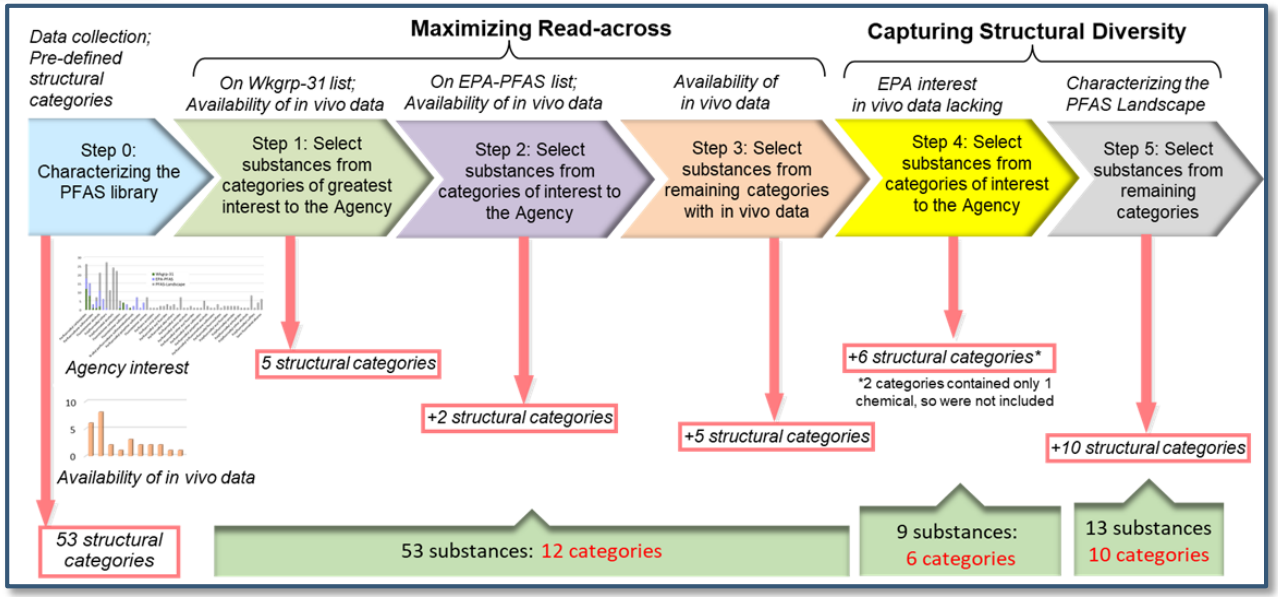
Published: 11 January 2019 | CID: 014501 | https://doi.org/10.1289/EHP4555

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



In Vitro Toxicity and Toxicokinetic Testing

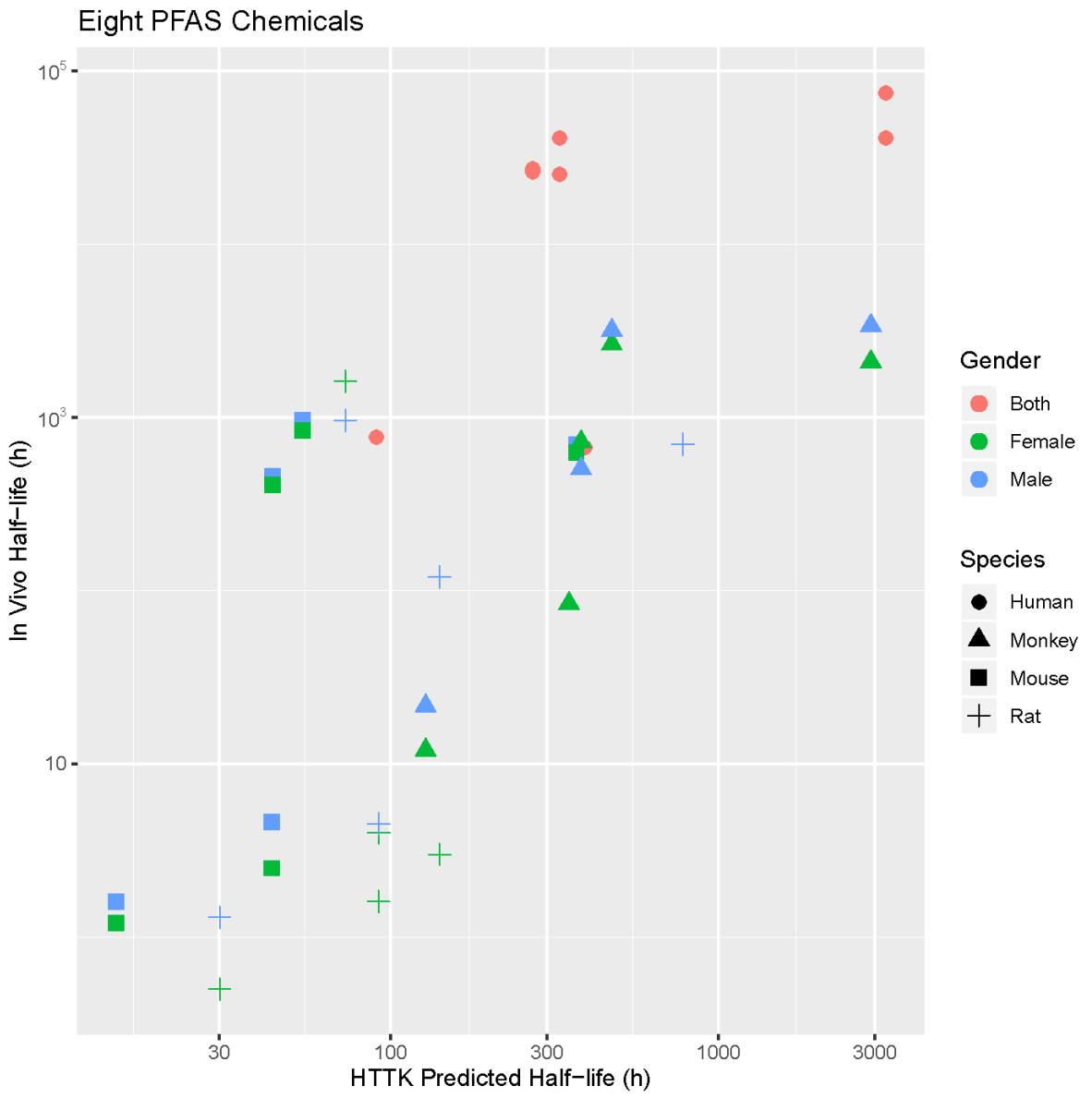
Toxicological Response	Assay	Assay Endpoints	Purpose
Hepatotoxicity	3D HepaRG assay	Cell death and transcriptomics	Measure cell death and changes in important biological pathways
Developmental Toxicity	Zebrafish embryo assay	Fertilization, lethality, and structural defects	Assess potential teratogenicity
Immunotoxicity	Bioseek Diversity Plus	Protein biomarkers across multiple primary cell types	Measure potential disease and immune responses
Mitochondrial Toxicity	Mitochondrial membrane potential and respiration (HepaRG)	Mitochondrial membrane potential and oxygen consumption	Measure mitochondrial health and function
Developmental Neurotoxicity	Microelectrode array assay (rat primary neurons)	Neuronal electrical activity	Impacts on neuron function
Endocrine Disruption	ACEA real-time cell proliferation assay (T47D)	Cell proliferation	Measure ER activity
General Toxicity	Attagene cis- and trans- Factorial assay (HepG2)	Nuclear receptor and transcription factor activation	Activation of key receptors and transcription factors involved in hepatotoxicity
	High-throughput transcriptomic assay (multiple cell types)	Cellular mRNA	Measures changes in important biological pathways
	High-throughput phenotypic profiling (multiple cell types)	Nuclear, endoplasmic reticulum, nucleoli, golgi, plasma membrane, cytoskeleton, and mitochondria morphology	Changes in cellular organelles and general morphology
Toxicokinetic Parameter	**Assay**	**Assay Endpoints**	**Purpose**
Intrinsic hepatic clearance	Hepatocyte stability assay (primary human hepatocytes)	Time course metabolism of parent chemical	Measure metabolic breakdown by the liver
Plasma protein binding	Ultracentrifugation assay	Fraction of chemical not bound to plasma protein	Measure amount of free chemical in the blood

*Assays being performed by NTP and EPA

In Vitro-In Vivo Concordance
In Vivo Half-Lives vs. HTTK – PFAS

Cross-Species and Cross-Gender Comparisons Possible

QSAR to predict $T_{1/2}$ bins holds potential for data-poor PFAS



PFAS Method Development & Stock Assessment

PFAS Stock assessment to verify stability, quality

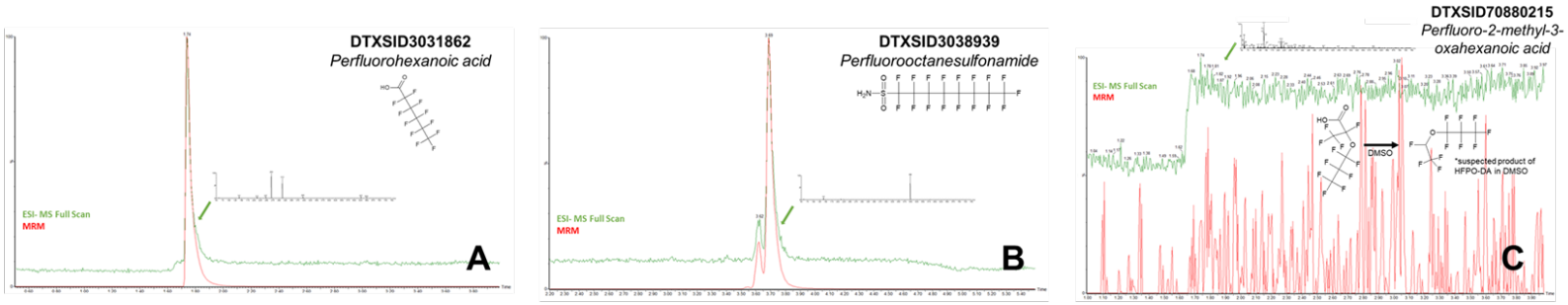


Figure 4. Comparison of 30 mM PFAS stocks in DMSO.
A: PFHxA; B: PFOSA; C: HFPO-DA

Over 50 PFAS detected in a single method; Most with LODs ≤ 0.05 pg/µL

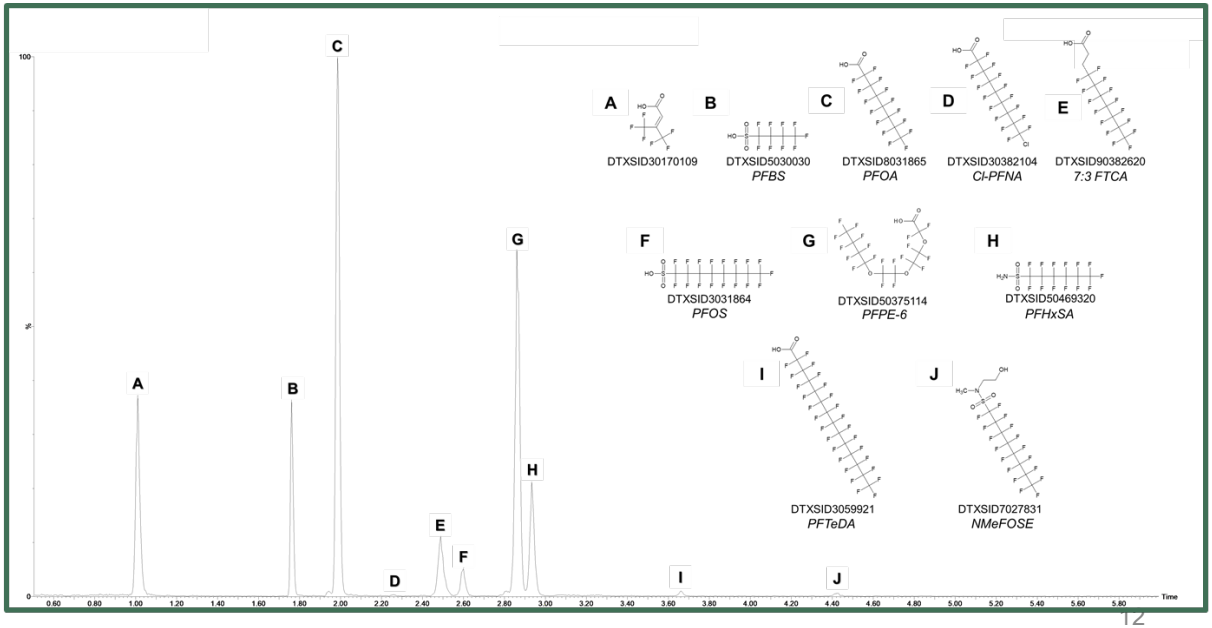
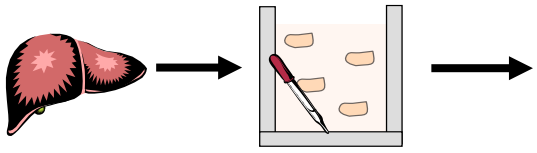
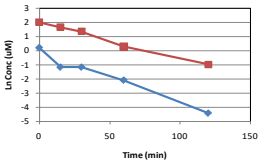


Figure 3. Total Ion Chromatogram of a 10 pg/µL Mixture of Ten PFAS of Different Classes.

In Vitro Toxicokinetic Assays and *In Vitro-In Vivo* Extrapolation



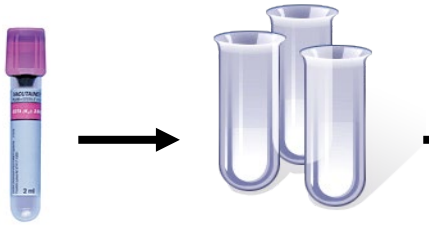
Hepatocytes



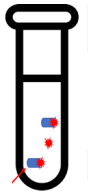
Hepatic Clearance (Cl_{int})

IVIVE

Relationship Between Administered Dose and Blood Concentration at Steady State



Plasma



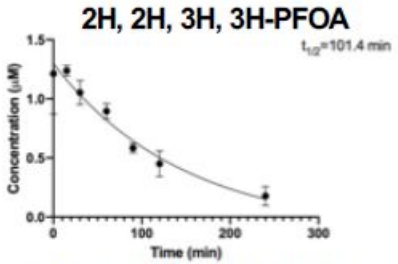
Plasma Protein Binding (F_u)

+ additional TK parameters (e.g., renal; in progress)

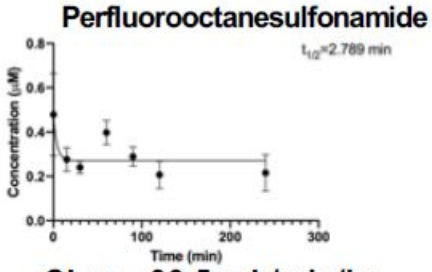
Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012
Wetmore *et al., Tox Sci.,* 2015
Smeltz *et al.*, in preparation

David Crizer
Michael DeVito
Marci Smeltz

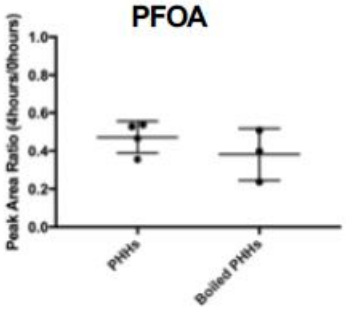
In Vitro Hepatic Clearance – Half-Life Assessments

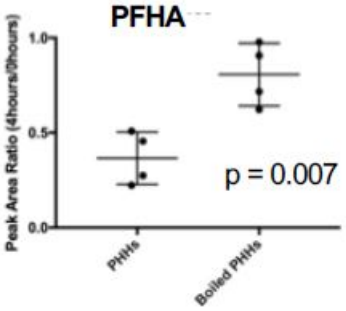


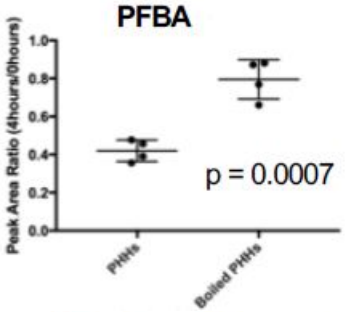
CL_{int} = 0.9 mL/min/kg



CL_{int} = 33.5 mL/min/kg

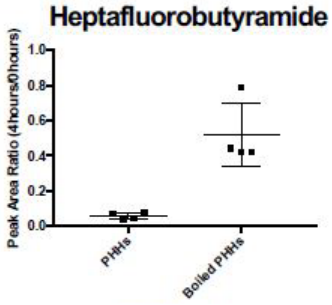


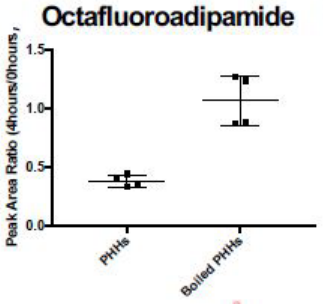


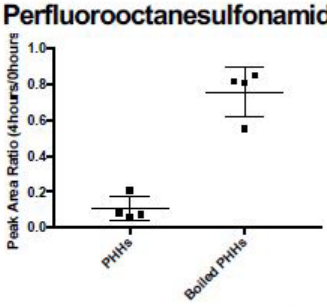


PHHs = primary human hepatocytes

PFAS - Amides





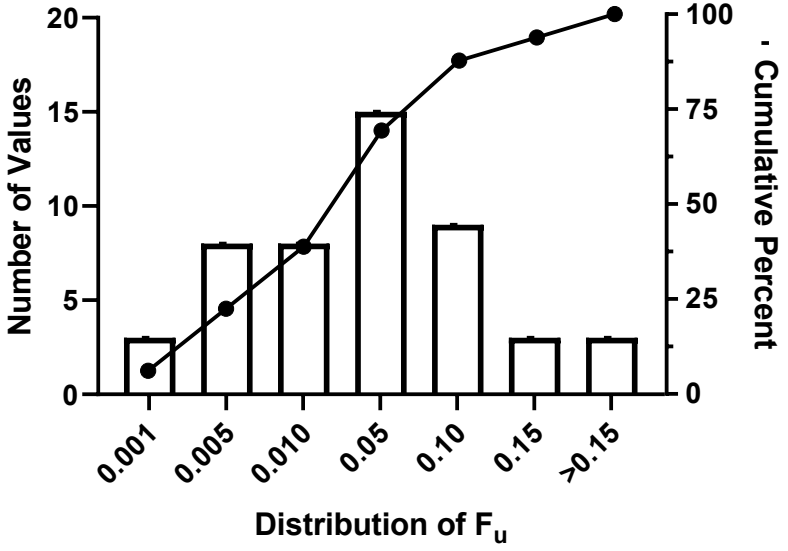


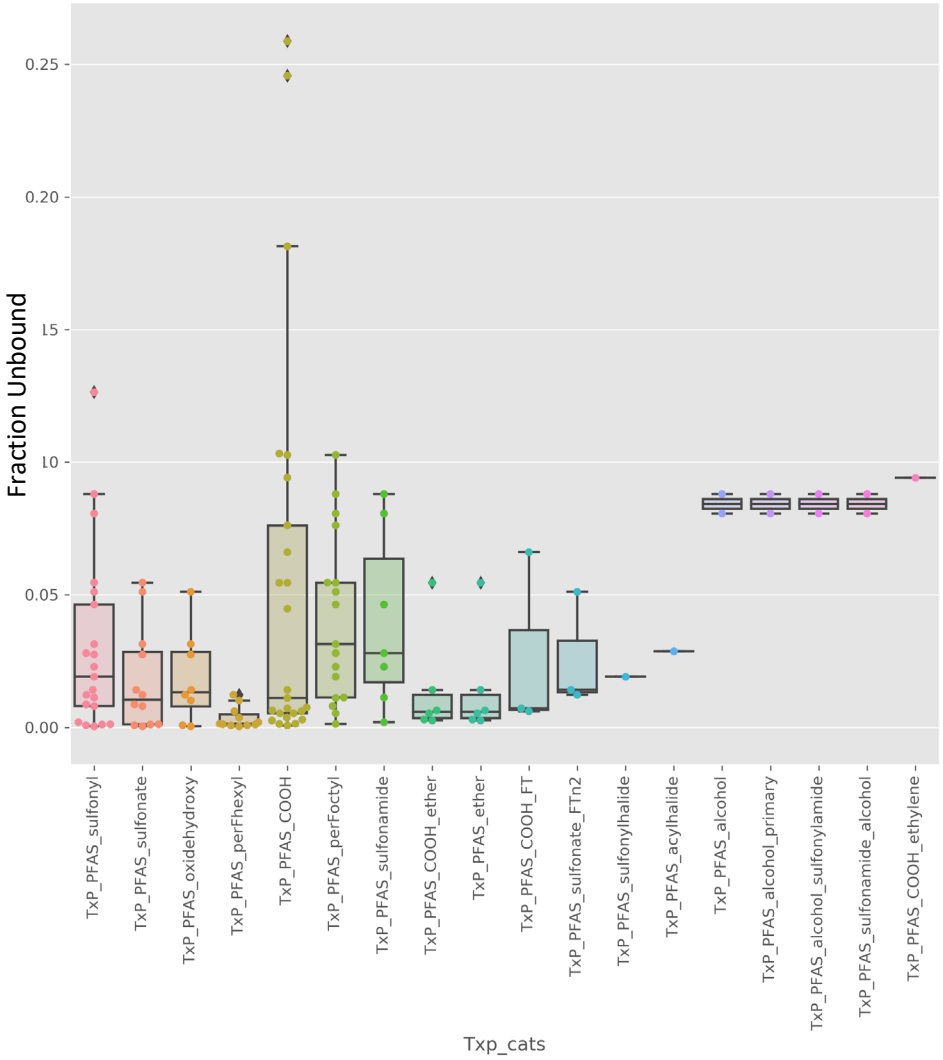
Chain length/functional groups associated with differing metabolic stabilities

Preliminary Category-Based Analyses of Toxicokinetic Data

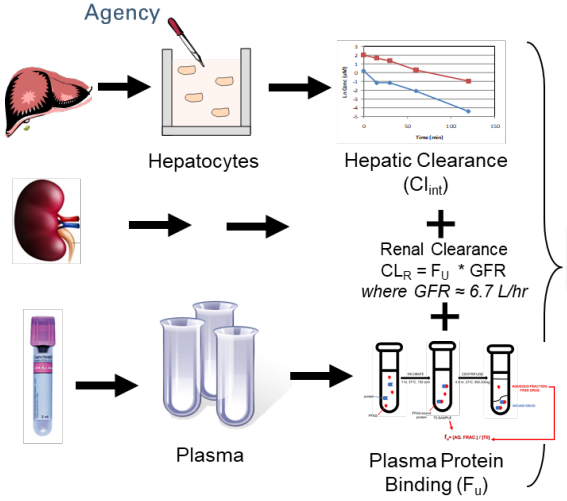
Preliminary set: Plasma protein binding data across 50+ PFAS

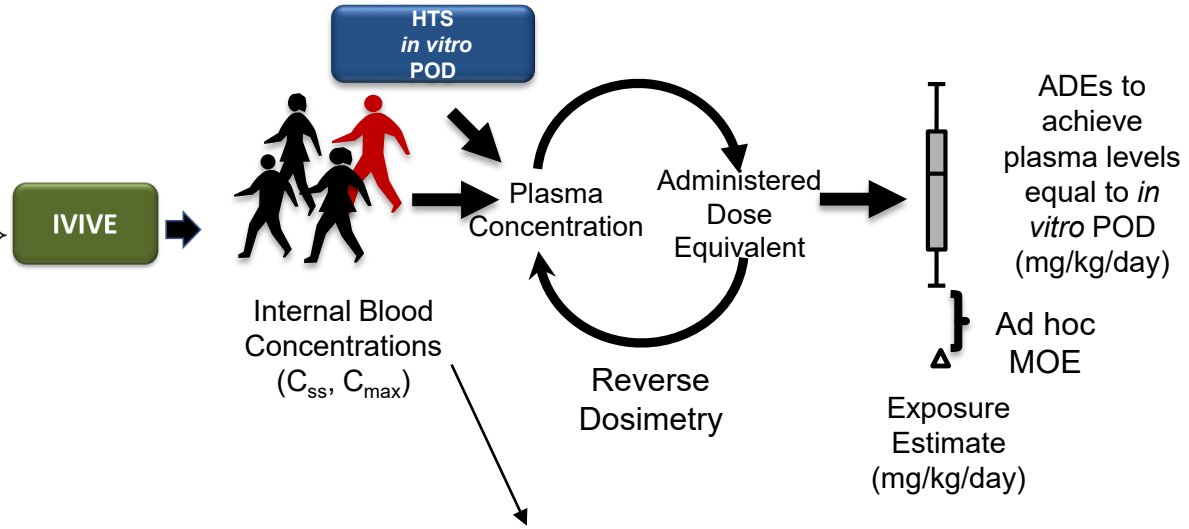
75% of PFAS: F_u<0.05





In Vitro-In Vivo Extrapolation

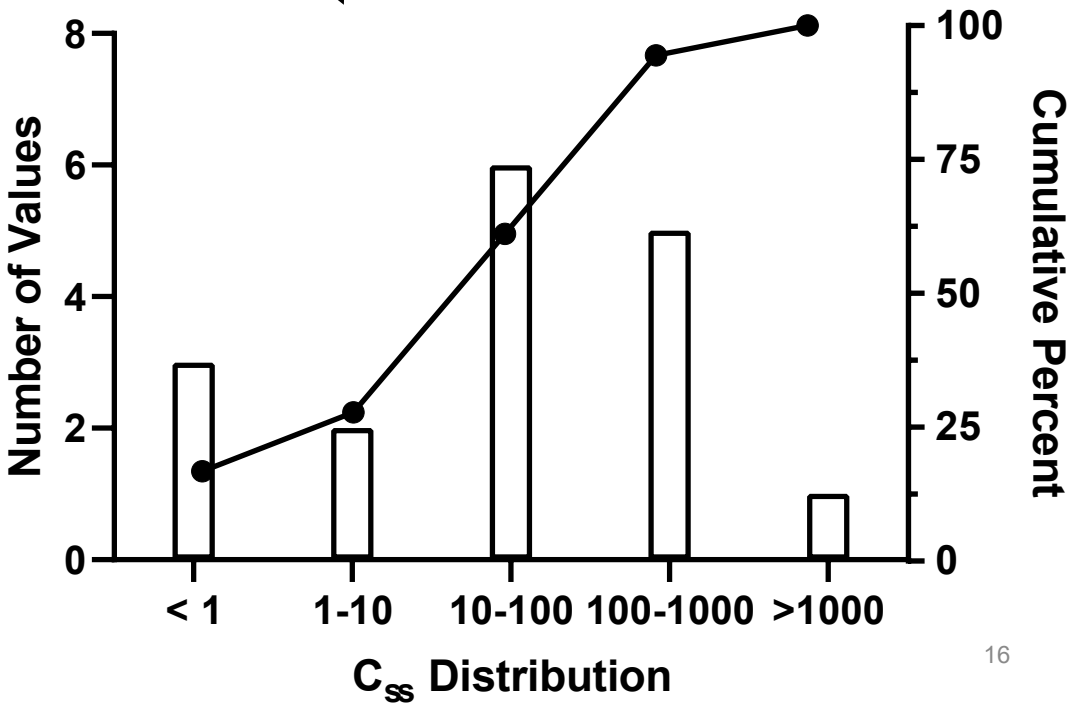




Preliminary IVIVE Modeling Across 17 PFAS

Steady-state blood concentrations (C_{ss}) predicted for adult population

Patterns are emerging that may be informative in predicting PFAS TK



Future Plans – PFAS Toxicokinetic Modeling

Method	Species-Specific Data	Species- and Chemical-Specific Data	Evaluation	Dosimetric Anchoring	Route Extrapolation	Interspecies Extrapolation	Inter-chemical Extrapolation	Number of PFAS Addressed
Empirical Toxicokinetics	Model animal tissue concentration vs. time (cvt) data (Sayre *et al.*)		Bayesian analysis using diffuse priors	Yes	No	No	No	~10
Physiologically-based Toxicokinetics	Physiology	Animal cvt data plus epidemiologically-observed human half-lives	Qualitative	Yes	Yes	No	No	2
HTTK	Physiology	*In vitro* plasma protein binding and hepatic clearance	R^2 ~ 0.8 for PFAS, four species	Yes	Yes	Yes	No	~60
Machine Learning	Physiology	Structure, QSPR-predicted phys-chem and HTTK		No	Maybe?	Yes	Yes	Hundreds

Sayre *et al.*, Database of pharmacokinetic time-series data
And parameters for 144 environmental chemicals. *Submitted*

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