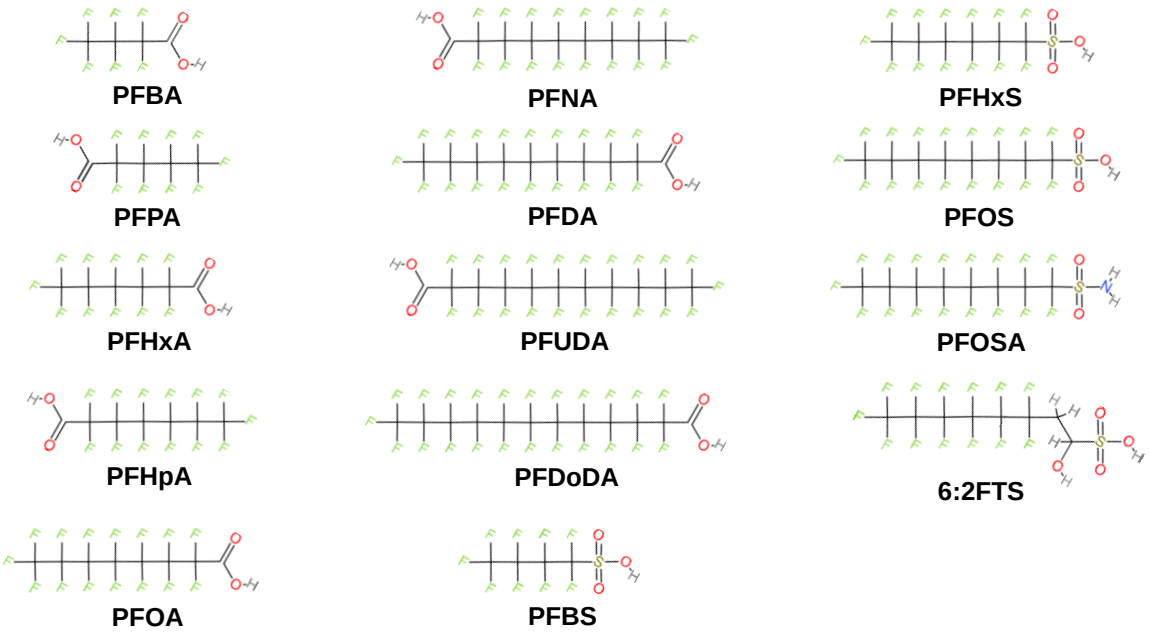
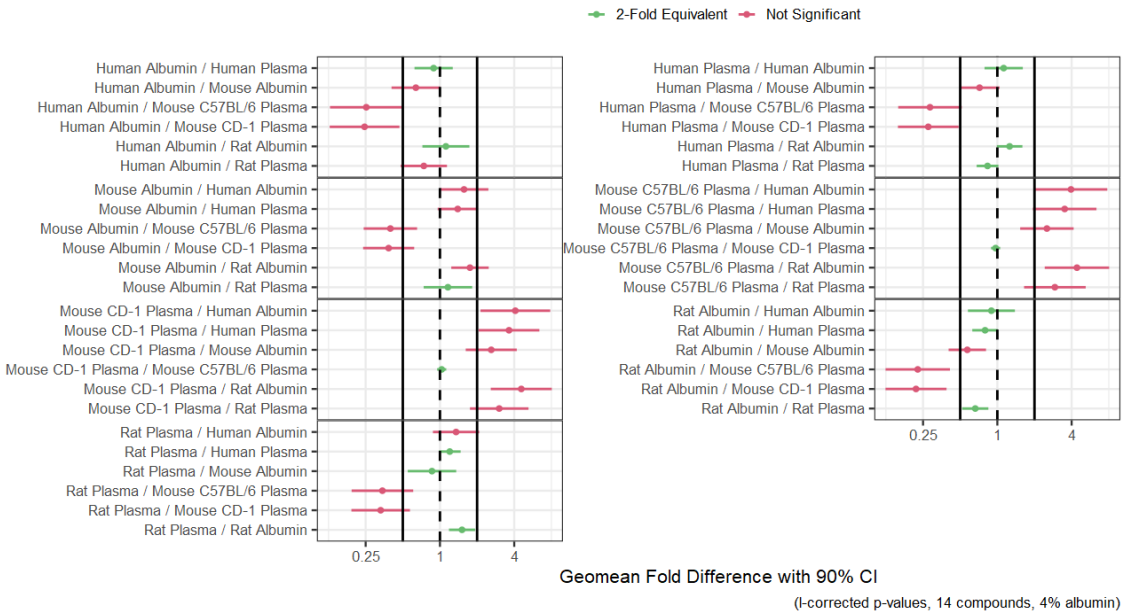
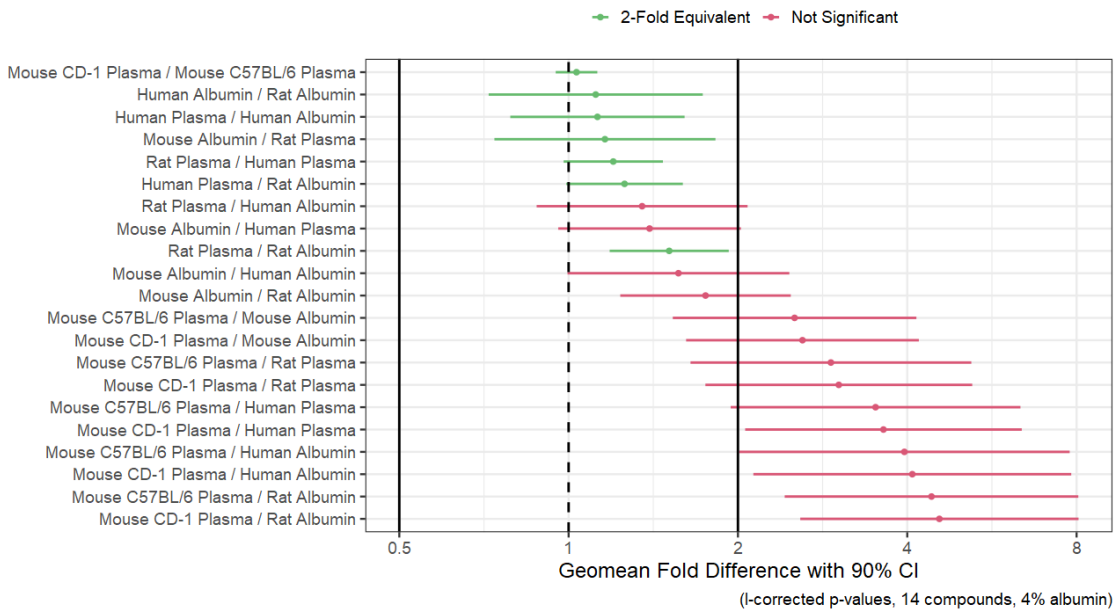


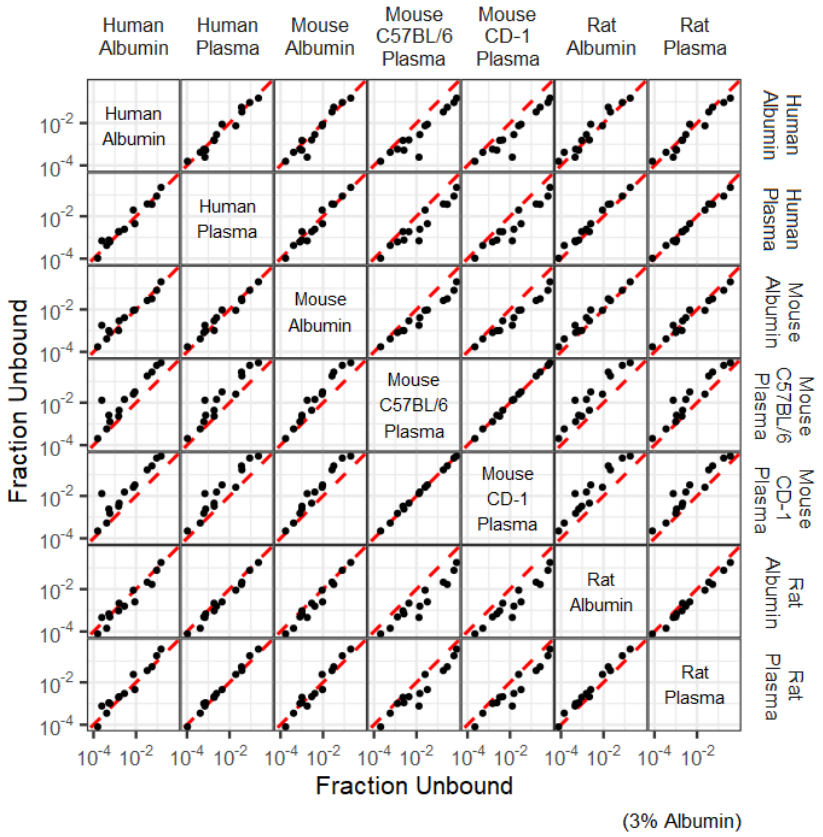
Supplementary Figure S1. Chemical structures of 14 PFAS selected in study.



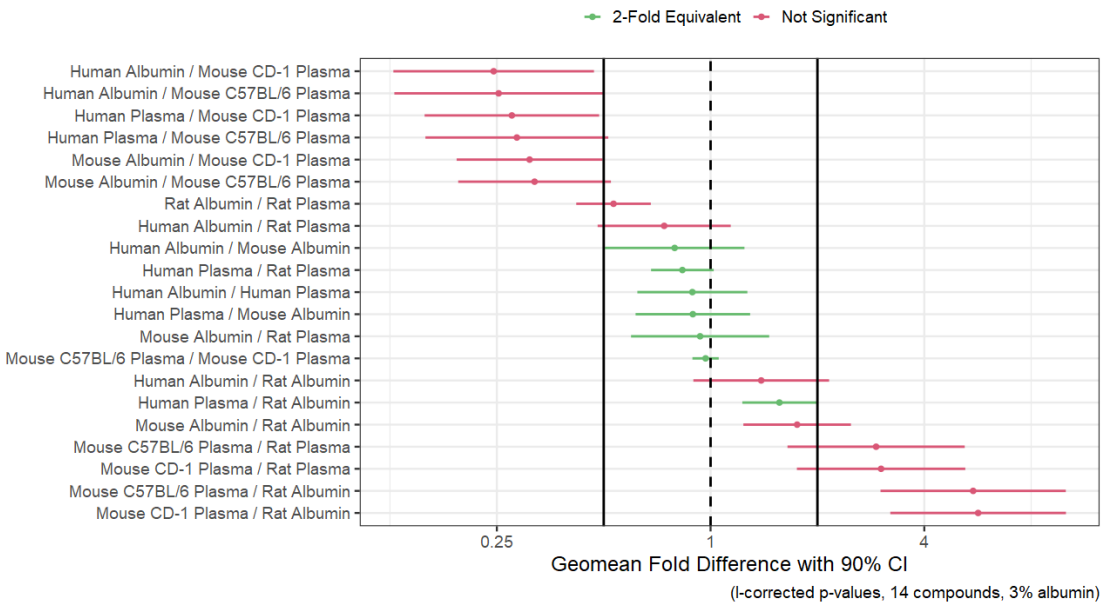
Supplementary Figure S2. ℓ-correction adjusted TOST equivalence tests conducted for within-matrix pairwise comparisons fu values for all species and plasma/ albumin combinations grouped by numerator. 2-fold difference threshold employed to determine equivalence accounting for established assay variance.



Supplementary Figure S3. ℓ-correction adjusted TOST equivalence tests conducted for within-matrix pairwise comparisons fu values for all species and plasma/ albumin combinations sorted by geomean fold difference with matrix ratios ordered such that the geomean fold difference is always greater than 1. 2-fold difference threshold employed to determine equivalence accounting for established assay variance.



Supplementary Figure S4. Pairwise fraction unbound (f_u) comparison for all species and subspecies for human, rat, and mouse (CD-1 and C57BL/6) plasma and 3% albumin for rodents.



Supplementary Figure S5. ℓ-correction adjusted TOST equivalence tests conducted for within-matrix pairwise comparisons fraction unbound (f_u) values for all species and plasma/ 3% albumin combinations organized from least to highest geomean fold difference. 2-fold difference threshold employed to determine equivalence accounting for established assay variance.

Supplementary Table S1. 14 total PFAS within the study including perfluoroalkyl carboxylates, perfluoroalkyl sulfonates, perfluoroalkyl sulfonamide, and fluorotelomer sulfonate with respective PFAS acronym, full PFAS name, number of carbons within carbon chain, molecular weight (g/mol), and logD.

PFAS	Full Name	Carbon Chain	Molecular weight	logD
Perfluoroalkyl Carboxylates			(g/mol)	
PFBA	Perfluorobutanoic acid	**4**	214.04	2.23
PFPA	Perfluoropentanoic acid	**5**	264.05	3.02
PFHxA	Perfluorohexanoic acid	**6**	314.05	3.81
PFHpA	Perfluoroheptanoic acid	**7**	364.06	4.59
PFOA	Perfluorooctanoic acid	**8**	414.07	5.38
PFNA	Perfluorononanoic acid	**9**	464.08	6.17
PFDA	Perfluorodecanoic acid	**10**	514.08	6.96
PFUDA	Perfluoroundecanoic acid	**11**	564.09	7.75
PFDoDA	Perfluorododecanoic acid	**12**	614.1	8.54
Perfluoroalkyl Sulfonates				
PFBS	Perfluorobutane sulfonic acid	**4**	300.1	3.73
PFHxS	Perfluorohexane sulfonic acid	**6**	400.12	5.30
PFOS	Perfluorooctane sulfonic acid	**8**	500.13	6.88
Perfluoroalkyl Sulfonamides				
PFOSA	Perfluorooctanesulfonamide	**8**	499.14	5.52
Fluorotelomer Sulfonates				
6:2 FtS	6:2 Fluorotelomer sulfonate	**8**	427.98	4.44

Supplementary Table S2. PFAS LC-MS/MS multiple reaction monitoring (MRM) conditions including, quadrupoles (Q1, Q3), polarity, declustering potential (DP), and collision energy (CE).

PFAS	MRM transitions		Polarity	DP	CE
	Q1	Q3			
PFBA	212.99	169.01	Negative	-30	-15
PFPA	262.99	219.02	Negative	-30	-15
PFHxA	312.99	269.04	Negative	-30	-15
PFHpA	363.01	319.06	Negative	-30	-15
PFOA	413.1	369.13	Negative	-30	-15
PFNA	463.04	419.15	Negative	-30	-15
PFDA	513.05	469.14	Negative	-30	-15
PFUDA	563.08	519.16	Negative	-40	-15
PFTeDA	713.03	669.09	Negative	-40	-15
PFBS	298.96	80.1	Negative	-80	-65
PFHxS	399.07	80.1	Negative	-20	-55
PFOS	499.1	80.1	Negative	-120	-35
6:2FTS	427.16	81	Negative	-80	-65
PFOSA	498.09	169.01	Negative	-120	-35

Supplementary Table S3. Species and matrix combination comparisons including mean absolute fold-difference (MAFD), spearman rank-correlation, and geometric mean fold difference.

1st Tissue	2nd Tissue	Mean Absolute Fold Difference	Spearman's Rank Correlation	Geometric Mean Fold Difference (1st Tissue/2nd Tissue)
Human Albumin (4%)	Human Plasma	1.52	0.96	0.89
Human Albumin (4%)	Mouse Albumin (3%)	1.81	0.95	0.79
Human Albumin (4%)	Mouse C57BL/6 Plasma	7.22	0.92	0.25
Human Albumin (4%)	Mouse CD-1 Plasma	7.21	0.93	0.24
Human Albumin (4%)	Rat Albumin (3%)	1.87	0.98	1.39
Human Albumin (4%)	Rat Plasma	1.81	0.99	0.74
Human Plasma	Human Albumin (4%)	1.52	0.96	1.13
Human Plasma	Mouse Albumin (3%)	1.58	0.97	0.89
Human Plasma	Mouse C57BL/6 Plasma	4.84	0.95	0.28
Human Plasma	Mouse CD-1 Plasma	4.88	0.96	0.28
Human Plasma	Rat Albumin (3%)	1.66	0.98	1.56
Human Plasma	Rat Plasma	1.32	0.99	0.83
Mouse Albumin (3%)	Human Albumin (4%)	1.81	0.95	1.26
Mouse Albumin (3%)	Human Plasma	1.58	0.97	1.12
Mouse Albumin (3%)	Mouse C57BL/6 Plasma	3.86	0.99	0.32
Mouse Albumin (3%)	Mouse CD-1 Plasma	3.90	1.00	0.31
Mouse Albumin (3%)	Rat Albumin (3%)	1.96	0.96	1.75
Mouse Albumin (3%)	Rat Plasma	1.75	0.97	0.93
Mouse C57BL/6 Plasma	Human Albumin (4%)	7.22	0.92	3.95
Mouse C57BL/6 Plasma	Human Plasma	4.84	0.95	3.51
Mouse C57BL/6 Plasma	Mouse Albumin (3%)	3.86	0.99	3.13
Mouse C57BL/6 Plasma	Mouse CD-1 Plasma	1.09	1.00	0.97

Mouse C57BL/6 Plasma	Rat Albumin (3%)	7.71	0.93	5.49
Mouse C57BL/6 Plasma	Rat Plasma	4.05	0.95	2.93
Mouse CD-1 Plasma	Human Albumin (4%)	7.21	0.93	4.08
Mouse CD-1 Plasma	Human Plasma	4.88	0.96	3.63
Mouse CD-1 Plasma	Mouse Albumin (3%)	3.90	1.00	3.23
Mouse CD-1 Plasma	Mouse C57BL/6 Plasma	1.09	1.00	1.03
Mouse CD-1 Plasma	Rat Albumin (3%)	7.73	0.94	5.67
Mouse CD-1 Plasma	Rat Plasma	4.08	0.95	3.02
Rat Albumin (3%)	Human Albumin (4%)	1.87	0.98	0.72
Rat Albumin (3%)	Human Plasma	1.66	0.98	0.64
Rat Albumin (3%)	Mouse Albumin (3%)	1.96	0.96	0.57
Rat Albumin (3%)	Mouse C57BL/6 Plasma	7.71	0.93	0.18
Rat Albumin (3%)	Mouse CD-1 Plasma	7.73	0.94	0.18
Rat Albumin (3%)	Rat Plasma	1.97	0.99	0.53
Rat Plasma	Human Albumin (4%)	1.81	0.99	1.35
Rat Plasma	Human Plasma	1.32	0.99	1.20
Rat Plasma	Mouse Albumin (3%)	1.75	0.97	1.07
Rat Plasma	Mouse C57BL/6 Plasma	4.05	0.95	0.34
Rat Plasma	Mouse CD-1 Plasma	4.08	0.95	0.33
Rat Plasma	Rat Albumin (3%)	1.97	0.99	1.88
Human Albumin (4%)	Human Plasma	1.52	0.96	0.89
Human Albumin (4%)	Mouse Albumin (4%)	2.08	0.95	0.64
Human Albumin (4%)	Mouse C57BL/6 Plasma	7.22	0.92	0.25
Human Albumin (4%)	Mouse CD-1 Plasma	7.21	0.93	0.24
Human Albumin (4%)	Rat Albumin (4%)	1.72	0.98	1.12
Human Albumin (4%)	Rat Plasma	1.81	0.99	0.74
Human Plasma	Human Albumin (4%)	1.52	0.96	1.13
Human Plasma	Mouse Albumin (4%)	1.73	0.97	0.72

Human Plasma	Mouse C57BL/6 Plasma	4.84	0.95	0.28
Human Plasma	Mouse CD-1 Plasma	4.88	0.96	0.28
Human Plasma	Rat Albumin (4%)	1.40	0.98	1.26
Human Plasma	Rat Plasma	1.32	0.99	0.83
Mouse Albumin (4%)	Human Albumin (4%)	2.08	0.95	1.57
Mouse Albumin (4%)	Human Plasma	1.73	0.97	1.39
Mouse Albumin (4%)	Mouse C57BL/6 Plasma	3.12	0.99	0.40
Mouse Albumin (4%)	Mouse CD-1 Plasma	3.14	1.00	0.38
Mouse Albumin (4%)	Rat Albumin (4%)	1.95	0.96	1.75
Mouse Albumin (4%)	Rat Plasma	1.78	0.97	1.16
Mouse C57BL/6 Plasma	Human Albumin (4%)	7.22	0.92	3.95
Mouse C57BL/6 Plasma	Human Plasma	4.84	0.95	3.51
Mouse C57BL/6 Plasma	Mouse Albumin (4%)	3.12	0.99	2.52
Mouse C57BL/6 Plasma	Mouse CD-1 Plasma	1.09	1.00	0.97
Mouse C57BL/6 Plasma	Rat Albumin (4%)	6.19	0.93	4.42
Mouse C57BL/6 Plasma	Rat Plasma	4.05	0.95	2.93
Mouse CD-1 Plasma	Human Albumin (4%)	7.21	0.93	4.08
Mouse CD-1 Plasma	Human Plasma	4.88	0.96	3.63
Mouse CD-1 Plasma	Mouse Albumin (4%)	3.14	1.00	2.60
Mouse CD-1 Plasma	Mouse C57BL/6 Plasma	1.09	1.00	1.03
Mouse CD-1 Plasma	Rat Albumin (4%)	6.21	0.94	4.56
Mouse CD-1 Plasma	Rat Plasma	4.08	0.95	3.02
Rat Albumin (4%)	Human Albumin (4%)	1.72	0.98	0.90
Rat Albumin (4%)	Human Plasma	1.40	0.98	0.80
Rat Albumin (4%)	Mouse Albumin (4%)	1.95	0.96	0.57
Rat Albumin (4%)	Mouse C57BL/6 Plasma	6.19	0.93	0.23
Rat Albumin (4%)	Mouse CD-1 Plasma	6.21	0.94	0.22
Rat Albumin (4%)	Rat Plasma	1.65	0.99	0.66

Rat Plasma	Human Albumin (4%)	1.81	0.99	1.35
Rat Plasma	Human Plasma	1.32	0.99	1.20
Rat Plasma	Mouse Albumin (4%)	1.78	0.97	0.86
Rat Plasma	Mouse C57BL/6 Plasma	4.05	0.95	0.34
Rat Plasma	Mouse CD-1 Plasma	4.08	0.95	0.33
Rat Plasma	Rat Albumin (4%)	1.65	0.99	1.51

Supplementary Table S4. Fraction unbound (f_u) and %CV for species specific 3% serum albumin [rat (RSA), and mouse (MSA)].

PFAS	RSA 3%	MSA 3%
PFBA	0.18 (3%)	0.206 (23%)
PFPA	0.0769 (4%)	0.0809 (11%)
PFHxA	0.0212 (4%)	0.0266 (11%)
PFHpA	0.00257 (27%)	0.00954 (12%)
PFOA	0.00157 (3%)	0.00411 (5%)
PFNA	0.00217 (2%)	0.00292 (6%)
PFDA	0.000494 (19%)	0.000839 (26%)
PFUDA	0.000147 (28%)	0.000417 (19%)
PFDoDA	0.0000777 (16%)	0.0001749 (15%)
PFBS	0.0168 (8%)	0.0321 (11%)
PFHxS	0.00047 (23%)	0.00181 (13%)
PFOS	0.000697 (25%)	0.00102 (19%)
PFOSA	0.000952 (11%)	0.00102 (11%)
6:2 FtS	0.00892 (4%)	0.0091 (9%)