Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supporting Information

Partition coefficients of four perfluoroalkyl acid alternatives between bovine serum albumin (BSA) and water in comparison to ten classical perfluoroalkyl acids

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Content

SI-1. Experimental Section

SI-1.1 Suppliers of individual PFAAs for dialysis experiments

Analyte		Company	Purity
Perfluorobutanoic acid	**PFBA**	Sigma Aldrich	99%
Perfluorohexanoic acid	**PFHxA**	Fluka	>97%
Perfluoroheptanoic acid	**PFHpA**	Sigma Aldrich	99%
Perfluorooctanoic acid	**PFOA**	ABCR	>97%
Perfluorononanoic acid	**PFNA**	Sigma Aldrich	97%
Perfluorodecanoic acid	**PFDA**	Fluka	>97%
Perfluoroundecanoic acid	**PFUnDA**	Sigma Aldrich	95%
Perfluorobutane sulfonic acid	**PFBS, TBA salt**	Fluka	>98%
Perfluorohexane sulfonic acid	**PFHxS, K-salt**	Fluka	98%
Perfluorooctane sulfonic acid	**PFOS, K-salt**	Fluka	98%

SI-1.2 Dialysis cells

SI-1.2.1 Preparation of buffer

For dialysis equilibrium experiments, Hank's balanced salt solution (HBSS) was used as a buffer and was supplied as a powder from Sigma Aldrich (Steinheim). The pH was adjusted to 7.4 after adding sodium carbonate (Sigma Aldrich; 0.35 g/L), TRIS (Roth, Karlsruhe; 1.21 g/L) and NaN_3 (Merck, Darmstadt; 0.3 g/L), the latter was used to prevent microbial activity. This buffer system was selected for consistency with former dialysis studies and exhibits the electrolyte concentration in blood.[1] The concentration of NaN_3 showed no effect on the experiments.[1, 2]

SI-1.2.2 Experimental setup

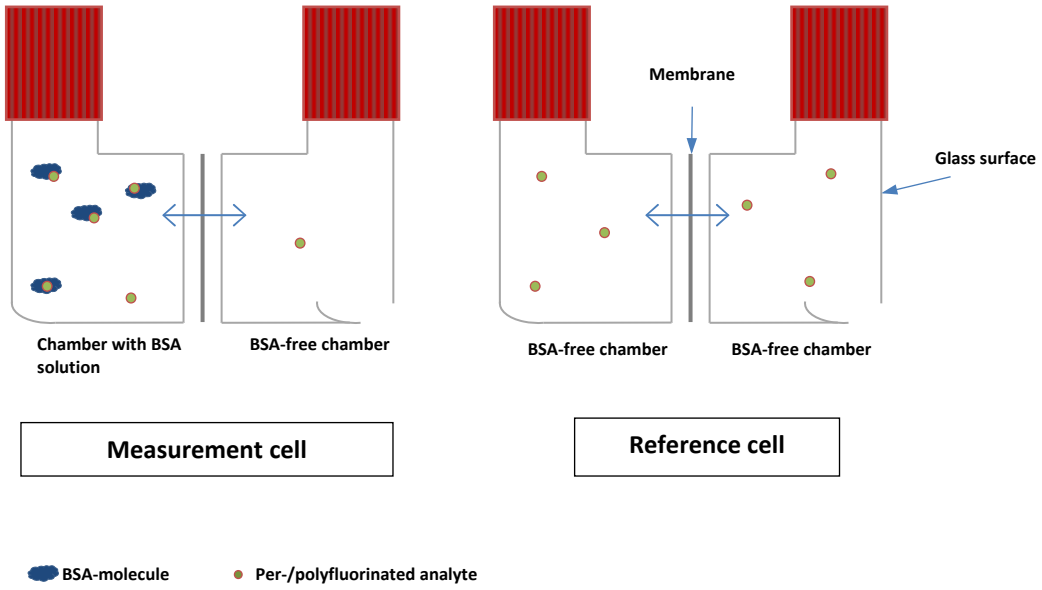

Figure SI-1.2.3. Schematic setup of a dialysis cell experiment. Three measurement cells and three reference cells were prepared for the determination of the partition coefficient for each compound. Initially, the stock solution of compound was added in the right chamber of both cells. In the measurement cell BSA-solution was added in one chamber, while in the reference cell no protein was added. To all cells of the setup, the same analyte concentration was added. Samples were taken from the initial concentration and, in equilibrium, from the BSA-free chambers. PFAAs/alternatives tend to sorb to a different degree to the glass surface of the cell leading to varying amounts of freely dissolved mass. Therefore, we used two approaches to determine total analyte mass ($m_{i,tot}$) within the system. For compounds where less than or equal to 10% of the $m_{i,tot}$ sorbed to the surface, reference cells were used. For compounds that sorb substantially (>10%) to the surfaces of the dialysis cells, reference cells could not be taken as the appropriate reference for the mass balance due to the variability of glass surfaces of the handmade cells (see main text). For those compounds an extraction step was added. The scheme was adapted from a version published in the supplementary information in Henneberger et al.[3]

SI-1.3 Instrumental analysis

Table SI-1.3.1. LC-Ramp for PFAA/alternatives analysis with Eluent A: 2 mM NH_4Ac in Milli-Q water and Eluent B: 2 mM NH_4Ac, 10 % acetonitrile and 2.5 % Milli-Q water in methanol. Column was a ACQUITY UPLC BEH Shield C18 (130 Å, 1.7 µm, 2.1 mm x 50 mm) supplied by Waters.

Time min	Flow mL/min	% A	% B
Initial	0.4	90	10
0.5	0.4	90	10
5	0.4	0.1	99.9
7.3	0.4	0.1	99.9
7.31	0.4	90	10
9	0.4	90	10

Table SI-1.3.2. MS parameters for PFAAs and alternatives measured with LC-MS/MS from Waters. Retention time (rt), collision energy (CE)

Analyte	rt (min)	Cone (V)	CE (V)	MRM transitions	Internal Standard IS	Cone (V)	CE (V)	IS MRM transitions	Qualifier transitions
PFBA	1.35	2	12	213 > 169	$^{13}C_4$PFBA	2	12	217 > 172	
PFHxA	4.01	2	12	313 > 269	$^{13}C_2$PFHxA	2	12	315 > 270	
PFHpA	4.43	2	12	363 > 319	$^{13}C_2$PFHxA	2	12	315 > 270	
PFOA	4.73	2	12	413 > 369	$^{13}C_4$PFOA	2	12	417 > 372	
PFNA	4.99	2	12	463 > 419	$^{13}C_5$PFNA	2	12	468 > 423	
PFDA	5.2	2	12	513 > 469	$^{13}C_2$PFDA	2	12	515 > 470	
PFUnDA	5.4	2	12	563 > 519	$^{13}C_2$PFUnDA	2	12	565 > 520	
PFBS	3.65	30	30	299 > 80	$^{13}C_2$PFHxA	2	12	315 > 270	299 > 99
PFHxS	4.57	30	32	399 > 80	$^{18}O_2$PFHxS	30	32	403 > 103	399 > 99
PFOS	5.05	30	38	499 > 80	$^{13}C_4$PFOS	30	38	503 > 99	499 > 99
DONA	5.63	4	14	377 > 251	$^{13}C_3$HFPO-DA	8	20	287 > 185	377 > 85
HFPO-DA	5.27	8	20	285 > 185	$^{13}C_3$HFPO-DA	8	20	287 > 185	285 > 169
9Cl-PF3ONS	5.49	8	26	531 > 350	$^{13}C_8$PFOS	30	38	507 > 99	531 > 83
PFECHS	5.08	54	32	461 > 381	$^{13}C_8$PFOA	4	10	421 > 376	461 > 99

SI-2. Results

SI-2.1 The importance of monitoring fraction bound

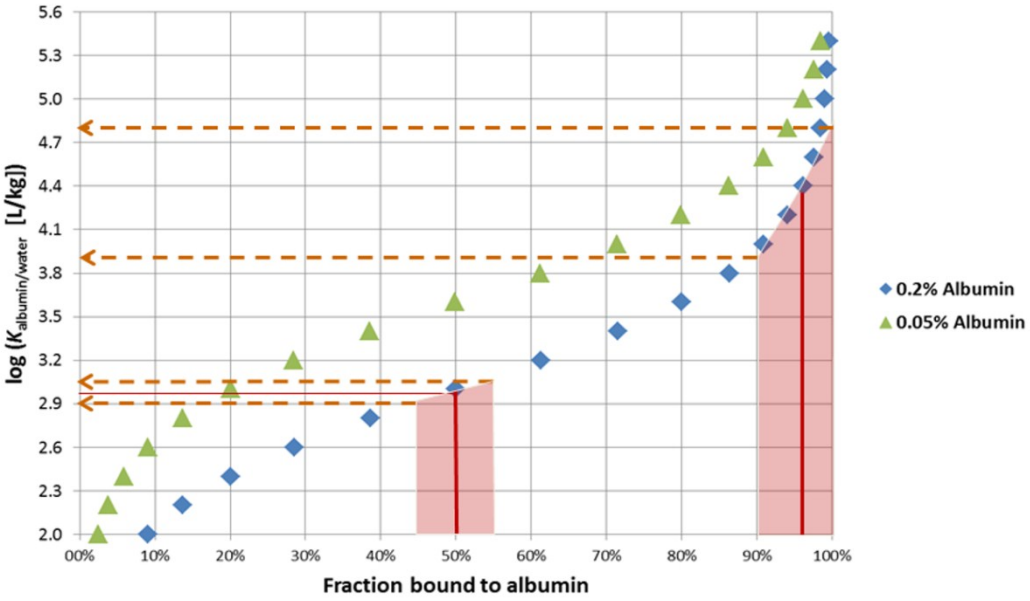

Figure SI-2.1. The relation between measuring error and fraction bound on the determination of the partition coefficient. To ensure the accuracy of the determined of the partition coefficient the fraction which is bound to the albumin should be between 20 – 80%. Below or above that, small measuring errors could have significant effects on the concluded partition coefficient, since in this range partition coefficients alter drastically.

SI-2.2 Precision experiments

Table SI-2.2. Week-to-week precision test for the determination of albumin/water partition coefficients. Three experiments were conducted with PFNA and both batches of BSA. Sampling was done after 72 h and 96 h of triplicates. Mean and standard deviation was calculated for albumin/water partition coefficients both sampling points (three x triplicates).

PFNA	**log $K_{albumin/water}$ [L/kg]**			
	BSA Batch A3803	**BSA Batch A7906**	**BSA Batch A3803**	**BSA Batch A7906**
1st experiment	**Sampling 72 h**		**Sampling 96 h**	
Vial1	4.39	4.50	4.25	4.49
Vial2	4.38	4.37	4.26	4.51
Vial3	4.38	4.52	4.28	4.49
2nd experiment				
Vial1	4.52	4.64	4.52	4.68
Vial2	4.54	4.64	4.54	4.69
Vial3	4.54	4.59	4.54	4.71
3rd experiment				
Vial1	4.46	4.53	4.46	4.57
Vial2	4.45	4.58	4.45	4.48
Vial3	4.49	4.40	4.49	4.52
Mean	**4.46**	**4.53**	**4.42**	**4.57**
SD	**0.07**	**0.10**	**0.12**	**0.10**

SI-2.3 Dialysis experiments

Table SI-2.3. Concentrations × of dialysis experiments in reference cells (c_{ref}) and freely dissolved in measurement cells (c_{free}) at equilibrium (*) analysed by LC-MS/MS. Values represent the mean and standard deviation (SD) of six measurements. Concentration of BSA solution c_{BSA} (batch A3803) used for dialysis experiments and amount of analyte/amount of BSA at equilibrium (*v*).

Analyte	c_{ref}* [ng/mL]	SD	c_{free}* [ng/mL]	SD	c_{BSA} [g/L]	*v* [mol/mol]
PFCAs						
PFBA	5.77	(0.30)	3.82	(0.25)	3×10^{0}	4×10^{-4}
PFHxA	5.72	(0.11)	2.39	(0.12)	1×10^{0}	1.4×10^{-3}
PFHpA	6.09	(0.50)	3.82	(0.32)	1×10^{-1}	7.4×10^{-3}
PFOA	10.56	(0.27)	5.86	(0.27)	1×10^{-1}	1.5×10^{-2}
PFNA	6.45	*(0.38)*	4.09	*(0.21)*	5×10^{-2}	1.2×10^{-2}
PFDA[a]	5.33	*(0.02)*	2.27	*(1.25)*	5×10^{-2}	1.6×10^{-2}
PFUnDA[a,b]	2.92	(1.30)	1.80	(0.60)	2.5×10^{-2}	1.2×10^{-2}
HFPO-DA	8.13	(0.55)	2.91	(0.11)	3×10^{0}	6.7×10^{-4}
DONA	7.06	(0.34)	5.15	(0.17)	1×10^{-1}	6.5×10^{-3}
PFSAs						
PFBS	10.50	(0.74)	5.86	(0.80)	1×10^{0}	2.1×10^{-3}
PFHxS	9.50	(0.45)	3.61	(0.15)	5×10^{-2}	3.8×10^{-2}
PFOS[a]	6.31	(0.33)	3.98	(0.30)	2.5×10^{-2}	2.5×10^{-2}
9Cl-PF3ONS[a]	4.76	(0.17)	2.09	(0.06)	2.5×10^{-2}	2.7×10^{-2}
PFECHS	8.37	(0.51)	2.85	(0.11)	1×10^{-1}	1.5×10^{-3}

[a]extraction step included due to glass sorption of the analyte, [b]only mean of five measurements taken

SI-2.4 Batch A7906

We determined albumin/water partition coefficients with another batch of BSA (A7906, Sigma). Both, A7906 and A3803, were lyophilized powders gained by heat shock fraction. According to the manufacturer, the extraction of A7906 was done without the additional removal of fatty acids, whereas A3803 has undergone a charcoal treatment to remove essentially all fatty acids.

We found some differences in the albumin/water partition coefficients measured with the different BSA batches (Table and Figure SI-2.4.1-2.4.4, below). Especially short-chain PFAAs and PFHxS showed a lower sorption strength of at least 0.3 log units when we used A7906 compared to the batch A3803 (main study). If these differences were caused either by potentially present fatty acids that were not removed in A7906 or by conformational changes evolved by the extraction and purification steps could not be examined. The exact fatty acid composition and their concentrations are not specified and quantification of fatty acids in the two batches was not possible due to the very low content of individual fatty acids. In conclusion we assume that different batches of BSA could affect the determined partition coefficients. The trend of increasing partition coefficients with increasing chain length of the PFCAs, however, was for both batches consistent. Variable extraction and purification steps could probably affect the conformation of the native serum albumin.[4] Overall, one has to consider that a determination of partition coefficients with serum albumin of exactly the same conformation cannot be achieved, since every withdrawal from the natural environment could influence the protein.

We decided to use essentially fatty acid free albumin to determine the partition coefficients of the PFAS to avoid potential interferences by impurities.

Table SI-2.4.1. Logarithmic albumin/water partition coefficients (log $K_{albumin/water}$ [L water/kg albumin]) determined for ten PFAAs and four alternative compounds with BSA batch A7906. Mean and standard deviation SD were taken from six measurements. HFPO-DA and DONA are not fully fluorinated, therefore no number is assigned. Fraction bound $f_{i,bound} = m_{i,bound}/m_{i,tot}$. K_a is the association constant ([PL]/([P] × [L])) with protein P and ligand L and is displayed to facilitate comparison with literature values.

Number of perfluorinated carbons	Analyte	log $K_{albumin/water}$ [L/kg]	SD	K_a [M^{-1}]	SD	f_{bound}	SD	Recovery (incl. Extracts)
PFCAs								
3	PFBA	1.90	(0.07)	5.4×10^3	(9.4×10^2)	29%	(4%)	104%
5	PFHxA	3.02	(0.07)	7.0×10^4	(1.1×10^4)	35%	(4%)	95%
6	PFHpA	3.79	(0.05)	4.1×10^5	(4.6×10^4)	62%	(3%)	98%
7	PFOA	4.17	(0.05)	1.0×10^6	(1.2×10^5)	43%	(3%)	94%
8	PFNA	4.48	(0.05)	2.1×10^6	(2.4×10^5)	45%	(3%)	101%
9	PFDA[a]	4.84	(0.05)	4.7×10^6	(5.6×10^5)	63%	(3%)	84% (119%)
10	PFUnDA[a,b]	4.72	(0.1)	3.6×10^6	(9.1×10^5)	44%	(6%)	59% (149%)
	HFPO-DA	2.71	(0.04)	3.4×10^4	(3.4×10^3)	44%	(2%)	92%
	DONA	3.80	(0.04)	4.2×10^5	(4.2×10^4)	61%	(2%)	91%
PFSAs								
4	PFBS	2.65	(0.12)	3.1×10^4	(7.5×10^3)	32%	(6%)	106%
6	PFHxS	4.49	(0.13)	2.2×10^6	(7.1×10^5)	44%	(7%)	96%
8	PFOS	4.83	(0.11)	4.7×10^6	(1.2×10^6)	77%	(4%)	92%
8	9Cl-PF3ONS[a]	5.02	(0.03)	7.2×10^6	(5.2×10^5)	58%	(2%)	81% (110%)
8	PFECHS	4.30	(0.03)	1.3×10^6	(8.6×10^4)	50%	(2%)	≈ 90%

[a]extraction step included due to glass sorption of the analyte, [b]only mean of four measurements taken

Table SI-2.4.2. Concentrations of dialysis experiments with BSA batch A7906 in reference cells (c_{ref}) and freely dissolved in measurement cells (c_{free}) at equilibrium (*) analysed by LC-MS/MS. Values represent the mean and standard deviation (SD) of six measurements. Concentration of BSA solution c_{BSA} (batch A7906) used for dialysis experiments and amount of PFAA/amount of BSA at equilibrium (*v*).

Analyte	c_{ref}* [ng/mL]	SD	c_{free}* [ng/mL]	SD	c_{BSA} [g/L]	*v* [mol/mol]
PFCAs						
PFBA	8.42	(0.42)	6.00	(0.29)	1×10^{1}	1.5×10^{-4}
PFHxA	8.49	(0.54)	5.54	(0.30)	1×10^{0}	1.2×10^{-3}
PFHpA	7.16	(0.35)	2.74	(0.19)	5×10^{-1}	3.1×10^{-3}
PFOA	12.10	(0.70)	6.93	(0.38)	1×10^{-1}	1.6×10^{-2}
PFNA	6.11	(0.36)	3.34	(0.17)	1×10^{-1}	1.5×10^{-2}
PFDA[a]	4.11	(0.26)	1.51	(0.12)	5×10^{-2}	1.4×10^{-2}
PFUnDA[a,b]	2.99	(0.44)	1.67	(0.26)	2.5×10^{-2}	1.0×10^{-2}
HFPO-DA	9.30	(0.44)	5.24	(0.23)	3×10^{0}	5.4×10^{-4}
DONA	9.70	(1.02)	3.79	(0.20)	5×10^{-1}	4.2×10^{-3}
PFSAs						
PFBS	8.42	(0.42)	6.00	(0.29)	2×10^{0}	7.0×10^{-4}
PFHxS	9.20	(0.49)	5.18	(0.75)	5×10^{-2}	2.7×10^{-2}
PFOS	5.71	(0.41)	1.32	(0.24)	1×10^{-1}	1.2×10^{-2}
9Cl-PF3ONS[a]	4.59	(0.10)	1.94	(0.11)	2.5×10^{-2}	2.5×10^{-2}
PFECHS	6.69	(1.09)	3.33	(0.11)	1×10^{-1}	9.6×10^{-4}

[a]extraction step included due to glass sorption of the analyte, [b]only mean of four measurements taken

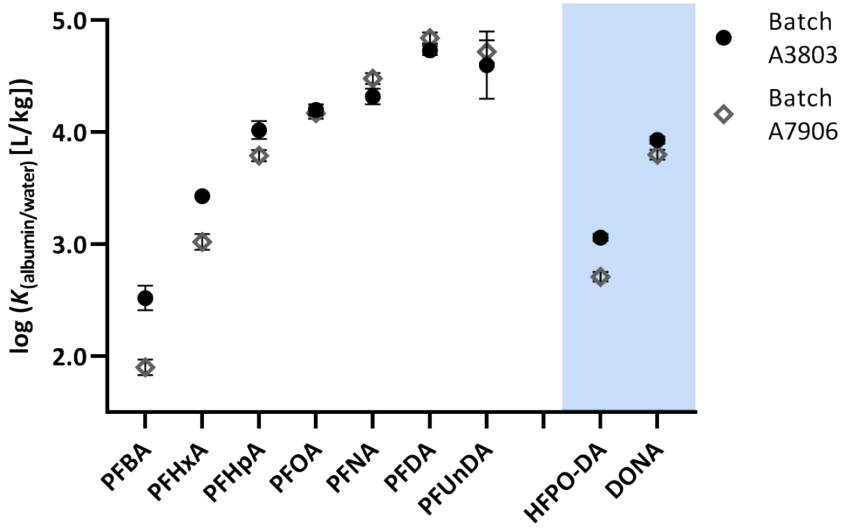

Figure SI-2.4.3. Comparison of albumin/water partition coefficients for the series of PFCAs/carboxylate alternatives determined with different batches of BSA (A3803 and A7906). When A7906 was used, lower

partition coefficients could be noted for shorter-chain carboxylates. Error bars of standard deviations are partly covered by symbols of data points.

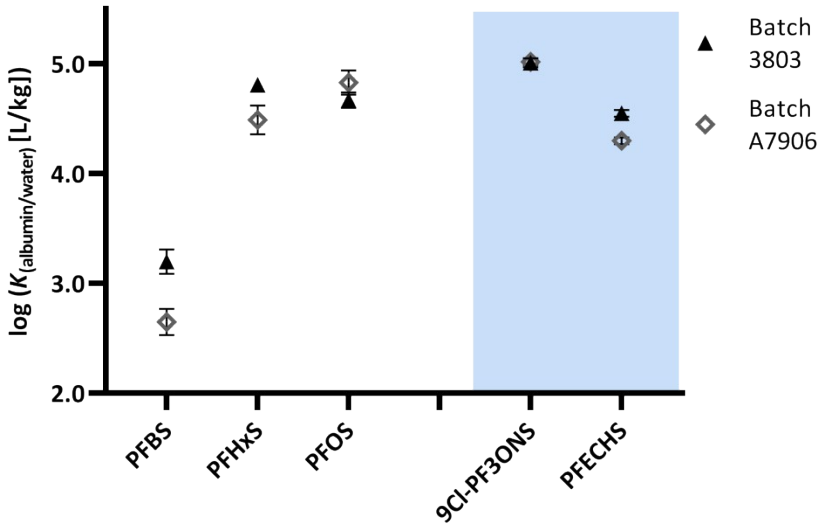

Figure SI-2.4.4. Comparison of albumin/water partition coefficients for the series of PFSAs/sulfonate alternatives determined with different batches of BSA (A3803 and A7906). When A7906 was used, lower partition coefficients could be noted for shorter-chain sulfonates, except for PFECHS. Error bars of standard deviations are partly covered by symbols of data points.

SI-2.5 Comparison with literature (Bischel et al. 2011)

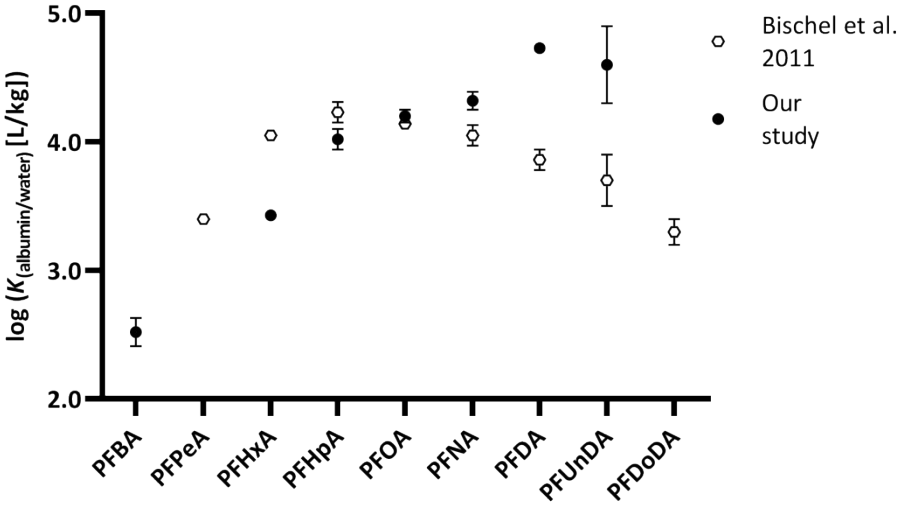

Figure SI-2.5.1. Comparison of albumin/water partition coefficients for the series of PFCAs determined by Bischel et al. (2011)[5] and in our study. Bischel measured additionally perfluoropentanoic acid (PFPeA with 5 perfluorinated carbons) and perfluorododecanoic acid (PFDoDA with 12 perfluorinated carbons) but not PFBA. They described partition coefficients with an increasing and a decreasing trend with increasing chain-length with a peak at PFHpA, whereas we see an increasing trend with increasing chain-length. Error bars of standard deviations are partly covered by symbols of data points.

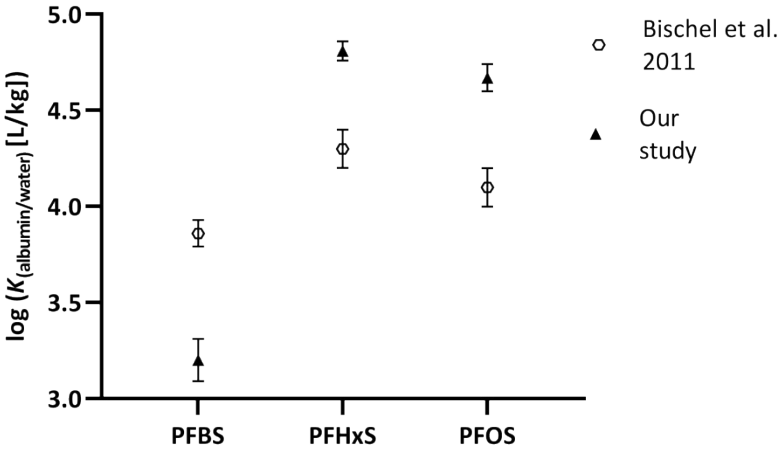

Figure SI-2.5.2. Comparison of partition coefficients for the series of PFSAs determined by Bischel et al. (2011)[5] and in our study. As seen for PFCAs, Bischel noted a similar trend with the highest partition coefficient for PFHxS. Regarding our results, we discuss that the trend is not clear, since PFOS sorbed stronger to the glass and increased therefore the experimental uncertainty.

SI-2.6 Conformer of PFHxA and PFOA in cross section

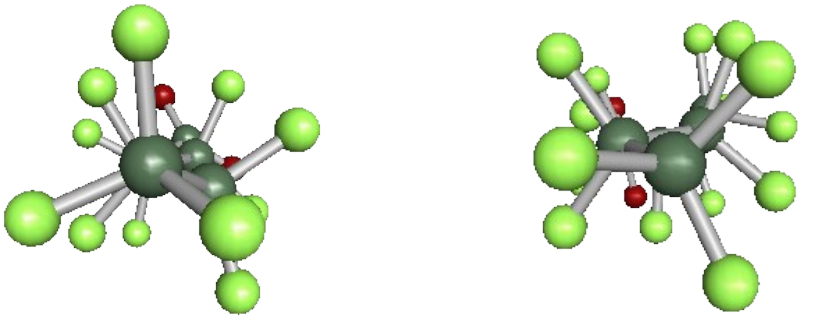

Figure SI-2.6. Conformers of PFHxA (five perfluorinated carbons) on the left and PFOA (seven perfluorinated carbons) on the right, calculated by COSMOconf/TURBOMOLE in cross-section.[6, 7] Literature discussed a conformeric change from a rather zig-zag form to a more helical structure occurring when the number of perfluorinated carbons exceeds six. This difference is not discernable here.

SI-2.7 Influence of fatty acid binding

Table SI-2.7. Logarithmic albumin/water partition coefficients for PFHxA or PFNA determined in presence of fatty acids (tridecanoic acid, TDA or heptadecanoic acid, HDA) at different molar ratios ([fatty acid] : [albumin]). Mean and standard deviations are taken by the displayed six measurements.

TDA Dialysis Experiments

[TDA] : [albumin]	2.5	1.2	0.3
	log $K_{albumin/water}$ for PFNA		
72h Vial1	4.34	4.54	4.63
72h Vial2	4.32	4.52	4.67
72h Vial3	4.40	4.50	4.58
96h Vial1	4.28	4.52	4.60
96h Vial2	4.32	4.55	4.61
96h Vial3	4.37	4.57	4.58
Mean	**4.34**	**4.53**	**4.61**
SD	0.04	0.02	0.03

[TDA] : [albumin]	2.3	1.1	0.6
	log $K_{albumin/water}$ for PFHxA		
72h Vial1	2.88	3.10	3.17
72h Vial2	2.87	3.04	3.19
72h Vial3	2.91	3.14	3.19
96h Vial1	2.82	3.16	3.16
96h Vial2	2.94	3.08	3.25
96h Vial3	2.95	3.07	3.13
Mean	**2.89**	**3.10**	**3.18**
SD	0.05	0.05	0.04

[TDA] : [albumin]	2.3	1.1	0
	log $K_{albumin/water}$ for PFHxA		
72h Vial1	3.15	3.19	3.35
72h Vial2	3.06	3.25	3.34
72h Vial3	3.00	3.13	3.41
96h Vial1	3.03	3.13	3.31
96h Vial2	3.00	3.31	3.30
96h Vial3	2.89	3.13	3.39
Mean	**3.02**	**3.19**	**3.35**
SD	0.09	0.08	0.04

HDA Dialysis Experiments

[HDA] : [albumin]	2.4	1.2	0.2
	log $K_{albumin/water}$ for PFNA		
72h Vial1	4.59	4.53	4.49
72h Vial2	4.55	4.58	4.56
72h Vial3	4.53	4.52	4.51
96h Vial1	4.56	4.59	4.51
96h Vial2	4.54	4.66	4.59
96h Vial3	4.54	4.65	4.55
Mean	**4.55**	**4.59**	**4.54**
SD	0.02	0.06	0.04

[HDA] : [albumin]	2.5	1.5	0.5
	log $K_{albumin/water}$ for PFHxA		
72h Vial1	3.26	3.27	3.31
72h Vial2	3.24	3.16	3.26
72h Vial3	3.25	3.23	3.24
96h Vial1	3.23	3.32	3.32
96h Vial2	3.25	3.16	3.23
96h Vial3	3.26	3.23	3.22
Mean	**3.25**	**3.23**	**3.26**
SD	0.01	0.06	0.04

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