Category-Based Toxicokinetic Evaluations of Data-Poor Per- and Polyfluoroalkyl Substances (PFAS) by Gas Chromatography Coupled with Mass Spectrometry

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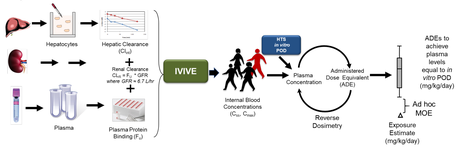
Background

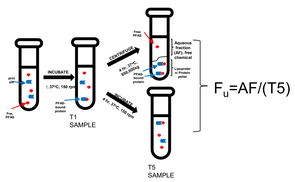
- Despite widespread concern over PFAS exposure, only limited biomonitoring, toxicity and toxicokinetic (TK) data are available.
- New approach methods (NAMs) that make use of *in vitro* and *in silico* methodologies are available to provide toxicity and TK data efficiently.
- Read-across approaches that use such data to categorize PFAS in specific groupings can be used to inform risk across the broader set of PFAS.
- This poster describes:
  - Evaluation and generation of *in vitro* TK data (plasma protein binding (PPB), hepatic clearance) of a set of 75 PFAS by gas chromatography mass-spectrometry.
  - Trends analyses to aid in developing grouping strategies
  - Preliminary findings with regard to *in vitro-in vivo* extrapolation (IVIVE) to predict *in vivo* TK.



TK NAM approach using *in vitro* TK data to predict *in vivo* TK. Using TK NAMs, *in vitro* points of departure (POD) can be converted to an administered equivalent dose (AED), useful in margin of exposure (MOE) evaluations employed during risk assessment.

In Vitro Toxicokinetic Assays

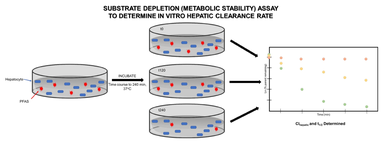
ULTRACENTRIFUGATION PLASMA PROTEIN BINDING ASSAY



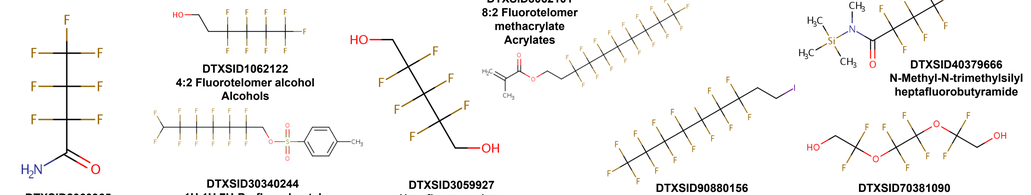
- Human plasma (10-donor pool, mixed sex) centrifuged at 850,000x*g* to separate aqueous fraction from plasma proteins, lipoproteins, and fatty acids
- Fraction unbound (f_u) derived by analyte quantitation in aqueous fraction (AF) and whole plasma, performed by gas chromatography-tandem mass spectrometry (GC-MS/MS) on Agilent 8890/7010B, 7890A/7010A, and 7890B/7010B instrumentation

- Substrate depletion approach using primary human hepatocytes (50-donor pool, mixed sex) at 1 µM PFAS.
- Time course: 0, 15, 30, 60, 90, 120, and 240 min with linear regression fit to determine intrinsic clearance (Cl_{int})
- Media only and heat-inactivated hepatocyte controls to monitor abiotic loss

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



PFAS Chemical Space Evaluated



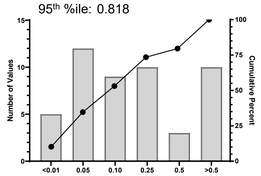
75 PFAS containing functional groups displayed above were selected for evaluation. All DMSO stocks passed an analytical QC check prior to study start. Targeted mass spectrometric methods have been developed for 62 to date. PPB, hepatic clearance, and associated stability assays have been conducted; TK data generated to date is discussed below.

Results

Category-Based Evaluations of PFAS Plasma Protein Binding (PPB)

Distribution of f_u values for 48 GC-MS PFAS

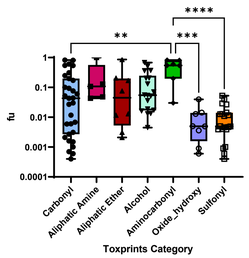
f_u Summary Statistics
25th %ile: 0.028
Median: 0.098
Mean: 0.218
75th %ile: 0.248
95th %ile: 0.818



Distribution of f_u values measured for 48 GC-MS PFAS.

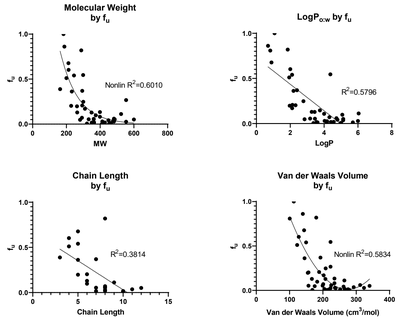
A parallel f_u study on 58 LC-MS PFAS showed a median f_u = 0.01; i.e., 10-fold higher binding compared to this set (75%ile: f_u<0.05; LCMS PFAS include PFOA, PFOS, PFHxS).
See Smeltz et al., poster 3093; P196

Grouping f_u based on ToxPrint ChemoTypes



Using ToxPrint ChemoTypes to evaluate f_u. A one-way ANOVA was performed on the major ToxPrint chemotypes using a Tukey test for multiple comparisons to determine between-group differences. *p<0.05, **p<0.01, ***p<0.001.
Note the ToxPrint ChemoType names are condensed for display purposes.

Identifying trends between f_u and physicochemical properties



Physicochemical properties trends analysis. Plots depict physicochemical properties that showed a significant correlation with f_u. Regression analyses were fit with simple linear, or non-linear regression, using whichever better fit the data. Chemicals were manually categorized for chain length and only included straightforward cases.

Results: IVIVE Modeling

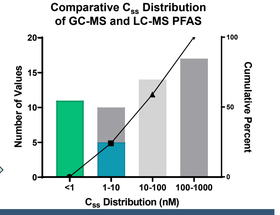
DTXSID	Compound Name	Avg MW (g/mol)	f_u	in vitro CL_{int} (µL/min/10^6 cells)	CL_{renal} (L/h)	CL_{hep} (L/h)	Css (nM)
DTXSID0059871	Pentafluoropropionamide	163.047	0.809	0	5.4203	0.000	3.33
DTXSID3059927	Hexafluoroamylene	212.091	0.677	0	4.5359	0.000	3.06
DTXSID50369896	1H,1H,10H,10H-Perfluorodecane-1,10-diol	462.13	0.004	56.6	0.0268	2.323	2.71
DTXSID00380798	1H,1H,11H,11H-Perfluorotetraethylene glycol	410.112	0.013	19.93	0.0871	2.649	2.62
DTXSID60379269	3-(Perfluoropropyl)propanol	228.11	0.203	2.69	1.3601	5.407	1.90
DTXSID30340244	1H,1H,7H-Perfluoroheptyl 4-methylbenzenesulfonate	486.27	0.029	23.85	0.1943	6.740	0.87
DTXSID90880156	1-Iodo-1H,1H,2H,2H-perfluorononane	524.012	0.1123	5.75	0.7524	6.324	0.79
DTXSID30396867	1H,1H,8H,8H-Perfluorooctane-1,8-diol	362.115	0.058	20.006	0.3886	10.761	0.73
DTXSID70381090	1H,1H,8H,8H-Perfluoro-3,6-dioxaoctane-1,8-diol	294.097	0.207	6.72	1.3869	12.601	0.71
DTXSID10382147	3-(Perfluoro-2-butyl)propane-1,2-diol	294.117	0.37	5.88	2.4790	18.266	0.48
DTXSID80310730	Octafluoroadipamide	288.097	0.82	2.95	5.4940	19.858	0.40
DTXSID1062122	4:2 fluorotelomer alcohol	264.091	0.1300	37.71	0.8703	32.796	0.33
DTXSID60400587	Nonafluoropentanamide	263.063	0.198	26.026	1.3266	33.860	0.32
DTXSID70366226	Perfluoropentanamide	245.072	0.54	14.68	3.6180	43.315	0.26
DTXSID2060965	Heptafluorobutyramide	213.055	0.803	19.95	4.0401	52.624	0.24
DTXSID40379666	N-Methyl-N-trimethylsilylheptafluorobutyramide	299.264	0.545	19.95	3.6515	50.397	0.18

C_{ss} values calculated for 16 PFAS for which both f_u and Cl_{int} values were available. Cl_{renal} = GFR x f_u.

Key Findings Compared to LC-MS PFAS, this set of 16 had much lower C_{ss} values, due to:

- higher f_u values (on average 10-fold higher);
- higher hepatic clearance rates (only 2 with no Cl_{int}; for LC-MS PFAS, most <1µL/min/10^6 cells)
- Highest C_{ss} for GC-MS PFAS 3.33 nM

Distribution of steady-state concentration (C_{ss}) values for GC-MS PFAS in comparison to LC-MS PFAS. The number of GC-MS PFAS (shown in color) are overlaid on top of the distribution of LC-MS PFAS (gray), which show much higher C_{ss}.



Summary

- PPB data on 48 GC-MS PFAS measured to date indicate lower binding than LC-MS PFAS, with f_u values ranging from 0.004 -1, and a mean f_u of 0.22.
- Evaluation of PFAS PPB using ToxPrint ChemoTypes showed variations based on presence of certain functional groups. Lower f_u was noted for sulfonyl/sulfur-containing PFAS and oxide hydroxy-containing PFAS. Higher f_u was noted for amino-carbonyl and nitrogen-containing PFAS.
- Correlations between increased PPB and higher molecular weight, chain length, log P_{OW}, and van der Waals volumes were noted during trends analyses.
- Due to lower PPB and higher hepatic clearance, these PFAS are less bioaccumulative than legacy PFAS with carboxylic acid and sulfonate functional groups (e.g., PFOA, PFOS).
- Given the relatively high rate of metabolic clearance for this set, ongoing work is now evaluating PFAS metabolic biotransformation through non-targeted and other analyses.
- Data generation across additional PFAS and toxicokinetic assays evaluating renal reuptake (i.e., *in vitro* transporter assays) are currently underway.

References

Smeltz, M., Clifton, M., McMillan, L., Wetmore, B. Application of a Quality Scoring System for Assessing Per- and Polyfluoroalkyl Substances (PFAS) in Organic Solvents for *In Vitro* Toxicokinetic Testing. 2022 SOT Annual Meeting, PFASI Poster Session, #3093, P.196.

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