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

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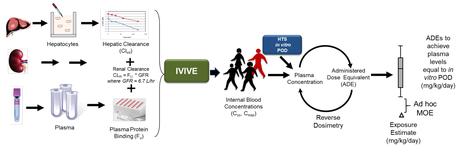
Progress for a Stronger Future

Background

New approach methodologies (NAMs) make use of *in vitro* high-throughput screening (HTS) data and *in silico* approaches to inform chemical safety assessment through the translation of bioactive *in vitro* assay concentrations to administered dose equivalents. This approach utilizes *in vitro* point of departure information from HTS studies where *in vitro* toxicokinetic (TK) data is applied to assigned administered dose equivalents, and to compare exposure estimates to define *ad hoc* margin of exposure.

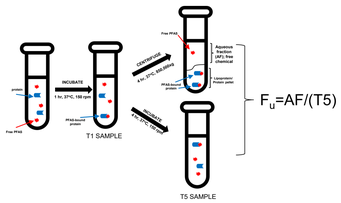


in vitro TK data and modeling → *in vivo* concentrations → ad hoc MOE

Per- and polyfluoroalkyl substances (PFAS) have become chemicals of concern for human health as more is learned about their widespread presence and persistence in the environment. Given the inclusion of 1,220 PFAS on the Toxic Substances Control Act (TSCA) inventory, the availability of *in vivo* toxicologic data and exposure information on only a subset is inadequate to provide an understanding of the potential exposures, TK, and toxicities across this structurally diverse domain. To address this deficiency, we are applying NAMs to a panel of PFAS, containing carboxylic acid, sulfonate, and ether functionalities, for use in IVIVE models to predict systemic concentrations and eventual application in read-across approaches.

In Vitro Toxicokinetic Assays

ULTRACENTRIFUGATION PLASMA PROTEIN BINDING ASSAY

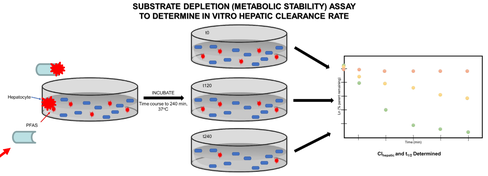


F_u=AF/(T5)

- Human plasma (10-donor pool, mixed sex) centrifuged at 850,000x*g* to separate aqueous fraction from albumin, lipoproteins, and fatty acids
- Analyte quantitation (multiple reaction monitoring, MRM) for aqueous fraction performed on Waters Xevo TQ-S (ultra-high-performance liquid chromatography-tandem mass spectrometry, UPLC-MS/MS)

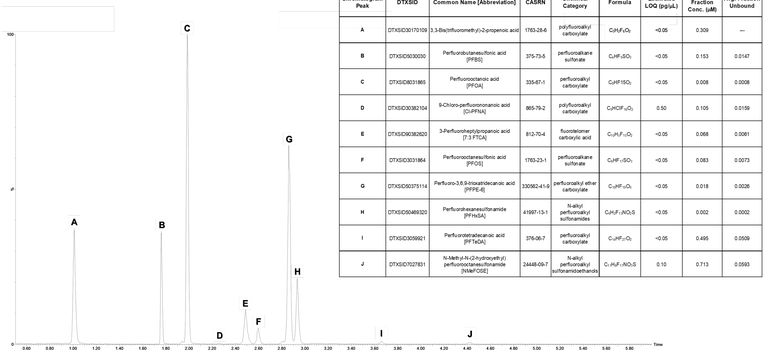
- Substrate depletion approach using primary human hepatocytes (50-donor pool, mixed sex) at 1 μM PFAS concentration
- Time course: 0, 15, 30, 60, 90, 120, and 240 min with non-linear regression fit to determine half-life ($T_{1/2}$)
- Single Ion Monitoring mode on Thermo Vanquish with Q Exactive Plus

SUBSTRATE DEPLETION (METABOLIC STABILITY) ASSAY TO DETERMINE IN VITRO HEPATIC CLEARANCE RATE

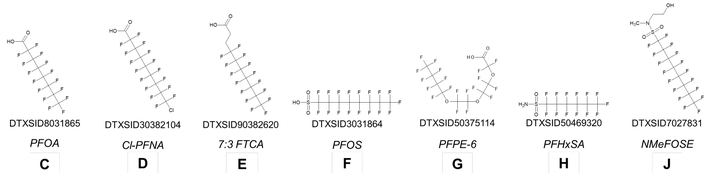


Determining Fraction Unbound by LC-MS/MS

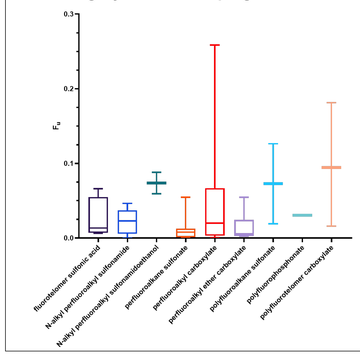
Fraction unbound (F_u) was determined by measuring PFAS concentration in aqueous fraction and plasma by Waters Xevo TQ-S micro (UPLC-MS/MS) in 6.5 min run with low ppb (pg/μL) sensitivity

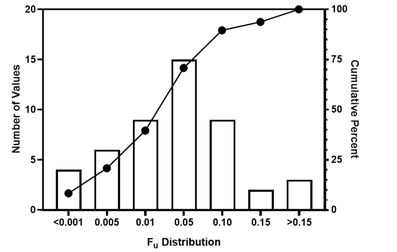


Chromatogram Peak	DTXSID	Common Name [Abbreviation]	CASRN	Chemical Category	Formula	Estimated LOQ (pg/μL)	Avg. Aqueous Fraction Conc. (μM)	Avg. Fraction Unbound
A	DTXSID30170109	3,3-Bis(trifluoromethyl)-2-propenoic acid	1763-28-6	polyfluoroalkyl carboxylate	$C_5H_4F_6O_2$	<0.05	0.309	---
B	DTXSID5030030	Perfluorobutanesulfonic acid [PFBS]	375-73-5	perfluoroalkane sulfonate	$C_4HF_9SO_3$	<0.05	0.153	0.0147
C	DTXSID80319865	Perfluorooctanoic acid [PFOA]	335-67-1	perfluoroalkyl carboxylate	C_8HF15O_2	<0.05	0.008	0.0008
D	DTXSID30382104	9-Chloro-perfluorononanoic acid [Cl-PFNA]	865-79-2	polyfluoroalkyl carboxylate	$C_9HClF_{16}O_2$	0.50	0.105	0.0159
E	DTXSID90382620	3-Perfluoroheptylpropanoic acid [7:3 FTCA]	812-70-4	fluorotelomer carboxylic acid	$C_{10}H_5F_{13}O_2$	<0.05	0.068	0.0061
F	DTXSID3031864	Perfluorooctanesulfonic acid [PFOS]	1763-23-1	perfluoroalkane sulfonate	$C_8HF_{17}SO_3$	<0.05	0.083	0.0073
G	DTXSID50375114	Perfluoro-3,6,9-trioxatridecanoic acid [PFPE-6]	330962-41-9	perfluoroalkyl ether carboxylate	$C_{10}HF_{17}O_5$	<0.05	0.018	0.0026
H	DTXSID50469320	Perfluorohexanesulfonamide [PFHxSA]	41997-13-1	N-alkyl perfluoroalkyl sulfonamides	$C_6H_2F_{13}NO_2S$	<0.05	0.002	0.0002
I	DTXSID3059921	Perfluorotetradecanoic acid [PFTDA]	376-06-7	perfluoroalkyl carboxylate	$C_{13}HF_{27}O_2$	<0.05	0.495	0.0509
J	DTXSID7027831	N-Methyl-N-(2-hydroxyethyl) perfluorooctanesulfonamide [NMeFOSE]	24448-09-7	N-alkyl perfluoroalkyl sulfonamidoethanols	$C_{11}H_8F_{17}NO_3S$	0.10	0.713	0.0593



Category-based Grouping and Distribution Analyses of PFAS Plasma Protein Binding Data



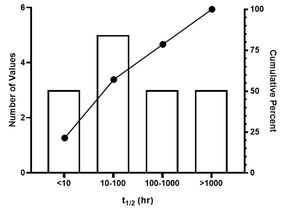


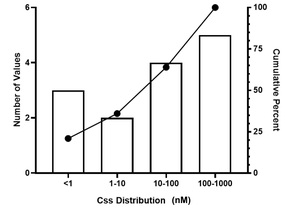
IVIVE Modeling Results

IVIVE modeling was performed using collected *in vitro* $Cl_{hepatic}$ and F_u data for a representative set of PFAS to predict steady-state blood concentrations (C_{ss}) for an adult population:

Compound Name	Fu	Cl_{renal} (L/hr)	$Cl_{hepatic}$ (L/hr)	Css (nM)
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

Assumptions: 1 μg/kg/day exposure, linear kinetics, 100% bioavailability, and nonmetabolic renal clearance only





Summary

- Experimental *in vitro* toxicokinetic data (F_u and $Cl_{hepatic}$) are being measured on over 100 PFAS for use in IVIVE modeling.
- Plasma protein binding data on 49 PFAS measured to date using UPLC-MS indicate high binding rates, with 75% exhibiting F_u values from 0.001 – 0.05.
- Preliminary category-based analyses of F_u values show perfluoroalkyl carboxylates have the greatest range, while other functional groups exhibit tighter distributions.
- 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. Half-life (T½) estimates tracked similarly, with 50% of tested PFAS having T½ s ≤ 100 hr and ~20% with T½ values exceeding 1000 hr.
- Data generation across additional PFAS and toxicokinetic assays for bioavailability and renal reuptake are currently underway.

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