Equilibrium sorption of perfluoroalkyl acids (PFAAs) and four of their alternatives in mammals

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften
(Dr. rer. nat.)

der
Naturwissenschaftlichen Fakultät II
Chemie, Physik und Mathematik

der Martin-Luther-Universität
Halle-Wittenberg

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Verteidigt am: 18. März 2021

Summary

Perfluoroalkyl acids (PFAAs) are the first mostly ionic organic compounds that are known to accumulate in organisms. This, together with their high persistency has caused great concern over the last two decades. In contrast to typical hydrophobic pollutants that accumulate primarily in fat tissues, the accumulation behavior of PFAAs is based on different physico-chemical properties and is still not fully understood. This makes the prediction of their bioaccumulation more complex. Potentially toxic PFAAs such as perfluorooctanoic acid (PFOA) have been replaced in industrial use by structurally modified alternative compounds which often contain more polar groups such as ether linkages in their structure but are still highly fluorinated. These alternatives have been shown to be as persistent as the classical PFAAs. However, the discussion whether they are less bioaccumulative than the PFAAs is ongoing.

The bioaccumulation of a compound depends, amongst others, on the sorption of that compound to all potentially relevant physiological matrices in an organism. In case of PFAAs these are the plasma protein albumin, membrane lipids, structural proteins and storage lipids. These matrices were either shown to have high binding affinities for PFAAs or they are generally abundant in an organism.

The purpose of this work was, therefore, to systematically investigate the sorption of PFAAs and alternatives to the described physiological matrices for the first time under physiological conditions, thereby improving the understanding of the bioaccumulative behavior of these compounds. To this end, equilibrium partition coefficients between the respective physiologically relevant matrix and water were determined by dialysis experiments for six perfluoroalkyl carboxylic acids (PFCAs), three perfluoroalkane sulfonic acids (PFSAs) and four compounds that have been increasingly produced as alternatives. A direct comparison of alternatives and PFAAs and an assessment of their bioaccumulative potential was possible by using one consistent method.

The obtained matrix/water partition coefficients ($K_{matrix/w}$) of the PFAAs are relatively high compared to other organic acids in the respective matrices. Log $K_{matrix/w}$ (with K in L_w/L_{matrix}) of PFAAs range from 2.5 to 4.8 for albumin, 2.3 to 4.9 for membrane lipids, and 0.5 to 3.4 for structural proteins. Sorption to storage lipids was rather low (log K < -0.5) for all compounds. For all of the investigated matrices, the trend in sorption behavior of the PFAAs was similar insofar as the respective matrix/water partition coefficient, $K_{matrix/w}$, increased with increasing number of

perfluorinated carbons and the PFSAs sorbed more strongly than their carboxylic counterparts (same number of perfluorinated carbons). Compared to the classical PFAAs all four alternatives showed similarly high $K_{matrix/w}$. Neither the ether linkages nor other structural modifications seem to substantially affect the sorption behavior to physiologically relevant matrices. This indicates that the alternatives – although structurally modified – are as bioaccumulative as the classical PFAAs.

The determined equilibrium partition coefficients were used together with data on the physiological composition to estimate the distribution of these compounds in the model organisms of human and rat. According to our calculations, albumin, membrane lipids and structural proteins are the matrices with the highest sorption capacities for PFAAs and alternatives. Thus, all three matrices should be included when modeling the accumulation of these compounds. The sorption to storage lipids is - despite the high proportion of this matrix in an organism – only of minor relevance in the accumulation of PFAAs and alternatives.

The approach was evaluated by comparing the predicted to measured PFAA distribution data in various organs reported in literature. For this, the relative PFAA concentrations were calculated in adipose tissue, blood, brain, gonads, gut, heart, kidney, liver, lungs, muscle tissue, skin and spleen. The evaluation focused mainly on PFOA as most data in the literature were available for this compound. For the majority of the organs, the predicted PFOA concentrations matched the reported concentrations with deviations of typically less than two times. The good correlation confirms that our approach represents all relevant sorption processes. For liver and brain, our calculations consistently under- and overpredicted the measured concentrations, respectively. Active transport processes likely affect the distribution of PFAAs in these two organs, underlining that future research should focus on tissue-specific kinetic studies.

This work provides a complete overview of all relevant physico-chemical distribution processes for PFAAs and four of their alternatives. The insights gained from this study can be incorporated and considered when building physiologically based toxicokinetic models of various complexity. This will help to assess the risk of PFAAs and structurally similar compounds to organisms, which is an urgent task, especially in light of the growing number of highly fluorinated alternatives.

Zusammenfassung

Perfluoroalkylsäuren (PFAAs) sind die ersten überwiegend ionisch vorliegenden organischen Verbindungen von denen bekannt ist, dass sie sich in Organsimen anreichern (bioakkumulieren). Dies und die Tatsache, dass PFAAs sehr persistent sind, haben in den letzten zwei Jahrzenten Anlass zur Besorgnis gegeben. Im Gegensatz zu den typischen hydrophoben Schadstoffen, die sich primär im Fettgewebe anreichern, beruht das Akkumulationsverhalten von PFAAs auf anderen physiko-chemischen Eigenschaften und ist immer noch nicht vollständig verstanden. Dies macht die Vorhersage ihres biokkumulativen Verhaltens komplexer. Potenziell toxische PFAAs wie die Perfluoroctansäure (PFOA) wurden im industriellen Gebrauch durch strukturell modifizierte Alternativstoffe ersetzt, die zwar oft mehr polare Gruppen wie zum Beispiel Etherbindungen in ihrer Struktur aufweisen, jedoch immer noch hoch fluoriert sind. Diese Alternativstoffe haben sich bereits als ähnlich persistent wie die klassischen PFAAs herausgestellt. Die Diskussion, ob sie ein geringeres bioakkumulatives Potenzial als die PFAAs besitzen, ist jedoch noch nicht abgeschlossen.

Die Akkumulation einer Verbindung hängt unter anderem davon ab, wie stark diese Verbindung an allen potenziell relevanten physiologischen Matrizen in einem Organismus sorbiert. Im Falle der PFAAs sind diese das Plasmaprotein Albumin, Membranlipide, Strukturproteine und Speicherlipide. Diese Matrizen könnten entweder relevant sein, da sie eine starke Bindungsaffinität für PFAAs aufzeigen oder da sie einen hohen Anteil im Organismus ausmachen.

Um unser Verständnis des bioakkumulativen Verhaltens von PFAAs und Alternativstoffen grundlegend zu verbessern, war es Ziel dieser Arbeit, die Sorption an den genannten physiologischen Matrizen für mehrere dieser Verbindungen zum ersten Mal systematisch unter physiologischen Bedingungen zu untersuchen. Dafür wurden zunächst die Verteilungskoeffizienten zwischen der jeweiligen Matrix und Wasser für eine Reihe an PFAAs, darunter sechs Perfluoroalkylcarbonsäuren (PFCAs), drei Perfluoroalkylsulfonsäuren (PFSAs), und darüber hinaus vier ihrer Alternativstoffe mittels Dialyseexperimenten bestimmt. Durch Anwendung einer konsistenten Methode war ein direkter Vergleich von Alternativstoffen und klassischen PFAAs – und damit eine Aussage über deren bioakkumulatives Potential – möglich.

Die ermittelten Matrix/Wasser-Verteilungskoeffizienten ($K_{matrix/w}$) der PFAAs sind im Vergleich zu anderen organischen Säuren in den jeweiligen Matrizen relativ hoch. Logarithmierte $K_{matrix/w}$ (mit K in L_w/L_{matrix}) der PFAAs liegen zwischen 2.5 und 4.8 für Albumin, 2.3 und 4.9 für Membranlipide, und 0.5 und 3.4 für Strukturproteine. Die Sorption an Speicherlipiden ist für alle Verbindungen

hingegen sehr niedrig (log K < -0.5). An allen untersuchten Matrizen zeigen die PFAAs einen ähnlichen Trend im Sorptionsverhalten, insoweit dass die jeweiligen Matrix/Wasser-Verteilungskoeffizienten, $K_{matrix/w}$, mit wachsender Anzahl an perfluorierten Kohlenstoffen steigen und PFSAs stärker an die jeweilige Matrix sorbieren als die entsprechenden PFCAs (mit gleicher Anzahl perfluorierter Kohlenstoffe). Für alle Alternativstoffe sind die ermittelten $K_{matrix/w}$ ähnlich hoch wie für die PFAAs. Weder die enthaltenen Ethergruppen noch andere strukturelle Modifikationen scheinen das Sorptionsverhalten an den physiologisch relevanten Matrizen stark zu beeinflussen. Dies deutet darauf hin, dass die Alternativstoffe – trotz struktureller Unterschiede – wahrscheinlich ein ähnlich hohes bioakkumulatives Potenzial wie die PFAAs aufweisen.

Die experimentell bestimmten Verteilungskoeffizienten wurden zusammen mit Daten zur physiologischen Zusammensetzung verwendet um die Verteilung der Verbindungen in den Modellorganismen Mensch und Ratte abzuschätzen. Den Berechnungen zufolge weisen Albumin, Membranlipide und Strukturproteine die höchsten Sorptionskapazitäten für PFAAs und Alternativstoffe auf. Aufgrund dessen sollten alle drei Bestandteile für die Vorhersage der Akkumulation dieser Verbindungen berücksichtigt werden. Die Sorption zu Speicherlipiden spielt trotz des hohen Anteils dieser Matrix am Organismus eine untergeordnete Rolle für die Verteilung von PFAAs und Alternativstoffen.

Um den verwendeten Ansatz zu validieren, wurde die Verteilung der PFAAs anhand von Rechnungen modelliert und mit gemessenen Verteilungsdaten in mehreren Organen aus der Literatur verglichen. Dafür wurden relative PFAA Konzentrationen in Blut, Fett, Gehirn, Geschlechtsdrüsen, Haut, Herz, Leber, Lunge, Magen, Milz, Muskel und Niere berechnet. Die Validierung bezog sich hauptsächlich auf PFOA, da für diese Verbindung die meisten Daten in der Literatur verfügbar waren. Für die Mehrzahl der Organe lagen die berechneten PFOA Konzentrationen mit Abweichungen von meist weniger als dem zweifachen nahe bei den Literaturwerten. Diese gute Korrelation belegt, dass in unserem Ansatz alle relevanten Sorptionsprozesse einbezogen sind. In Gehirn und Leber wurden die PFOA Konzentrationen durch unseren Ansatz jeweils konsistent über- beziehungsweise unterschätzt. Dies deutet darauf hin, dass aktive Transportprozesse die Verteilung von PFAAs in diesen beiden Organen beeinflussen. Zukünftige Forschung sollte sich deswegen auf gewebespezifische kinetische Untersuchungen konzentrieren.

Insgesamt stellt die vorliegende Arbeit wesentliche Informationen bereit, die in Modellierungsansätzen wie zum Beispiel in physiologisch basierten toxikokinetischen Modellen verschiedenster Komplexität genutzt oder berücksichtigt werden können. Dies trägt somit zur Risikobewertung von PFAAs und strukturell ähnlichen Verbindungen bei, was insbesondere im Hinblick auf die wachsende Anzahl von hoch fluorierten Alternativstoffen eine drängende Aufgabe darstellt.

Preface

The present work was performed from August 2017 to August 2020 at the Helmholtz Centre for Environmental Research Leipzig in the Department Analytical Environmental Chemistry. The thesis is written as a monograph and is based on the following articles.

Allendorf F., Berger U., Goss K.-U., and Ulrich N., Partition coefficients of four perfluoroalkyl acid alternatives between bovine serum albumin (BSA) and water in comparison to ten classical perfluoroalkyl acids. Environmental Science: Processes and Impacts. 2019, 21(11):1852-1863.
The first author conducted the experiments, analyzed the data and wrote the manuscript. N. Ulrich and K.-U. Goss contributed to the design of the experiments. N. Ulrich, U. Berger and K.-U. Goss critically revised the manuscript.

Ebert A., Allendorf F., Berger U., Goss K.-U., and Ulrich N., Membrane/water partitioning and permeabilities of perfluoroalkyl acids and four of their alternatives and the effects on toxicokinetic behavior. Environmental Science and Technologies. 2020, 54 (8):5051–5061.
The first two authors contributed equally to the manuscript. The first stated author conducted the permeability experiments and the second stated author conducted the dialysis cell experiments, determining the sorption of PFAAs to membrane lipids. Both N. Ulrich and K.-U. Goss contributed to the design of the experiments. Together with U. Berger they critically revised the manuscript.

Allendorf F., Goss K.-U., and Ulrich N., Estimating the equilibrium distribution of perfluoroalkyl acids (PFAAs) and four of their alternatives in mammals. 2021, Environmental Toxicology and Chemistry. 40 (3): 910-920.
The first author conducted the experiments, analyzed the data and wrote the manuscript. N. Ulrich and K.-U. Goss contributed to the design of the experiments and the distribution calculations and critically revised the manuscript.

Note that text passages, tables and figures in this monograph are partly taken from the above listed publications without further indication. The abstracts of the original publications were included in Chapter 6.

Content

1. Introduction

Long-chain perfluoroalkyl acids (PFAAs) are structurally defined by their acidic functional group attached to an alkane chain in which all hydrogen atoms are substituted by fluorine. PFAAs include both perfluoroalkyl carboxylic acids (PFCAs, $C_nF_{2n+1}COOH$, $n \geq 7$) and perfluoroalkane sulfonic acids (PFSAs, $C_nF_{2n+1}SO_3H$, $n \geq 6$).[1] The high electronegativity of fluorine is responsible for the strong acidic nature (p*K*a is estimated below 1)[2,3] as well as for the extremely stable C-F bonds. Their physico-chemical stability coupled with their surface active property have led to their use in a wide range of applications. Since their introduction in the 1950s they have been used as processing aids for the fluoropolymer manufacture, components in aqueous film-forming fire-fighting foams, or as mist suppressants in the chromium plating industry, amongst others. Additionally, other highly fluorinated compounds that have been or are still used in surface treatment of textiles, leather, paper and board can degrade in the environment to form PFAAs.[4] As a result these compounds are ubiquitously present in the environment where they have been found to accumulate in wildlife and humans.[5-7] PFAAs are also highly persistent in the environment and have thus been identified as contaminants of high concern.[8,9] Compounds such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are further suspected to be organ and developmental toxic to human as well as carcinogenic among highly exposed members of general populations.[10,11]

Due to these concerns, the use of long-chain PFAAs has been restricted by voluntary and regulatory measures, leading to a shift in production towards short-chain PFAAs and alternative compounds over the last two decades.[12] These alternatives are often ether-based polyfluorinated structures that exhibit a strong similarity to PFAAs. Chemical structures of two prominent PFAAs and four representatives of alternatives are shown in Figure 1. While it was shown that these alternatives are still persistent,[13-17] their bioaccumulative and toxic potential compared to long-chain PFAAs is still under discussion.[16,18-21] Recently, for one of the alternatives (tetrafluoro-2-(heptafluoropropoxy)-propanoate, HFPO-DA) similar adverse effects as for PFOA were reported in pregnant mice and their offspring at same dose levels.[20] Yet, it was shown that the elimination half-live of HFPO-DA (i.e. the bioaccumulative potential) was significantly smaller than that of PFOA in rats.[16] Other monitoring studies have shown that the alternative 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS) accumulates strongly in the crucian carp.[22]

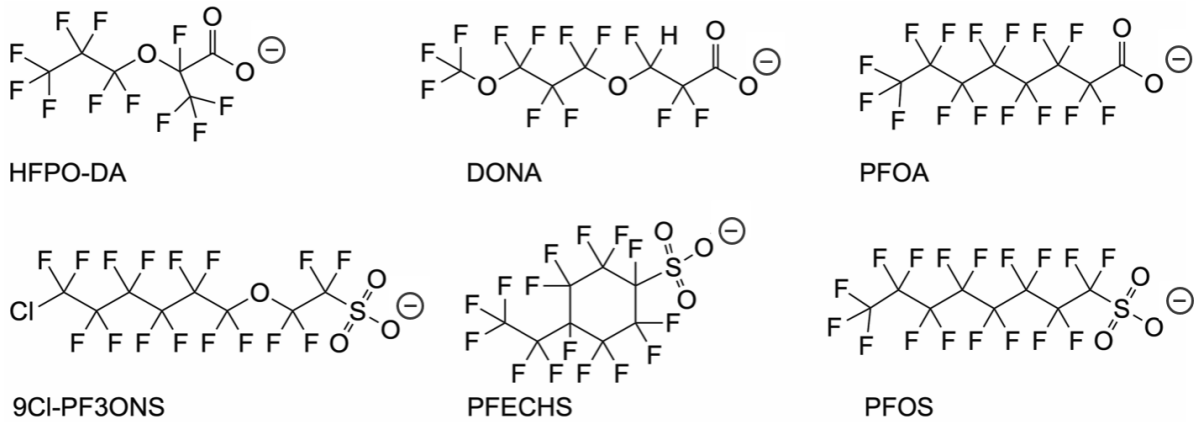

Figure 1. Chemical structures of four alternatives to long-chain perfluoroalkyl acids and two of the PFAAs that were included in this work. Above: tetrafluoro-2-(heptafluoropropoxy)-propanoate (HFPO-DA) and 4,8-dioxa-3H-perfluorononanoate (DONA), compared to perfluorooctanoate (PFOA). Below: 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS) and perfluoro-4-ethylcyclohexanesulfonate (PFECHS) compared to perfluorooctanesulfonate (PFOS). Indeed, PFECHS and 9Cl-PF3ONS were already produced before the phase-out of long-chain PFAAs was initiated[23] and were not originally synthesized to replace PFAAs. Both compounds are included as alternatives in the study since they have been increasingly used over the last decade.[24-26]

For risk assessment, characterization of the bioaccumulative potential of PFAAs and their alternatives is crucial. Models that allow accurate predictions to which extent PFAAs and their alternatives accumulate in organisms can contribute to bioaccumulation assessment with a reduced number of animal testing. The accumulation of a compound in an organism depends on several toxicokinetic factors involving the absorption, distribution, metabolism and excretion (ADME). Physiologically based toxicokinetic (PBTK) models of various complexities are used to describe and predict concentration-time curves based on the ADME processes. An important input information for such models are equilibrium partition coefficients between various physiological matrices (e.g. lipids, proteins) and water. While the required partition coefficients can be predicted quite well for most neutral chemicals,[27] such predictions become much more difficult for ionizable chemicals and even the more so for perfluorinated acids.[28,29] The partition behavior of PFAAs differs strongly from other compounds due to their unique chemical properties. Highly fluorinated compounds have much smaller van der Waals interactions compared to non-fluorinated molecules of the same size.[30,31] Hence, to improve the prediction of the partition behavior for the whole substance class and consequently to contribute to valid PBTK models, the experimental determination of partition coefficients to physiologically relevant matrices for some of these compounds is essential.[32-34]

A number of physiological matrices with high sorption capacities for PFAAs have been reported. These were serum albumin as a transport protein in blood, phospholipids as the major component of cellular membranes (hereinafter termed membrane lipids) and alpha globulins as well as liver fatty acid binding proteins (FABPs).[35-37]

For albumin and storage lipids, the equilibrium partitioning of PFAAs and alternatives to these matrices might be affected under certain conditions. For albumin, it has been claimed in literature that PFAAs compete with other ligands such as fatty acids for specific binding sites on that protein.[35,38] A competition for specific binding sites would affect the albumin/water partition coefficients of PFAAs. It was additionally reported that this competitive behavior depends on the chain-length of the PFAA.[39,40]

In the past, storage lipids have only been considered for neutral compounds as a possible sorbing matrix while they are usually neglected for ions. Yet, in two cases, storage lipids could become relevant. These highly acidic compounds are predominantly anionic. If they form ion pairs with a cation the resulting net-neutral molecule could sorb preferably to neutral storage lipids. Another possibility that could affect the sorption to storage lipids involves the high surface activity of PFAAs that could lead to a sorption to the interfacial area between the storage lipids and the surrounding aqueous phase.

Based on these considerations the following research objective of this work was formed.

2. Research Objective

The aim of this research is to improve the understanding and prediction of the bioaccumulative behavior of PFAAs and the increasing number of highly fluorinated alternatives. To this end, partition coefficients towards relevant biological matrices (albumin, membrane lipids, structural proteins, storage lipids) were determined experimentally for a set of PFAAs and four alternatives to long-chain PFAAs in a first part. In a second part, the accumulation for PFAAs and alternatives in mammals using physiologically based distribution calculations was investigated.

In the first part, the sorption behavior of a series of six PFCAs, three PFSAs and four alternatives to albumin, membrane lipids, structural proteins and storage lipids is systematically determined to each of the matrices under physiological conditions. The use of one consistent method for each matrix allows direct comparison of the sorption behavior of alternatives and PFAAs. Thus, this first part also addresses the question whether alternative compounds are less concerning than the classical PFAAs in regard to their bioaccumulative potential.

In the second part, focus is on predicting the distribution of PFAAs and alternatives considering all physiologically relevant matrices in mammals using the corresponding partition coefficients. These physiologically based distribution calculations enable us to identify matrices and organs that show a high sorption capacity for these compounds. Our approach can be further used to calculate relative concentrations of PFAAs and alternatives in individual organs.

The comparison of calculated with measured organ concentrations in rats and humans allows to evaluate our approach, meaning to what extent we can assess bioaccumulation of PFAAs and alternatives by accounting for the equilibrium partitioning. In addition, knowledge gaps can be identified that facilitate to narrow down open research questions improving overall the prediction of bioaccumulation for these compounds.

3. Experimental determination of equilibrium partition coefficients

3.1. Materials and Methods

3.1.1. Investigated compounds

Sodium dodecafluoro-3H-4,8-dioxanonanoate (DONA), 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-)propanoic acid (HFPO-DA/GenX), potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS, main component of F-53B), potassium perfluoro-4-ethylcyclohexanesulfonate (PFECHS) and a mixture of PFAA calibration standards (PFAC-MXB) and internal standards (MPFAC-MXA) were supplied by Wellington Laboratories (Ontario, Canada). PFAAs were purchased individually from various manufacturers (Table 1). For each compound, a stock solution was prepared in methanol and stored at -18°C.

Table 1. Overview of all investigated compounds including a series of PFAAs and four alternatives to long-chain PFAAs, along with their abbreviations, suppliers and purities.

Compound	Abbreviation	Supplier	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[a]	**PFBS**	Fluka	> 98 %
Perfluorohexane sulfonic acid[b]	**PFHxS**	Fluka	98 %
Perfluorooctane sulfonic acid[b]	**PFOS**	Fluka	98 %
Tetrafluoro-2-(heptafluoropropoxy)-propanoic acid	**HFPO-DA/GenX**	Wellington Laboratories	> 98 %
Dodecafluoro-3H-4,8-dioxanonanoate[c]	**DONA**	Wellington Laboratories	> 98 %
9-chlorohexadecafluoro-3-oxanonane-1-sulfonate[b]	**9Cl-PF3ONS**	Wellington Laboratories	> 98 %
Perfluoro-4-ethylcyclohexanesulfonate[b]	**PFECHS**	Wellington Laboratories	> 98 %

[a]purchased as a tetrabutylammonium hydroxide salt [b]purchased as a potassium salt [c]purchased as a sodium salt

3.1.2. Buffer solutions

For dialysis experiments with serum albumin, membrane lipids and storage lipids, Hank's balanced salt solution (HBSS) was supplied as a powder from Sigma Aldrich (Munich). To mimic physiological conditions[41] sodium carbonate (Sigma Aldrich; 0.35 g/L) and TRIS (Roth, Karlsruhe; 1.21 g/L) were added and pH was adjusted to 7.4.

For dialysis experiments with structural proteins, 30 mM phosphate buffer was prepared with potassium dihydrogen phosphate (2.42 g/L; Sigma Aldrich, Munich), dipotassium hydrogen phosphate trihydrate (2.78 g/L; Sigma Aldrich) in bidestilled water. After a pH of 7.7 was adjusted, 10 mL of 1 N potassium hydroxide (KOH) was added to neutralize organic acids (e.g. lactic acids).[42]

To both buffer solutions, NaN_3 (Merck, Darmstadt) was added as an antimicrobial agent to the concentration of 0.3 g/L at which no effects have been reported.[41,43]

3.1.3. Investigated physiological matrices

In the following, the preparation of the different physiological matrices are described that were used to determine albumin/water partition coefficients ($K_{alb/w}$), membrane lipid/water partition coefficients ($K_{ml/w}$), structural protein/water partition coefficients ($K_{sp/w}$) and storage lipid/water partition coefficients ($K_{sl/w}$).

Albumin

Lyophilized powders of bovine serum albumin (BSA) were supplied by Sigma Aldrich (Munich) with the product numbers A3803 (heat shock fraction, essentially fatty acid free, ≥98 %) and A7906 (heat shock fraction, pH 7, ≥98 %). Partition coefficients presented in Table 2, Section 3.2.1. were measured only with batch A3803, essentially fatty acid free. A3803 was the same batch used in experiments where partition coefficients of other anionic compounds were determined.[29] Solutions of albumin were prepared freshly with HBSS for each experiment.

Membrane lipids

Synthetic powder of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC; Avanti Polar Lipids (Alabaster, Alabama; > 99 %)) was used to prepare spherical vesicles of phospholipid bilayers (liposomes). Experiments with membrane lipids composed of POPC allowed a direct comparability to results from Bittermann et al.[44] Liposomes were prepared as described before.[45,46] POPC was weighed and dissolved in chloroform. A thin film of the suspension was formed using a rotary evaporator and was dried overnight. Buffer solution (HBSS) was added and multilamellar vesicles

were formed under gentle agitation. The suspension was subjected to a freeze-and-thaw cycle (10x) with liquid nitrogen to produce intermediate-sized unilamellar vesicles. The suspension was then extruded tenfold through a polycarbonate membrane with 0.1 µm pore size (Whatman, Maidstone) in a mini-extruder (Avanti Polar Lipids) at room temperature, generating homogeneous POPC-liposomes.[47] Liposome stock solutions were subsequently used for the dialysis experiments. The POPC content was determined indirectly measuring the phosphorus content using ion chromatography (ICS-6000, Thermo Scientific, Waltham, Massachusetts). After addition of potassium persulfate, the solution was incubated at 90°C overnight, according to a protocol by Huang et al.[48] The recovery of the initially weighted POPC was analyzed for each experiment. Recovery ranged from 72 – 120 %. The resulting error in the corresponding $K_{ml/w}$ was small compared to the error resulting from the experimental setup and quantification (which is in the range of ± 0.2 log units).

Structural proteins

Structural proteins were extracted from chicken filet. In brief, these proteins were extracted, freeze-dried, ground, defatted and stored at -25°C until use.[42,49] For dialysis experiments, the extract was weighed directly into one of the dialysis chambers (see below) and diluted with phosphate buffer on the day of the experiment.

Storage lipids

Either olive oil with a high fraction of unsaturated fatty acid (cold pressed, extra native, Bioplanet) or fresh milk with 3.8 % fat content (homogenized, pasteurized) was bought from a local grocery store.

3.1.4. Dialysis experiments for the determination of $K_{alb/w}$, $K_{ml/w}$ and $K_{sp/w}$

Individual methanol stock solutions of poly- and perfluorinated compounds were diluted in buffer solution (≈20 µg/L) and samples of these dilutions were quantified to determine the exact concentration. Dialysis cells were used as described in detail before.[29,50] They were composed of two glass chambers (custom-made) separated by a cellulose membrane (see Figure 2). These dialysis membranes (Spectrum Laboratories Inc., Rancho Dominguez, California) exhibit specific pore sizes allowing permeation of the poly- and perfluorinated compounds with retention of the matrix. For albumin and membrane lipids, the dialysis membranes were used with a molecular cutoff of more than 12-14 kDa, for structural proteins comprising diverse molecule sizes the dialysis membranes were used with a cutoff of 3.5 kDa. Test and reference cells were prepared

for each compound in triplicates. For the test cells, the solution of the investigated matrix was added in one chamber (final volume of 5 mL), while for the reference cells, this chamber contained only buffer solution. In both, test and reference cells, 5 mL compound buffer solution were added into the second chamber. The reference cells were used to check for equilibrium between the two chambers and for determining the freely dissolved compound mass in total (mass balance was applied due to loss of compounds by adsorption on the glass surface, see calculations below).

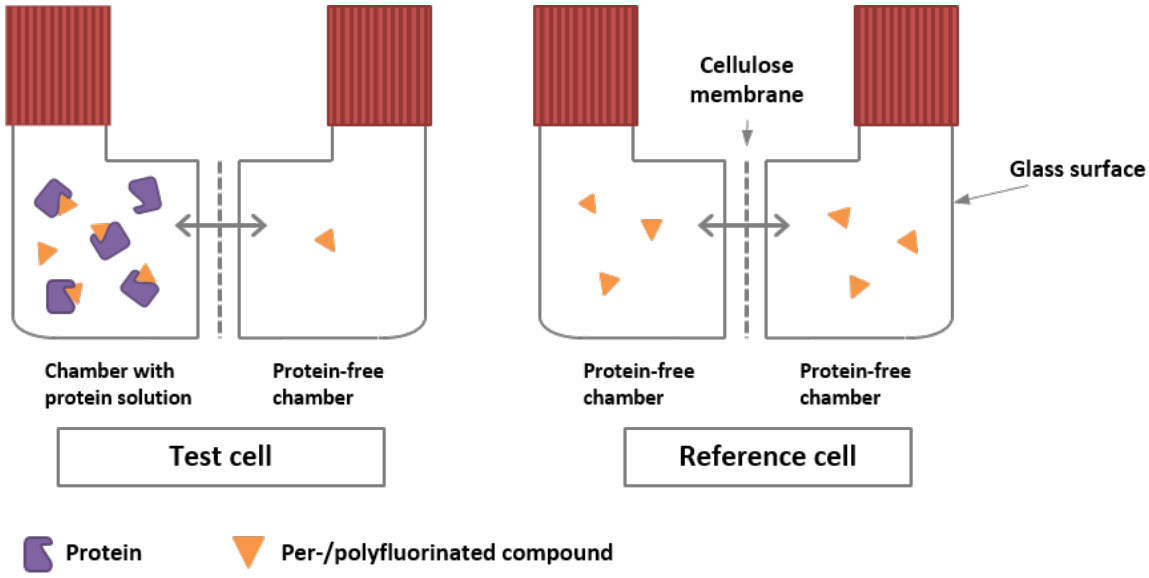

Figure 2. Setup of dialysis cells that were used for investigating the sorption to albumin, structural proteins and membrane lipids. Here, proteins are depicted as an example. Dialysis cells composed of two glass chambers separated by a cellulose membrane. The latter has a specific molecular cutoff value that prevents the proteins from permeating freely. There are two types of dialysis cells, test cells and reference cells. At the beginning of the experiment, protein solution was added to one chamber while compound solution was added to the other chamber. For reference cells, one chamber initially received only buffer while the other chamber received the compound solution. Equilibration time was 96 h for long-chain PFAAs such as PFDA, PFUnDA, PFOS, 9Cl-PF3ONS and PFECHS, for all other compounds it was 72 h.

Dialysis cells were incubated at 37°C in darkness under constant agitation (470 rpm). Matrix-free chambers of both measurement and reference cells were sampled on two consecutive days at least and partition coefficients were determined. When both values matched, equilibrium could be assumed. Most of the compounds required an equilibrating time of 72 h, except for longer-chain PFAAs like PFDA, PFUnDA and PFOS, and 9Cl-PF3ONS and PFECHS which needed 96 h to equilibrate. For each compound, the fraction sorbed to the matrix was kept between 20 – 80 % to reduce measuring uncertainty (Figure 3). To this end, the concentration of matrix in solution was adapted with respect to the sorption behavior of the investigated compound (see results section

for individual concentrations). For all matrices, it was assumed that the equilibrium partition coefficients were determined within the linear range of the sorption isotherm and that for the investigated proteins, no ligand oversaturation of the protein binding sites occurred.[42-44,50]

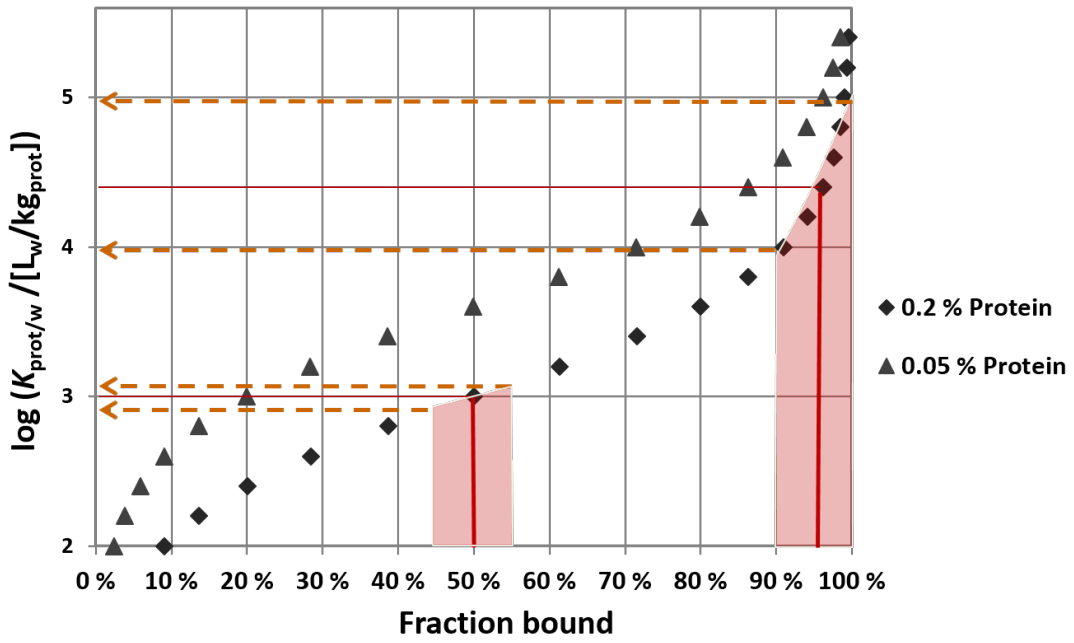

Figure 3. The relation between measuring uncertainty and fraction bound on the determination of the partition coefficient, exemplified for determination of a protein/water partition coefficient. To ensure the accuracy of the determined partition coefficient the fraction bound to the proteins should be between 20 – 80 %. Below or above that fraction, small measuring uncertainties could have significant effects on the determined partition coefficient, since in this ranges partition coefficients alter drastically.

Albumin: Competition experiments with fatty acids

In the context of the determination of $K_{alb/w}$, we conducted dialysis experiments with PFHxA or PFNA adding known amounts of two different fatty acids (tridecanoic acid (TDA, Fluka, Munich) or heptadecanoic acid (HDA, Fluka)) to test if free fatty acids and PFAAs would compete for specific albumin binding sites. For that, essentially fatty acid free BSA (batch A3803) was first equilibrated with the respective fatty acid for 24 h before adding the perfluorinated compound. Each setup had a specific molar ratio of fatty acid to albumin with approximate [fatty acid] : [albumin] ratios of 2.5, 1 and below 1, respectively. Samples of the BSA-free chambers were taken separately for fatty acid analysis and PFAA analysis at the same time points (after 72 h and 96 h).

Furthermore, a test was performed to determine week-to-week precision for the determination of the $K_{alb/w}$ for PFNA. For that, dialysis experiments were carried out three times within seven

months based on the setup described above, each time with three replicates. The precision was calculated by taking the standard deviation of nine partition coefficients into account.

3.1.5. Batch experiments for determination of $K_{sl/w}$

Experiments for the determination of $K_{sl/w}$ were conducted with solutions consisting of olive oil and an aqueous phase. For the aqueous phase, buffer solution in three dilutions (100 %, 10 %, or 1 % HBSS, Section 3.1.2) or pure water (bidistilled) was chosen to test whether the compounds form ion pairs with physiological cations. In these batch experiments all PFAAs and alternatives were added to the aqueous phase in a mixture. The partitioning process of individual compounds should not be affected in a mixture because the intermolecular interactions in the bulk phases are nonspecific (van der Waals forces) and the compounds do not have to compete for binding sites.

Oil and the respective aqueous phase was added to a total volume of 1 mL in polypropylene vials (Labsolute, Renningen). Vials with oil and aqueous phase (test vials) and vials without oil (reference vials) were prepared in triplicates each, similar to the set-up of the dialysis experiments. Vials were shaken at 250 rpm for 24 h at room temperature. Sampling of the aqueous phase was done after phase separation.

To check whether partitioning of PFAAs in a storage lipids/water system is dominated by partitioning into the interface between the storage lipids and the surrounding aqueous phase, calculated and experimentally determined milk/water partition coefficients ($K_{milk/w}$) were compared. Homogenized milk is an emulsion of water and dispersed fat globules. These globules contain storage lipids, but also membrane lipids and proteins and show a high interfacial area to the aqueous phase.[51] $K_{milk/w}$ for three PFCAs (PFOA, PFNA, PFDA) were determined directly with fresh milk by conducting dialysis experiments similar to the protocol described above. For comparison, $K_{milk/w}$ for these PFCAs were calculated based on the bulk phase partition coefficients to all milk components and the milk composition. If the experimental overall partition coefficient would be higher than the one calculated based on all known absorption processes, then this would be an indication that an additional sorption process – likely adsorption at the storage lipids/water interface – played a role. Experimental details, milk composition along with the calculations can be found in Appendix (App.) A.1. Milk composition

3.1.6. Chemical quantification

The analysis of PFAAs and alternatives was performed using Ultra Performance Liquid Chromatography with tandem mass spectrometry (UPLC-MS/MS; Xevo TQ-S Waters Corporation, Milford, Massachusetts) as described elsewhere.[52,53] To all samples, ammonium acetate (NH_4Ac) in methanol and internal standard solution were added to a final concentration of 2 mM and 1 ng/mL, respectively. Separation was achieved with the column ACQUITY UPLC BEH Shield C18 (1.7 µm particles; 2.1 mm x 50 mm). A "PFC isolator column" (Waters) was used to retain and separate method blanks originating from the mobile phases or the HPLC-system. The detector was operated in negative electrospray ionization mode (ESI^-) and subsequent multiple reaction monitoring for quantification. Instrumental parameters, the assignment of internal standards to compounds and MS/MS transitions and are reported in App. A.2. Quantification method Data acquisition, processing and analysis were done by the Water's proprietary software MassLynx (version 4.1). Quantification was achieved by an external linear calibration.

3.1.7. Determination of equilibrium partition coefficients

We used the following formula to calculate the matrix/water partition coefficients ($K_{alb/w}$ in L_w/kg_{alb}, $K_{ml/w}$ in L_w/kg_{ml}, $K_{sp/w}$ in L_w/kg_{sp} and $K_{sl/w}$ in L_w/L_{sl}) for a compound:

$$K_{i,matrix/w} = \frac{c_{i,matrix}\ *}{c_{i,w}\ *} \qquad \text{Eq. 1}$$

where i refers to the compound and *states the equilibrium condition. $c_{i,matrix}$ is the compound concentration in the physiological matrix (g/kg_{alb}, g/kg_{ml}, g/kg_{sp} or g/L_{oil}, respectively) and $c_{i,w}$ is the concentration in aqueous phase (g/L_w).

$c_{i,matrix}$ cannot be measured directly. Therefore, a mass balance approach was used to determine $c_{i,matrix}$ indirectly (Eq. 2). The freely dissolved compound mass quantified in the test cells or vials ($m_{i,w}$) is compared to that mass in the reference cells or vials without matrix ($m_{i,ref}$)

$$\mathrm{m}_{i,\mathrm{matrix}} = \mathrm{m}_{i,\mathrm{ref}} - \mathrm{m}_{i,\mathrm{w}} \qquad \text{Eq. 2}$$

PFAAs and their alternatives sorb to the glass[54] and membrane surfaces of the dialysis cells as determined in pre-tests. For PFDA, PFUnDA, PFOS and 9Cl-PF3ONS that sorbed 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. For these compounds an extraction step was added to fulfil the total mass balance criterion. To this

end, all fluids were discarded after the last sampling and the interior of the cells was extracted with 2 mL of methanol. The compound mass was analyzed and $m_{i,matrix}$ was then calculated taking into account the loss by adsorption. Considering these adsorption effects, overall recoveries varied between 82 – 115 % dependent on the investigated matrix. The individual recoveries are listed in result sections for the respective matrices. (The polypropylene vials for determining $K_{sl/w}$ were identically shaped. The adsorption to the surface of the vials could therefore be compensated by using the reference cells.)

For determination of $K_{i,sl/w}$, mean and standard deviation of three measurements were taken. For determination of the other $K_{i,matrix/w}$, mean and standard deviation of six measurements (three measurements on each of two days) were taken.

3.2. Results and Discussion

In the following four sections, results of the sorption of the series of PFAAs to the individual physiological matrices are discussed in details. In these sections, results for the investigated alternatives are only shortly described.

The discussion concerning the sorption behavior of alternatives compared to PFAAs can be found in the last section of this chapter.

3.2.1. Albumin/water partition coefficients $K_{alb/w}$

$K_{alb/w}$ were determined by dialysis experiments using bovine serum albumin (BSA). We expect similar partition coefficients for BSA and human serum albumin (HSA) and used the cheaper BSA in our studies. BSA and HSA share a sequence homology of 76 %.[55] They differ in length by two amino acid residues and fold into structurally similar globular tertiary structures.[56] Previously it was shown that sorption to BSA compared to HSA is in the same order of magnitude[57] for compounds like fatty acids as well as for PFOA and PFNA.[58,59]

Log $K_{alb/w}$ range from 2.5 to 4.8 (K_a =2.3 × 10^4 – 4.4 × 10^6 M^{-1}; Table 2 and Figure 5 in Section 3.2.5). When considering the sorption behavior of other organic anions to albumin, the partition coefficients of PFAAs are comparably high but in the same range as ibuprofen and 2-naphthaleneacetic acid (log $K_{alb/w}$ of 3.9 and 4.8, respectively).[29] Furthermore, PFCAs sorb more strongly to albumin than their hydrocarbon counterparts (C4-C11, measured at pH 7.6, 23°C).[60,61]

Table 2. Experimental logarithmic albumin/water partition coefficients (log $K_{alb/w}$ (with K in L water/kg albumin)) of a series of perfluoroalkyl acids and four of their alternatives. Association constants *(K_a)* are displayed to facilitate comparison with literature values.

Number of perfluorinated carbons	Compound	log ($K_{alb/w}$/ [L_w/kg_{alb}])[a]	K_a /[M^{-1}][b]	f_{bound}[c]	Recovery (including extracts)	c_{BSA}/ [g/L][d]
PFCAs						
3	**PFBA**	2.52 ± 0.11	2.3E+04 ± 4.8E+03	34 % ± 5 %	98 %	3.0E+00
5	**PFHxA**	3.43 ± 0.04	1.8E+05 ± 1.6E+04	58 % ± 2 %	98 %	1.0E+00
6	**PFHpA**	4.02 ± 0.08	7.2E+05 ± 1.4E+05	34 % ± 4 %	99 %	1.0E-01
7	**PFOA**	4.20 ± 0.05	1.1E+06 ± 1.1E+05	44 % ± 3 %	94 %	1.0E-01
8	**PFNA**	4.32 ± 0.07	1.4E+06 ± 2.2E+05	37 % ± 4 %	99 %	5.0E-02
9	**PFDA**[e]	4.73 ± 0.04	3.6E+06 ± 3.1E+05	57 % ± 2 %	57% (106%)	5.0E-02
10	**PFUnDA**[e,f]	4.60 ± 0.30	3.1E+06 ± 1.4E+06	35 % ± 13 %	40% (115%)	2.5E-02
	HFPO-DA[g]	3.06 ± 0.03	7.7E+04 ± 5.0E+03	64 % ± 1 %	96 %	3.0E+00
	DONA	3.93 ± 0.03	5.7E+05 ± 4.7E+04	30 % ± 2 %	93 %	1.0E-01
PFSAs						
4	**PFBS**	3.20 ± 0.11	1.1E+05 ± 2.8E+04	44 % ± 6 %	101 %	1.0E+00
6	**PFHxS**	4.81 ± 0.05	4.4E+06 ± 3.5E+05	62 % ± 2 %	92 %	5.0E-02
8	**PFOS**[e]	4.67 ± 0.07	3.2E+06 ± 4.9E+05	37 % ± 4 %	78% (104%)	2.5E-02
8	**9Cl-PF3ONS**[e]	5.01 ± 0.04	6.9E+06 ± 6.2E+05	56 % ± 2 %	75% (106%)	2.5E-02
8	**PFECHS**	4.55 ± 0.03	2.4E+06 ± 1.4E+05	66 % ± 1 %	95 %	1.0E-01

[a] ± standard deviation of six measurements (three measurements on two days). [b] Note that the association constant (K_a) is converted from $K_{alb/w}$ at one molar ratio and not determined by measuring the binding isotherms. [c] Fraction bound represents the mass of compound that is bound to albumin divided by the total available mass of compound in the reference cells ($m_{i,alb}/m_{i,ref}$). [d] Concentration of bovine serum albumin (BSA) used to determine $K_{alb/w}$. [e] Compounds sorbed substantially to the glass surface of the dialysis cells. For total mass balance, an extraction step with methanol was included and the partition coefficient and fraction bound was calculated with the mass that was not adsorbed to glass surface. [f] Mean and standard deviation of only five measurements. [g] HFPO-DA and DONA are not fully fluorinated, therefore no number of perfluorinated carbons is assigned.

Partition coefficients of the PFCAs increase with increasing chain-length, i.e. number of perfluorinated carbons. PFBA (3 perfluorinated carbons) and PFDA (9 perfluorinated carbons) have log $K_{alb/w}$ of 2.5 and 4.7, respectively. If this trend continues between PFDA and PFUnDA cannot be concluded from our experiments, due to the relatively higher uncertainty in the measurement for PFUnDA (SD > 0.3 log units). Also, $K_{alb/w}$ of longer chain-length analogues than of the PFCAs could not be determined. About 60 % of PFUnDA was adsorbed to the glass surface during the dialysis experiment. The required extraction with methanol for such long-chain

compounds increased the error in the resulting $K_{alb/w}$ and also limited the compounds that could be analyzed with our setup.

Among the PFSAs, PFBS has the lowest log $K_{alb/w}$ of 3.2, while PFHxS and PFOS show relatively high log $K_{alb/w}$ of 4.8 and 4.6, respectively. The log $K_{alb/w}$ values for PFHxS and PFOS are in the same order, however, a clear trend of increasing $K_{alb/w}$ with increasing molecular weight, which was observed for the PFCAs up to PFDA, cannot be seen for the investigated sulfonic acids.

PFSAs have in general higher $K_{alb/w}$ compared to PFCAs with the same number of perfluorinated carbons. Stronger sorption of PFSAs compared to their PFCA-counterparts is consistent with other studies.[62,63] The log $K_{alb/w}$ value of PFHxS is 0.8 higher compared to the log $K_{alb/w}$ of PFHpA whereas the partition coefficients of PFOS and PFNA differ in 0.4 log units. For PFBS, the corresponding carboxylic acid was not analyzed. We calculated 3D structures by the quantum chemical software COSMOconf/TURBOMOLE[63,64] to compare the conformers of PFHxS and PFOS, but no obvious differences were discernible. Reasons why binding of PFSAs to albumin is more favorable than PFCAs are still unclear and discussion is ongoing.[61,65-67]

Log $K_{alb/w}$ were 3.1 and 3.9 for the carboxylic acids HFPO-DA and DONA and 5.0 and 4.6 for the sulfonic acids 9Cl-PF3ONS and PFECHS, respectively and thus sorb in the same range as determined for the classical PFAAs.

Additionally, we performed a test with PFNA to assess week-to-week precision for the determination of partition coefficients (App. B.1. Precision experiments). PFNA was chosen as the test compound since it is a long-chain PFAA but still with less than 10 % sorption losses to cell surfaces. It was shown that within one experimental setup the standard deviation is smaller than that between the week-to-week setups, where standard deviation was determined to be 0.1 log units.

Another batch of BSA (A7906) was used to test if the determined $K_{alb/w}$ are dependent on the albumin selected for the experiments. See App. B.2. Batch A7906 for details. We found differences in the $K_{alb/w}$ up to 0.3 log units especially for short-chain PFAAs. Approximately the same overall standard deviation was determined for PFNA with both batches of BSA. In conclusion we assume that different batches of BSA could affect the value of determined partition coefficients. Variable extraction and purification steps could probably affect the conformation of the native serum albumin which could be a reason for the observed differences in the log $K_{alb/w}$ values.[68]

Albumin/water partition coefficients of PFAAs in literature

Over the last 20 years association constants (K_a) to albumin especially for PFOA were determined by electrophoresis[69], ^{19}F NMR[35,58], surface tension[69,70] and dialysis[66,71,72] and vary from 10^2 to 10^6 M^{-1}.[58,61,69,71,72] Further, studies using fluorescence spectroscopy included other PFAAs, deriving site-specific association constants on albumin.[65,73,74] Discrepancies were shown to exist not only between different methods, determined K_a also varied substantially within one method.[35,58,69,71] Reasons are probably indirect measuring methods and inconsistent experimental settings, such as temperature variations[58,61,72,74] and the highly variable molar ratios of [PFAA] : [albumin] (often >> 1)[35,58,61,69,71]. Sorptive losses to laboratory devices affecting the determination of K_a were checked or reported only rarely.[58,72] Additionally, the reported fractions bound were often greater than 80 %, a range where the propagated error in $K_{alb/w}$ could amount to one or two log units (Figure 3). Our association constants for PFOA and PFNA were 1.1×10^6 M^{-1} and 1.4×10^6 M^{-1}, respectively. Oversaturation can lead to an overestimation of the freely dissolved concentration of the compound and hence to an underestimation of the partition coefficient. In comparison, approximate physiological PFOA/albumin molar ratios in a worst case situation (with a albumin concentration of 35 g/L) would be 9×10^{-5} in general population and 2×10^{-3} in a high exposed population, i.e. in both cases far below a saturation of serum albumin, even when a mixture of PFAAs is taken into account.[75-78] Considering this, we conclude that other methods may underestimate the sorption of PFAAs to albumin in physiological systems.

Bischel et al. (2010 and 2011)[66,72] determined $K_{alb/w}$ of the same series of PFAAs (PFPeA-PFDoA and PFBS, PFHxS and PFOS) by a comparable equilibrium dialysis approach by means of direct quantification (LC-MS/MS). They used a molar ratio of [PFAA] : [albumin] < 1, but did their experiments at room temperature. According to their measurements, the highest $K_{alb/w}$ among the tested PFCAs was shown for PFHpA (log $K_{alb/w}$ = 4.2) and among the tested PFSAs for PFHxS (log K = 4.3; App. B.3. Comparison of $Kalb_{/w}$ with literature. For higher analogues they describe a decrease of the log $K_{alb/w}$ with increasing number of perfluorinated carbons (e.g. PFUnDA: log $K_{alb/w}$ = 3.7, PFOS: log $K_{alb/w}$ = 4.1). In contrast to Bischel et al. we found that the $K_{alb/w}$ of PFCAs increase with increasing number of perfluorinated carbons up to at least PFDA, which is also plausible on theoretical grounds.[30,79] Since the $K_{alb/w}$ of Bischel et al. were measured with very high fractions bound (> 95 %), their results are more likely affected by the measuring error (as discussed before). Bischel et al. (2011)[66] supported their observed trend by the following mechanistic explanation: PFCAs are supposed to undergo a conformeric change when the number of perfluorinated carbons exceeds six. While PFCAs below seven perfluorinated carbons show a zig-zag geometry, longer-

chain PFCAs adopt a helical twist in their structure.[80] However, we did not see such a difference in the respective 3D-structures for PFHxA and PFOA generated by mentioned quantum chemical-calculations with COSMOconf/TURBOMOLE.[63,64] Figures are included in App. B.3. Comparison of $Kalb_{/w}$ with literatureOur finding of a positive correlation with increasing number of perfluorinated carbons for PFCAs is supported by several other studies (including only a few PFAAs), although they measured in very high [PFAA] : [albumin] molar ratios.[40,58,65,74] Other organic ions also show an increase in the $K_{alb/w}$ with increasing alkyl chain length.[29] A similar sorption behavior was also observed for other compound classes such as saturated long-chain fatty acids to HSA.[81,82]

Influence of fatty acid binding

In literature it is often postulated that PFAAs compete for the specific albumin binding sites with fatty acids.[39,73] Serum albumin is the major transport protein for especially long-chain fatty acids and the structural similarity of perfluoroalkyl acids to fatty acids suggests a potential competition. D'eon et al.[38] showed that PFHxA is able to displace oleic acid even at low concentrations whereas this effect does not occur for PFOA, stressing that competing potency could be dependent on chain-length.

There are two different cases for a potential competition: (A) binding of the fatty acids to serum albumin is influenced by the presence of PFAAs and (B) binding of the PFAAs to serum albumin is influenced by the presence of fatty acids. Case (A) is not reasonable, the partition coefficients of the fatty acids are higher compared to the partition coefficients of the PFAAs and the concentration of fatty acids in blood is higher compared to concentration of PFAAs in the blood. For these reasons, a quantitative competition as described for case (A) and investigated by D'eon et al.[38] could not occur in humans. However, the sorption of PFAAs to serum albumin could be affected by fatty acids, which are present at high concentrations in the blood (case B). To investigate this potential competition and to answer the question whether our experimentally determined partition coefficients (with purified serum albumin) are representative for a physiological situation, where fatty acids are present in higher concentrations, PFHxA and PFNA were used as examples for PFCAs and tridecanoic acid (TDA) and heptadecanoic acid (HDA) as examples for fatty acids. Both fatty acids have an uneven number of carbons and therefore do not occur naturally. For this reason dialysis experiments and analysis could not be affected by impurities of ubiquitous fatty acids. HDA is a long-chain fatty acid and has higher physiological relevance (medium-chain fatty acids are barely measurable in human blood)[56] while TDA with its

higher solubility and a lower sorption capacity to serum albumin was more suitable for the experimental approach and analysis.

$K_{alb/w}$ for PFHxA and PFNA were measured in dependence of three different amounts of TDA or HDA, respectively. Fatty acid molar concentrations were adjusted to the physiological situation in the human blood ([fatty acid] : [albumin] ≈ 2.5 : 1)[83] and two smaller concentrations of the fatty acid to meet approximately equimolarity and below saturation (i.e. a ratio below 1). Detailed description on experimental setup can be found in App. B.4. Influence of fatty acids

For both compounds, PFNA and PFHxA, a slight decrease of determined $K_{alb/w}$ was indicated with increasing amounts TDA, but no concentration dependent alteration in $K_{alb/w}$ was observed with HDA. The determined log $K_{alb/w}$ of PHxA and PFNA remain close to 3.25 and 4.55, respectively with increasing amounts of this long-chain fatty acid (see App. B.4. Influence of fatty acids for a detailed discussion). Due to the higher relevance of HDA, it is expected that there is no significant competition of PFCAs and long-chain fatty acids in the human body and that the experimentally determined $K_{alb/w}$ (Table 2) are representative for a physiological situation. But one should be aware that only two representatives were selected in the conducted experiments (with odd number of carbons), which does not completely cover all physiological situations.

3.2.2. Membrane lipid/water partition coefficients $K_{ml/w}$

$K_{ml/w}$ were determined by equilibrium dialysis experiments using the neutral phosphatidylcholine lipid POPC as a representative for membrane lipids. This phospholipid contains a saturated and an unsaturated long-chain fatty acid which is typical for eukaryotic membranes.[84] Phospholipid/water partition coefficients were shown to be an appropriate surrogate for biomembrane/water partition coefficients – for the neutral and ionic species.[85]

Results of the dialysis experiments are listed in Table 3 and shown in Figure 5 in Section 3.2.5. Log $K_{ml/w}$ of PFAAs range from 2.3 to 4.9. It can be seen that for both PFCAs and PFSAs, $K_{ml/w}$ increase with increasing chain length, because more surface area for van der Waals interaction becomes available.[31]

PFUnDA is the only outlier to this trend. A deviation from linearity in $K_{ml/w}$ with increasing number of carbon atoms in the side-chain was reported in literature for 1-alkyl-3-methylimidazolium derivatives.[86] The so called “cut-off effect” was supposedly caused by a reduced diffusibility due to the size and flexibility of the longer alkyl side-chains. Yet, the experimental uncertainty for

PFUnDA allows no direct conclusion whether the decreased $K_{ml/w}$ is a consequence of the "cut-off effect". Long-chain compounds such as PFUnDA and PFOS show relatively high standard deviations. More than 10 % of PFOS and more than 70 % of PFUnDA was adsorbed to the glass surface during the dialysis experiment (Table 3). An additional extraction step needed to be performed, increasing the error in the determination of $K_{ml/w}$, and also limiting the compounds that could be analyzed with the setup. Similar to the experiments with albumin, PFUnDA is therefore the highest PFCA analogue for which $K_{ml/w}$ was determined.

Table 3. Experimental logarithmic membrane lipid/water partition coefficients (log $K_{ml/w}$ (with K in L water/kg membrane lipid)) of a series of perfluoroalkyl acids and four of their alternatives.

Number of perfluorinated carbons	Compound	log ($K_{ml/w}$/ [L_w/kg_{ml}])[a]	f_{bound}[a,b]	Recovery (including extracts)	c_{POPC}/ [g/L][c]
PFCAs					
3	PFBA	< 1.7[d]			
5	PFHxA	2.32 ± 0.08	30 % ± 4 %	100 %	2.0E+00
6	PFHpA	2.91 ± 0.06	45 % ± 3 %	93 %	1.0E+00
7	PFOA	3.52 ± 0.08	46 % ± 4 %	93 %	2.5E-01
8	PFNA	4.25 ± 0.04	65 % ± 2 %	92 %	1.0E-01
9	PFDA	4.82 ± 0.11	63 % ± 6 %	94 %	2.5E-02
10	PFUnDA[e]	4.54 ± 0.30	63 % ± 15 %	32 % (95 %)	5.0E-02
	HFPO-DA[f]	2.41 ± 0.13	35 % ± 6 %	100 %	2.0E+00
	DONA[f]	3.03 ± 0.07	36 % ± 3 %	92 %	5.0E-01
PFSAs					
4	PFBS	2.86 ± 0.06	59 % ± 3 %	101 %	2.0E+00
6	PFHxS	4.13 ± 0.05	57 % ± 3 %	102 %	1.0E-01
8	PFOS[e]	4.89 ± 0.30	55 % ± 17 %	87 % (104 %)	5.0E-03
8	9Cl-PF3ONS[e]	5.14 ± 0.03	52 % ± 2 %	87 % (110 %)	2.5E-03
8	PFECHS	4.53 ± 0.05	35 % ± 2 %	92 %	5.0E-03

[a] ± standard deviation of six measurements (three measurements on two days). [b] Fraction bound represents the mass of compound that is bound to the membrane lipids divided by the total available mass of compound in the reference cells ($m_{i,ml}/m_{i,ref}$). [c] Concentration of the membrane lipid 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) used to determine $K_{ml/w}$. [d] $K_{mem/w}$ could not be determined accurately for PFBA as the shortest chain compound because the required concentration of liposomes (> 10 g/L) was above the maximal concentration which can be used in the extruder (see 3.1.3). [e] Compounds sorbed substantially to the glass surface of the dialysis cells. For total mass balance, an extraction step with methanol was included and the partition coefficient and fraction bound was calculated with the mass that was not adsorbed to glass surface. [f] HFPO-DA and DONA are not fully fluorinated, therefore no number of perfluorinated carbons is assigned.

For PFBA as the shortest investigated PFCA homologue, the $K_{ml/w}$ was lower than what could be experimentally determined (log $K_{ml/w}$ < 1.7), because the manually operated extruder for preparing the liposomes could not be used for higher concentrations than 10 g/L POPC due to the resulting higher pressure. The lipid concentration could not be increased any further because the fraction bound would drop below 20 %, in turn increasing the measuring uncertainty (Figure 3). The two alternatives with carboxylic groups, HFPO-DA and DONA display log $K_{ml/w}$ of 2.4 and 3.0, and the two alternatives with sulfonate groups, 9Cl-PF3ONS and PFECHS display log $K_{ml/w}$ of 5.1 and 4.5, respectively and are thus in the range of the classical PFAAs.

Membrane lipid/water partition coefficients of PFAAs in literature

When compared to our experimental results for $K_{ml/w}$, all values reported in a study by Droge[87] matched our data within the boundaries of the typical experimental error of 0.2 log units (App. B.5. Comparison of $K_{ml/w}$ with literature). The comparison comprises our complete series of PFAAs except for PFUnDA. Several other studies have described the sorption of one or more PFAAs to membrane lipids determined with various methods.[87-95] From most of these methods only qualitative trends were derived by measuring and comparing a series of PFAAs. In accordance with our results these studies show that the sorption of PFAAs to the membrane lipids is increasing with increasing number of perfluorinated carbons/chain-length[87,93,95] and that PFSAs sorb stronger to the membrane lipids than PFCAs.[87,93-95]

3.2.3. Structural protein/water partition coefficients $K_{sp/w}$

$K_{sp/w}$ were determined by equilibrium dialysis experiments using structural proteins extracted from chicken muscle tissue. Differences in sorption to structural proteins from different species (chicken, fish or pig) were reported to be small for neutral organic compounds.[49] In addition, while sorption to other proteins such as albumin is generally caused by the ligand-protein interaction at specific binding sites, this is not the case for sorption of a compound to structural proteins out of fibers and filaments.[49] It is therefore assumed that the determined $K_{sp/w}$ can be used independent of species also for anionic compounds such as PFAAs.

Log $K_{sp/w}$ for PFAAs and alternatives range between 0.5 to 3.4 (Table 4 and Figure 5 in Section 3.2.5) and sorb to a similar extent to structural proteins as other anionic organic compounds.[42] As already shown for the sorption behavior to albumin and membrane lipids, $K_{sp/w}$ increase with increasing number of perfluorinated carbons for PFCAs and PFSAs, respectively, and PFSAs display

higher $K_{sp/w}$ than their corresponding carboxylic counterpart (same number of perfluorinated carbons). Moreover, alternatives sorb similarly strongly to structural proteins compared to the PFAAs. PFAAs (and alternatives) have in general lower binding affinities to structural proteins compared to the sorption to membrane lipids and to albumin, which is consistent with observations from monitoring studies.[33] The variability in the determined $K_{sp/w}$ for all compounds was relatively high with a standard deviation of about 0.3 log units compared to the results from the other dialysis cells experiments that generally deviated by less than 0.1 log units. This could be due to the more heterogeneous biological extract. The $K_{sp/w}$ for PFAAs were measured here for the first time and no direct comparison to literature values can be made.

Table 4. Experimental logarithmic structural protein/water partition coefficients (log $K_{sp/w}$ (with K in L water/kg structural protein)) of a series of perfluoroalkyl acids and four of their alternatives.

Number of perfluorinated carbons	Compound	log ($K_{sp/w}$/ [L_w/kg_{sp}])[a]	f_{bound}[a, b]	Recovery (including extracts)	c_{sp} /[g/L][c]
PFCAs					
3	PFBA	n.a.[d]			
5	PFHxA	0.50 ± 0.18	15 % ± 4 %	106 %	100
6	PFHpA	1.17 ± 0.11	31 % ± 5 %	100 %	60
7	PFOA	1.48 ± 0.15	24 % ± 6 %	95 %	20
8	PFNA	2.04 ± 0.06	54 % ± 4 %	83 %[e]	20
9	PFDA[f]	2.83 ± 0.15	77 % ± 6 %	61 % (82 %)	10
10	PFUnDA[f]	3.28 ± 0.14	67 % ± 6 %	27 % (89 %)	2
	HFPO-DA[g]	0.67 ± 0.23	20 % ± 7 %	105 %	100
	DONA[g]	1.08 ± 0.05	27 % ± 2 %	107 %	60
PFSAs					
4	PFBS	0.60 ± 0.10	17 % ± 3 %	100 %	100
6	PFHxS	1.59 ± 0.17	29 % ± 8 %	86 %[e]	20
8	PFOS[f]	2.80 ± 0.15	40 % ± 8 %	72 % (91 %)	2
8	9Cl-PF3ONS[f]	3.36 ± 0.16	70 % ± 8 %	72 % (98 %)	2
8	PFECHS	2.44 ± 0.30	23 % ± 8 %	92 %	2

[a] ± standard deviation of six measurements (three measurements on two days). [b] Fraction bound represents the mass of compound that is bound to the structural proteins divided by the total available mass of compound in the reference cells ($m_{i,sp}$/$m_{i,ref}$). [c] Concentration of protein extract used to determine $K_{sp/w}$. [d] log $K_{sp/w}$ for PFBA could not be determined because we were not able to apply a mass balance. Mass fraction that was dissolved freely and the mass in the reference cells were approximately equal, indicating a very low sorption to structural proteins. [e] No methanol extraction conducted. [f] Compounds sorbed substantially to the glass surface of the dialysis cells. For total mass balance, an extraction step with methanol was included and the partition coefficient and fraction bound was calculated with the mass that was not adsorbed to glass surface. [g] HFPO-DA and DONA are not fully fluorinated, therefore no number of perfluorinated carbons is assigned.

3.2.4. Storage lipid/water partition coefficients $K_{sl/w}$

$K_{sl/w}$ of PFAAs and alternatives were determined in solutions containing olive oil and either different dilutions of a physiological buffer solution (100 %, 10 % and 1 % HBSS; Section 3.1.2) or pure water. Previously, olive oil was shown to be a suitable surrogate to investigate the partitioning into mammalian storage lipids for various neutral compounds.[96]

In general, the determined $K_{sl/w}$ are low (log $K_{sl/w}$ < -0.5) for all compounds. In some cases (see App. B.6. Sorption to storage lipids), the sorption to oil was not detectable at all.

Experiments with different dilutions of the buffer solution (10 % and 1 % HBSS) and pure water were supposed to answer the question whether PFAAs form ion pairs under physiological conditions. For ion pair formation, the compounds require a cationic counterpart from the buffer solution. The so formed ion pairs would have no net charge and, by this, might show stronger partitioning into olive oil than the negatively charged PFAAs without cationic counterparts. For decreasing salt concentration, i.e. less cations available for ion pair formation, we would expect a decreasing sorption of PFAAs and their alternatives to oil. In case no ion pair formation occurs, the determined $K_{sl/w}$ for a compound should be unaffected by the buffer concentration and remain constant at different salt concentrations. Contrary to these theoretical considerations an unexpected effect was observed: $K_{sl/w}$ of the tested compounds increased with decreasing salt concentration (Figure 4 and App. B.6. Sorption to storage lipids). Highest $K_{sl/w}$ were measured with pure water or 1 % HBSS for all compounds. This observation could not be explained mechanistically. However, the data show that ion pair formation does not play a role and the overall sorption to storage lipids is very small for PFAAs and alternatives.

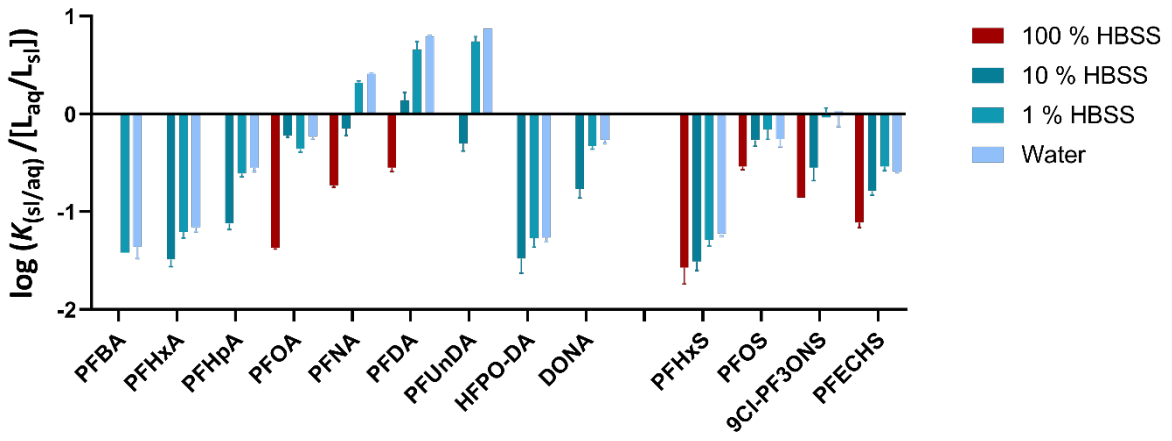

Figure 4. Logarithmic partition coefficients between olive oil and different salt solutions (buffer solution HBSS) in bidestilled water (log $K_{sl/aq}$ (with K in L aqueous solution/L storage lipid). The dilutions of buffer solution were used to investigate ion pair formation. 100 % HBSS represents physiological relevant salt concentrations and ionic strength.[41]

Possible partitioning of PFAAs into the interface between the storage lipids and the surrounding aqueous phase was investigated as follows. Experimentally determined $K_{milk/w}$ for PFOA, PFNA, and PFDA were compared to the respective $K_{milk/w}$ that were calculated without the consideration of possible interfacial effects (App. B.6. Sorption to storage lipids). The experimentally determined $K_{milk/w}$ for the three PFCAs are one order of magnitude higher compared to their respective calculated $K_{milk/w}$. This indicates that the partitioning of PFAAs and alternatives to the storage lipids of milk is increased by additional sorption at the interface between lipids and aqueous phase.

In contrast to the fat globules in milk, the storage lipids in adipose tissue of an organism show a much lower interfacial area compared to their volume. We assume therefore that the sorption to the interface should be of low relevance regarding the overall bioaccumulation of PFAAs and alternatives and was not considered in the distribution calculations described in Chapter 4. This is further supported by rather low enrichment of PFAAs in adipose tissue in rat.[33] However, this additional – and so far overlooked – sorption process can be essential in the investigation of bioaccumulation in newborns when PFAAs are passed on to the offspring by breast milk. Further experimental studies are needed to be able to predict the accumulation of PFAAs in such cases.

Additional dialysis experiments were conducted to determine $K_{milk/w}$ of the complete series of PFAAs and the four alternatives (App. B.6. Sorption to storage lipids) in view of the potential relevance for the bioaccumulation of PFAAs and alternatives in newborns.

3.2.5. Matrix/water partition coefficients of alternative compounds

HFPO-DA, DONA, 9Cl-PF3ONS, PFECHS

Due to their environmental significance, four alternative compounds were included to long-chain PFAAs in our analysis.[13,18,23,97-99] Matrix/water partition coefficients ($K_{matrix/w}$) of the alternatives for albumin, membrane lipids and structural proteins are listed within the tables in previous sections and shown in Figure 5. Results for the sorption of the alternatives to storage lipids (App. B.6. Sorption to storage lipids1) were limited due to low binding affinity as discussed for the PFAAs above.

For each of the investigated matrices, the determined $K_{matrix/w}$ of the alternatives were in range of those of the PFAAs. It is thus concluded that all alternatives examined in this study show a very similar sorption behavior to the physiologically relevant matrices as the PFAAs although they are structurally modified. These results indicate that alternatives are likely to be not less bioaccumulative as the classical PFAAs.

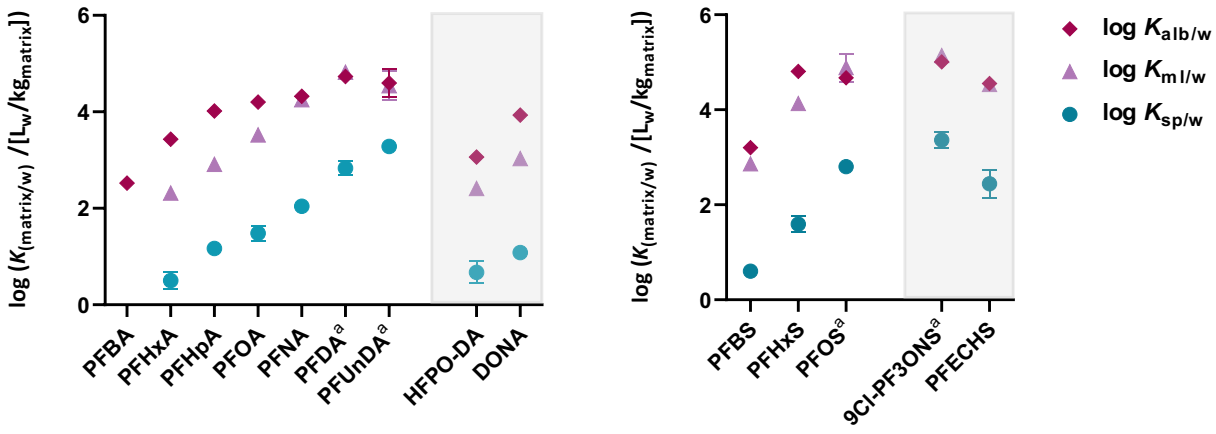

Figure 5. Logarithmic matrix/water partition coefficients (log $K_{matrix/w}$ (with K in L water/kg matrix)) with the matrices albumin, membrane lipids and structural proteins for the series of PFCAs and the two alternatives HFPO-DA and DONA with a carboxylate head group (on the left) and for the three PFSAs and two alternatives 9Cl-PF3ONS and PFECHS with a sulfonate head group (on the right). The order of PFAAs corresponds to an increasing chain-length. Error bars representing standard deviations (three measurements on each of two days) are partly covered by symbols of data points. For all compounds marked with a, an extraction step was included because of high adsorption to glass surfaces.

To estimate the sorption of alternatives compared to PFAAs, it is often suggested to categorize the compounds based on the number of perfluorinated carbons. For HFPO-DA and DONA

however, a categorization based on the number of perfluorinated carbons is difficult because some carbons are not fully fluorinated. By chemical structure HFPO-DA may be compared to PFHxA and DONA to PFHpA. Determined partition coefficients of these two alternatives are similar to the partition coefficients of the respective PFAAs and for respective matrix (Figure 5). Neither the incorporated ether groups nor the non-perfluorinated carbon atom next to the ether group in DONA or the side-chain in HFPO-DA seem to significantly affect the sorption behavior to the matrices. The two alternatives to the perfluorinated sulfonic acids, 9Cl-PF3ONS and PFECHS, can be compared directly to PFOS based on their chemical structure. They have two additional structural characteristics, i.e. the chlorine on the terminal position of 9Cl-PF3ONS and the cyclic structure of PFECHS. 9Cl-PF3ONS shows the highest $K_{matrix/w}$ of all compounds in each matrix respectively. The chlorine substituent on the terminal carbon in 9Cl-PF3ONS appears to cause the increase in sorption which is well known from many other organic pollutants. PFECHS and PFOS have similar matrix/water partition coefficients for each matrix respectively indicating that the cyclic structure of PFECHS does not affect the interactions with the matrix (e.g. by steric hindrance).

The structural characteristics of the four alternatives, especially the ether linkages, were investigated by using the TURBOMOLE software (included in COSMOconf)[63,64] as a quantum-chemical tool to calculate and visualize conformer of a compound with the most favorable (i.e. lowest) energy in 3D. For each conformer, so-called sigma surfaces or COSMO surface polarization charge densities are provided describing the abilities to undergo various intermolecular interactions, such as electrostatic, hydrogen bonding and van der Waals interactions. 3D-structures with the corresponding sigma surfaces are shown in Figure 6 and colors are assigned to charge densities of the surface regions. The compound di-*n*-butylether was included for comparison.

Surprisingly, there are no negative charge densities (red color) to be seen around the ether groups of HFPO-DA, DONA and 9Cl-PF3ONS as compared to di-*n*-butylether which stands here as a representative for typical ethers. Instead the surface charge densities of the perfluorinated chains that possess one or two ether functionalities look quite similar to those of PFOA without any ether functionality. Within the -CF_2-O-CF_2- group, fluorine has the highest electronegativity and induces a strong positive dipole at the neighboring carbons. For this reason, the dipol moment of the carbon – oxygen bond for the PFAAs should be smaller compared to the dipol moment of di-*n*-butylether, resulting in a lower electron density at the oxygen in the ether bond. This can explain

the rather unexpected experimental finding that the inclusion of ether functionalities into the perfluorinated chain did not render these molecules to be more polar and less sorbing.

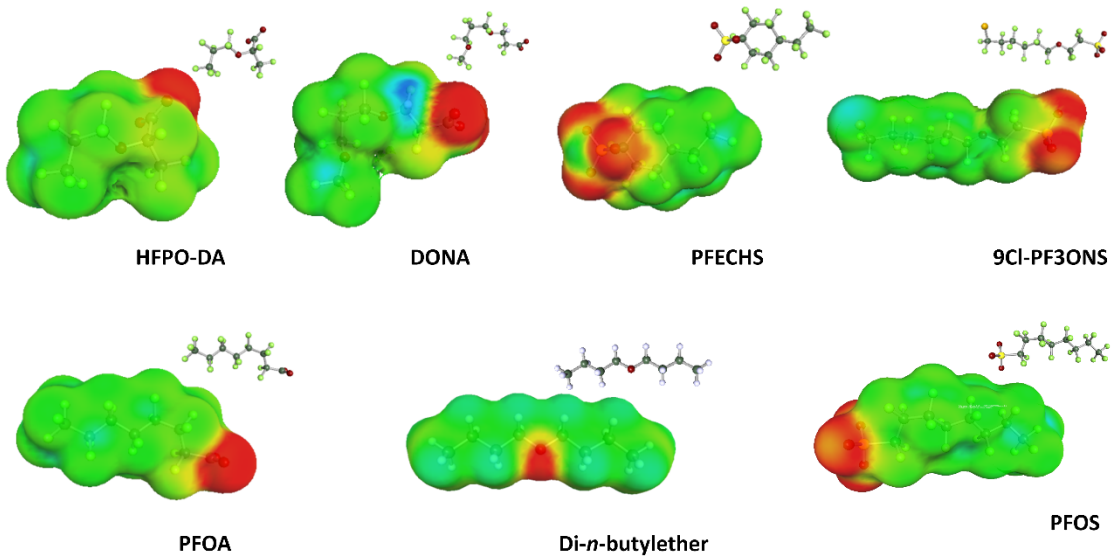

Figure 6. Electron surface charge densities (sigma surfaces) of lowest-energy conformations of the investigated alternatives and of PFOA, PFOS and Di-n-butylether for comparison calculated by COSMOconf/TURBOMOLE software[63,64]. The surface charge density indicates possible interaction sites and is color-coded sites: red: negative charge density (acidic), green: neutral, blue: positive charge density (basic). Color code in conformers: carbon is shown in dark green, fluorine in bright green, oxygen in red, sulfur in bright yellow, chlorine in dark yellow, and hydrogen in white.

4. Physiologically based distribution

4.1. Distribution calculations

4.1.1. Calculation approach

Our model organism comprised adipose, blood, brain, gonads, gut, heart, kidney, liver, lungs, muscle, skin and spleen as separate organs.

The physiological composition for rat and human was based on Ruark et al. 2014 that considers fractional volumes of relevant matrices (storage lipids, membrane lipids, structural proteins and water) in each tissue (App. C.1. Physiological data).[100] Volumes of FABP were included in the composition of liver and kidney due to the proposed relevance.[37,101] Furthermore, albumin fractions were added in interstitial space of each organ. Albumin as a component of the interstitium is usually not considered in physiologically based models for other compounds (compare for example Ruark et al. 2014 or Schmitt et al. 2008)[100,102] but could be of relevance due to the high sorption capacity of albumin for PFAAs.[101]

To provide a better overview, all experimentally determined matrix/water partition coefficients of PFAAs and alternatives that were used in the calculations (and discussed in previous Chapter 3) are summarized in Table App. C.2. Overview of the equilibrium partition coefficients.1. The partition coefficients to liver FABP were reported elsewhere (App. C.2. Overview of the equilibrium partition coefficients.2).[103-106] The approach is based on the assumption that partition coefficients to physiological matrices resulting from *in vitro* experiments can be directly transferred to *in vivo* situation and that all components of the organism are at equilibrium with each other.

The organ composition and the corresponding partition coefficients allow the calculation of the relative sorption capacity of the respective matrix (water – w, albumin – alb, structural proteins – *sp*, membrane lipid – ml, storage lipid – sl, or FABP). Eq. 3 represents the calculation of the relative sorption to albumin as an example. Analogue to this, formulas were compiled for each matrix separately (App. C.3. Calculations of relative sorption capacities of physiological matrices in an organ).

$$f_{i,\mathrm{alb}} = \frac{1}{1 + K_{i,\mathrm{w/alb}} \frac{\mathrm{V_w}}{\mathrm{V_{alb}}} + K_{i,\mathrm{sp/alb}} \frac{\mathrm{V_{sp}}}{\mathrm{V_{alb}}} + K_{i,\mathrm{ml/alb}} \frac{\mathrm{V_{ml}}}{\mathrm{V_{alb}}} + K_{i,\mathrm{sl/alb}} \frac{\mathrm{V_{sl}}}{\mathrm{V_{alb}}} \left(+ K_{i,\mathrm{FABP/alb}} \frac{\mathrm{V_{FABP}}}{\mathrm{V_{alb}}}\right)} \qquad \text{Eq. 3}$$

where $f_{i,\mathrm{alb}}$ is the fraction of the compound *i* that is sorbed to the investigated matrix (albumin) at equilibrium, V is the volume of the respective matrix (App. C.1. Physiological data) and $K_{\mathrm{i,matrix/alb}}$ is the partition coefficient of a compound between another matrix and albumin (in L_{alb}/L_{matrix}). For kidney and liver a term was inserted in the equations to account for the partitioning of PFAAs to FABP.

For each organ, organ/water partition coefficients were calculated for a compound *i* ($K_{i,\mathrm{organ/w}}$) with the formula:

$$K_{i,\mathrm{organ/w}} = f_{i,\mathrm{alb}}\, K_{i,\mathrm{alb/w}} + f_{i,\mathrm{sp}}\, K_{i,\mathrm{sp/w}} + f_{i,\mathrm{ml}}\, K_{i,\mathrm{ml/w}} + f_{i,\mathrm{sl}}\, K_{i,\mathrm{sl/w}} + f_{i,\mathrm{FABP}}\, K_{i,\mathrm{FABP/w}} + f_{i,\mathrm{w}} \qquad \text{Eq. 4}$$

4.1.2. Approach evaluation

The calculated organ partition coefficients were evaluated by comparison with measured PFAA concentrations in organs of human and rat reported in literature. Due to time variant exposure conditions only the relative partitioning between various organs could be evaluated with the equilibrium distribution calculations. Hence, from each study, one reported organ concentration was used as the reference value ($c_{i,\mathrm{ref}}$) in order to predict all other organ concentrations based on our calculated organ partition coefficients ($K_{i,\mathrm{organ/ref}}$):

$$c_{i,\mathrm{organ}} = K_{i,\mathrm{organ/ref}} * c_{i,\mathrm{ref}} \qquad \text{Eq. 5}$$

The measured and calculated PFAA organ concentrations were then compared (except for the reference organ) in order to evaluate the performance of our calculated organ partition coefficients.

4.2. Results and Discussion

4.2.1. Physiologically based distribution

The distribution is based on matrix/water partition coefficients (App. C.2. Overview of the equilibrium partition coefficients) and on physiological data for human or rat (App. C.1. Physiological data). For the physiological data, data by Ruark (2014) was used which listed component fractions for human and rat tissues separately.[100] This differentiation of the organ composition between mammalian organisms is not always done for physiologically based models[102,107] but is considered here to investigate the species-specific accumulation of PFAAs. The membrane lipid fraction in rat brain had to be revised in the Ruark collection because it was not consistent with the data in original references (see App. C.1. Physiological data.2).

While the calculated distribution specifically distinguish between human and rat tissue compositions the same $K_{matrix/w}$ for PFAAs were applied in both species (App. C.2. Overview of the equilibrium partition coefficients). This is justified as similar sorption behavior of PFAAs are expected for a matrix of mammalian. As stated in the corresponding sections of Chapter 3, proteins such as albumin show a close homology between human, bovine and rat in its primary and tertiary structure.[83] Furthermore, only small differences were found for $K_{matrix/w}$ determined with protein of different species for various compounds, including PFOA and PFNA.[35,49,59,103,105,106,108,109] Similar sorption properties have also been demonstrated for storage lipids from different origin (various animals and plants)[96] and phospholipids.[85,87]

Sorption to human and to rat FABP were reported for nine of the investigated PFAAs and two alternatives (App. C.2. Overview of the equilibrium partition coefficients.2).[103,105,106,109] If more than one $K_{FABP/w}$ for a compound was specified, the highest $K_{FABP/w}$ were used for the respective compound to depict the 'worst' case with the conducted distribution calculations. For the same reason FABP at the highest concentration was included in the liver and kidney composition reported in the literature (0.4 mM).[103]

Relative sorption capacities of the matrices were calculated for each organ in human and rat (Figures in App. C.4. Relative sorption capacities of matrices). For PFBA, the calculations were not conducted since no $K_{matrix/w}$ for this compound could be determined except for $K_{alb/w}$ (App. C.2. Overview of the equilibrium partition coefficients.1). The analysis of PFBA indicates that its sorption to all other matrices is rather low.

In general, high fractions (> 40 %) of the compounds are sorbed to membrane lipids and albumin and – to a minor degree – to structural proteins in all organs according to our calculations. High sorption of PFAAs to albumin and membrane lipids was already reported independently.[7,36] Previously published physiologically based models used only one of these two matrices, but disregarded the possible partitioning of PFAAs to other physiological matrices such as structural proteins and storage lipids.[101,110] Our results display that both matrices should be considered in order to predict adequately the distribution of PFAAs in an organism. Besides membrane lipids and albumin, also structural proteins should be included in such models because this physiological matrix showed relatively high sorption capacities for PFAAs especially in protein-rich tissues such as heart, lung, muscles, and spleen (App. C.4. Relative sorption capacities of matrices). Storage lipids and water represent rather low sorption capacities, even in adipose tissue which is composed of ~ 80 % storage lipids.

The sorption to FABP was proposed to play a key role for the accumulation that was observed for PFOA in the (rat) liver.[101] According to the calculations, sorption to FABP actually seems to be relevant for PFOA and short-chain compounds such as PFHxA and HFPO-DA (relative sorption capacity > 30 %, > 20 % and > 40 %, respectively) in kidney and liver of human and rat (App. C.4. Relative sorption capacities of matrices.2).

The proposed relevance of the sorption to FABP for PFOA could, however, not be definitely concluded from these calculations since the accuracy of the applied approach is dependent on the input data. For FABP, one has to take into account that not only physiological data vary between species but also reported $K_{FABP/w}$ vary by one log unit in literature (App. C.2. Overview of the equilibrium partition coefficients.2). With the lowest reported $K_{FABP/w}$, only around 4 % of PFOA would be sorbed to these proteins according to the calculations, and even for the highest $K_{FABP/w}$, combined with the highest FABP content, the sorption to FABP would still contribute less than the membrane lipids to the accumulation in the liver.

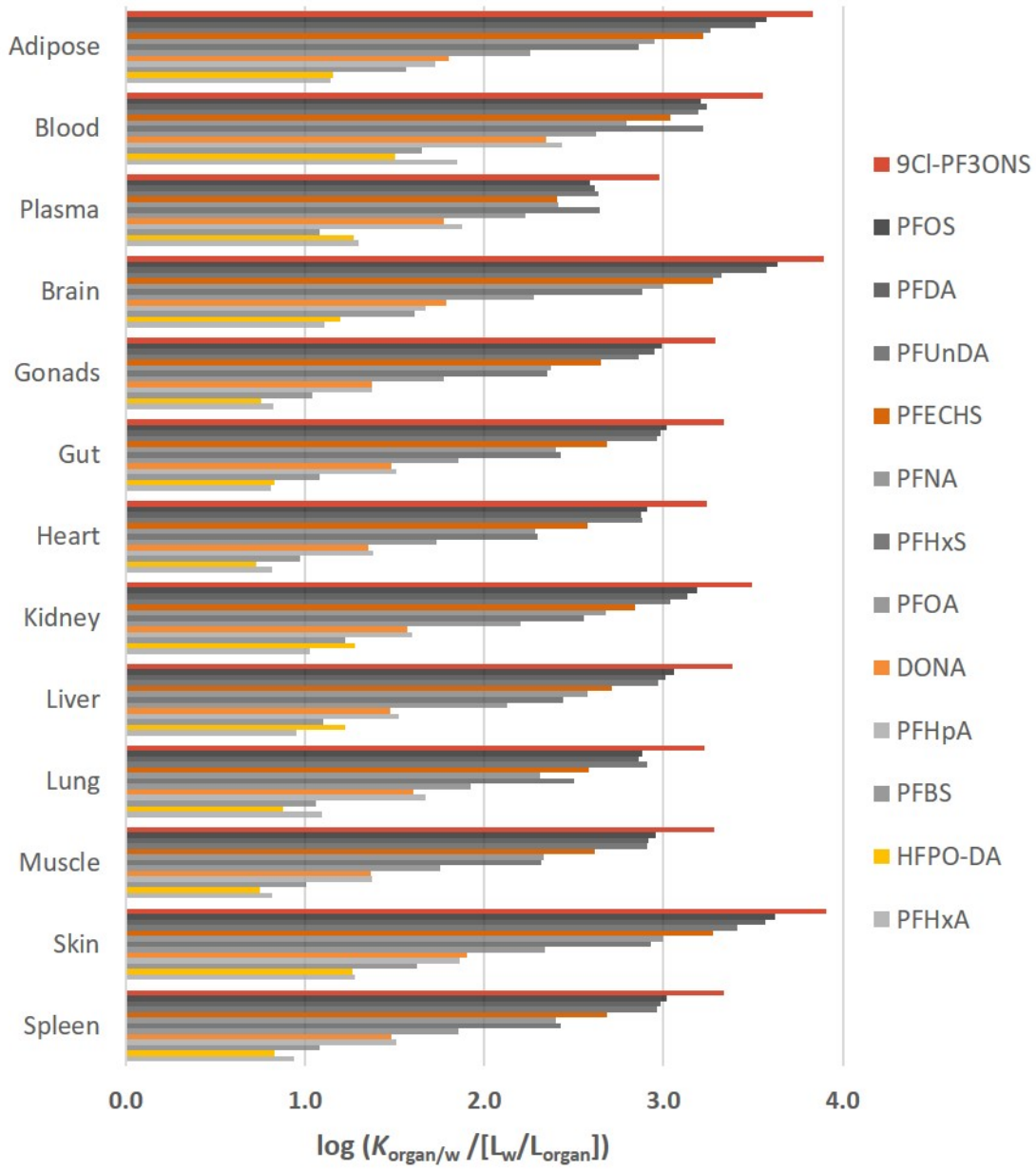

Figure 7. Logarithmic organ/water partition coefficients (log $K_{organ/w}$ (with K in L water/L organ)) calculated for a series of perfluoroalkyl acids and four of their alternatives. Calculations are based on human physiology. Compounds are intentionally arranged to facilitate comparison. For the same reason, alternatives to long-chain PFAAs are highlighted in bright colors.

Organ/water partition coefficients $K_{i,organ/w}$ were calculated based on the organ composition and the corresponding partition coefficient $K_{i,matrix/w}$ (Figure 7 and App. C.5. Organ/water partition coefficients $Korgan_{/w}$). Organs with highest $K_{organ/w}$ were different for human and rat. In human, adipose, brain and skin show the highest $K_{organ/w}$ directly followed by blood, liver and kidney. In rat, highest $K_{organ\ /w}$ were calculated for brain, kidney, liver and blood in that order.

Observed differences in $K_{organ/w}$ between human and rat are mainly caused by the higher fractions of membrane lipids in human organs compared to the rat organs (App. C.1. Physiological data). Overall, the range of $K_{organ/w}$ in different organs is quite narrow for any investigated compound (e.g. the log $K_{organ/w}$ of 9Cl-PF3ONS range between 3.2 – 3.9). Hence, we do not expect specific accumulation in any single organ for any of the investigated PFAAs. Regarding compounds, the relative sorption behavior of PFAAs and alternatives is similar in all organs with highest $K_{organ/w}$ for 9Cl-PF3ONS, PFOS and PFDA and lowest for PFHxA or HFPO-DA for both species.

4.2.2. Evaluation with experimental distribution data

The modeled distribution was evaluated by comparing calculated and measured organ concentrations with 11 data sets in human (App. D.1 Reported PFAA organ concentrations in literature - Human)[111,112] and 57 (male), 30 (female) data sets for different PFAAs in rats from literature (App. D.3 Reported PFAA organ concentrations in literature – Rat).[113-127] Deviations were grouped into the following categories: up to twofold, two- to fourfold and over fourfold deviation. Deviations of more than fourfold were distinguished in overestimations and underestimations. Data sets were included in the evaluation when more than three organ concentrations of PFAAs had been measured. One reported organ concentration of each data set was used as the reference value (‘reference organ’) in order to calculate the relative concentrations in all other organs. Reference organs were chosen according to their availability in the data sets and were either blood, plasma/serum, or lung in that order. These are the most commonly investigated organs which have not been associated with active transport.

Comparison to measured organ concentrations - Human

Organ concentrations for PFAAs in human were available from two references.[111,112] Maestri et al. (2006) measured PFOA and PFOS in adipose, blood, brain, gonads, kidney, liver, lung and muscle (2 data sets) whereas Perez et al. (2013) measured additional PFAAs (PFHxA-PFUnDA and PFBS, PFHxS; in total 9 data sets) in only five organs (brain, kidney, liver, lung and bone). Organ concentrations and the corresponding reference organ of each data set are displayed in App. D.1 Reported PFAA organ concentrations in literature - Human.

The number and overlap of available data sets in human was limited, so only the results for PFOA and PFOS could be validated (App. D.2 Comparison to measured organ concentrations in literature - Human). In liver, the concentrations of PFOA and PFOS were in accordance with the calculated

values (< 2x deviation). For both compounds, a consistent overestimation of their concentrations in brain was observed which might indicate active transport of these PFAAs out of the organ, as it is discussed in detail for the rat (see below). For all other investigated organs and for the series of PFAAs, no clear trend of over- or underestimation can be seen in the calculated values (App. D.2 Comparison to measured organ concentrations in literature - Human). For long-chain PFCAs (PFNA – PFUnDA), but not for the PFHxS and PFOS, the liver concentrations were overestimated. Kidney concentrations were in some cases slightly underestimated, except for PFHxA (> 4x overestimation).

Due to the limited availability of comparable data sets in humans the approach was additionally validated on PFAA concentrations measured in organs of the rat.

Comparison to measured organ concentrations - Rat

The majority of the available studies investigated PFOA and PFOS in males (27 and 24 data sets, respectively) and females (13 and 14 data sets, respectively). Smaller data sets on other PFAAs involved PFHxA, PFNA, PFBS and PFHxS. Reference organs for most of our calculations were blood or plasma values (App. D.3 Reported PFAA organ concentrations in literature – Rat).

Initially, reported PFAA concentrations were used to test the relevance of albumin in the interstitial space for estimating the distribution of PFAAs (Figure 8 and App. D.4 Comparison to measured organ concentrations in literature – Rat). When the approach accounted for albumin in interstitial space the accuracy of calculated concentrations for PFOA was improved in both sexes compared to the distribution that did not consider albumin in interstitial space. This improvement was particularly noticeable in well perfused organs such as kidney, spleen, heart and lung. For PFOS, no effect could be observed. Results are examined in more details in App. D.4 Comparison to measured organ concentrations in literature – Rat. In the following only the calculated concentrations which considered interstitial albumin are presented and discussed.

The evaluation focuses on the results for PFOA because various studies reported organ concentrations of this compound (App. D.3 Reported PFAA organ concentrations in literature – Rat.1 and App. D.3 Reported PFAA organ concentrations in literature – Rat.4). For other PFAAs, evaluation of the estimated distributions in rat was limited by the small number of available data sets and more importantly by a small number of involved labs (App. D.3 Reported PFAA organ concentrations in literature – Rat): It was often observed that systematically high deviations between measured and calculated concentrations occurred when data sets originated from the

same source (e.g. PFOS in female rats, App. D.4 Comparison to measured organ concentrations in literature – Rat). Some of it may be explained by analytical difficulties. In females for example, concentrations are often very low and close to the limit of the used detection method. Other reasons could be systematic errors in the studies.

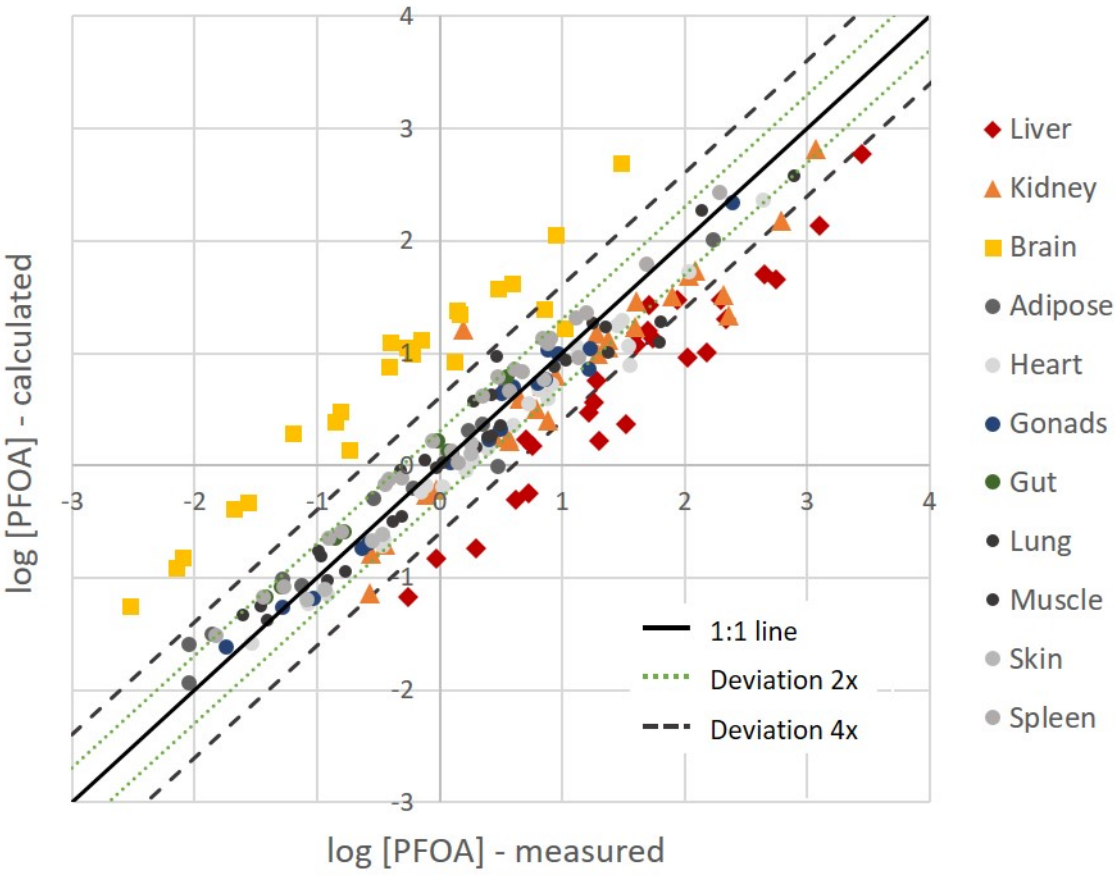

Figure 8. Comparison between measured and calculated relative PFOA concentrations in various organs for male rat. We defined two categories to evaluate deviations between the measured and calculated concentrations, up to twofold (green dotted line) and up to fourfold deviation (dashed line). A deviation of more than fourfold represents either an under- or an overestimation of the calculated PFOA organ concentration that is likely not due to uncertainties in the used approach (e.g. $K_{matrix/w}$ and physiological data on the composition of organs). Such significant deviation can be seen here for the liver and the brain, respectively. Concentrations were calculated using the rat model which considered albumin in interstitial space of organs. Concentration units are dependent on the units of the measured concentrations and were not standardized. See App. D.4.5 for individual values.

For PFOA, the estimations were generally quite accurate for various organs, as calculated PFOA concentrations matched with a deviation lower than twofold to measured concentrations in majority of investigated organs (Figure 8). This good agreement indicates that all significant sorption processes have been considered and were quantified correctly by adding up the sorption capacities for all matrices in an organ.

For other organs, however, calculations differed from reported PFOA concentrations as the concentrations were either underestimated (kidney and liver) or overestimated (brain). The observed differences for kidney, liver and brain indicate that other processes such as active transport might affect the distribution of PFOA in these organs. This was at least partly expected for kidney and liver because active transport was already described to occur at their membranes.[128,129] Especially membrane uptake transporters of two gene families, organic anion transporting polypeptides (Oatp) and organic anion transporters (Oat), were shown to actively transport PFOA *in vitro*.[129-131]

For kidney, the calculated PFOA concentrations slightly underestimated the accumulation in this organ in male rats as the actually measured concentrations are two- to fourfold higher (Figure 8 and App. D.4 Comparison to measured organ concentrations in literature – Rat5). For females, a more distinct underestimation in kidney was noticeable in the majority of the data sets for PFOA (App. D.4 Comparison to measured organ concentrations in literature – Rat8). The higher amount of PFOA could be due to the active uptake that was shown to be different for males and females.[129] However, underestimations of PFOA for females were not observed consistently in all data sets. No unambiguous conclusions are possible because the observed small deviations can also be caused by uncertainties in physiological data.

For liver, a distinct underestimation in males and only small differences in females between our calculated to measured PFOA concentrations suggested the influence of active uptake in this tissue. In literature, PFOA has been described to be actively transported into freshly isolated female and male rat hepatocytes, and involved transporters were found for several PFAAs (PFOA, PFNA and PFSAs). [128,130,131] Transporters included Oatps and sodium dependent taurocholate cotransporting polypeptide (Ntcps). According to our observations, hepatocytes in males seem to have a higher PFOA uptake than female hepatocytes because deviations to measured concentration is more distinct in males. This might indicate a sex-specific expression of transporters similar to kidney. Indeed, sex-specific distribution in liver for PFOA and PFOS was reported in a rat study, supporting our findings.[124]

For brain, the used approach overestimated the concentrations for PFOA consistently in both sexes by a factor 10 or more. It is noteworthy that this consistent overestimation is also observed in the data sets for all other investigated PFAAs except for PFHxA (App. D.4 Comparison to measured organ concentrations in literature – Rat5 – D.4 Comparison to measured organ concentrations in literature – Rat10). The high calculated PFOA concentrations were mostly due

to high membrane lipid content in brain and the high affinity of PFOA to this matrix (App. C.1. Physiological data and C.2. Overview of the equilibrium partition coefficients).

Three explanations could cause the significantly lower PFOA concentrations that were measured in brain: either passive diffusion of PFOA into the brain is limited across the blood-brain-barrier, or the sorption behavior of PFOA to brain is (negatively) affected by acidic phospholipids, or efflux occurs causing PFOA to be actively transported out of the brain back into blood.

Efflux of PFAAs in brain endothelia has not been investigated specifically, but some potential transporters candidates can be found in literature.[132] These include efflux transporters in the blood-facing membrane such as Mrp2 and Bcrp for which interaction with PFSAs was already described for human protein[131,132] or Mrp4. Also typical uptake transporters such as Oat3 or Oat4 at the brain-facing membrane could be involved in the transport out of the brain.[129] The influence of acidic phospholipids was suggested as another explanation for the overestimation of PFOA concentrations in brain because the estimated distribution into brain was based on experimental $K_{ml/w}$ for neutral phospholipids only.[87,133] In an organism, however, also acidic phospholipids are present although generally in much lower fractions.[134] These phospholipids are net-negatively charged and could therefore trigger a repellent effect on the also negatively charged PFAAs, lowering the overall sorption behavior of PFAAs. Consequently, a higher fraction of acidic phospholipids in brain could explain the measured PFAA concentrations. Yet, the brain has a similarly high fraction of acidic phospholipids to neutral phospholipids as the kidney, liver and heart[134] and when applying our approach, no differences were observed similar to those found for the brain concentrations. Finally, passive diffusion of PFOA into the brain might be limited across the blood-brain-barrier, preventing the accumulation of this compound in brain. The possibility that passive diffusion of PFAAs across the blood-brain-barrier is the limiting factor was excluded because anionic permeability through membrane lipids was shown to be sufficiently high.[133] Based on these considerations active efflux transporters in brain tissue are the most probable cause for the overestimation of PFOA concentrations in the brain.

Compared to PFOA, similar effects for PFOS were observed. Concentrations were underestimated in liver (more distinct in males and rather inconclusive in females) and, as mentioned above, PFOS brain concentrations were overestimated by our calculations. Similar transport processes as for PFOA could explain these deviations. In contrast to PFOA, calculated PFOS concentrations in kidney matched measured values in males and females, indicating that distribution is based on the equilibrium sorption processes considered in the approach. Since half-lives of PFOS are higher

than for PFOA,[25] one would assume that also here reabsorption in kidney occurs. For this, more research regarding potential transporters needs to be done.

Cheng and Ng published a physiologically based toxicokinetic model for PFOA in male rats in 2017 which included sorption solely to albumin and FABP but additionally accounted for more complex active transport and membrane permeability.[101] They predicted the PFOA concentrations in adipose, blood, kidney, liver, gut and muscle as a function of time, differing between administration routes. Their modeled concentrations have a similar or even higher accuracy for the investigated organs as estimated with our distribution calculations. Their model worked, therefore, similarly well although it did not consider membrane lipids as a sorbing matrix. This is contradictory regarding our finding that membrane lipids account for a major sorption site for PFAAs. We cannot explain this discrepancy yet. Overall, the herein presented physiologically based distribution calculations were able to estimate the PFOA concentrations in the majority of investigated organs with similar accuracy but much less computational effort than the toxicokinetic model, thereby providing a helpful tool to identify organs that need more attention in future research.

5. Conclusion

The work presented herein aimed to investigate the equilibrium distribution of PFAAs and alternatives to assess their bioaccumulative potential. Thereby, sorption behavior of a series of PFAAs and four of their alternatives to relevant matrices in an organism (albumin, membrane lipids, structural proteins and storage lipids) were determined by dialysis experiments and batch experiments under physiological conditions.

The PFAAs sorb in a similar manner to all matrices: $K_{matrix/w}$ increased with increasing number of perfluorinated carbons, and PFSAs show higher partition coefficients than their respective carboxylic counterparts (with the same number of perfluorinated carbons). Despite their structural differences the alternatives sorb to the same extent to each of the matrices as the conventional PFAAs. Along with the similar high membrane permeabilities of alternatives and PFAAs,[133] this indicates that alternatives show generally similar physico-chemical properties as PFAAs. In addition, alternatives are not biotransformed.[16,17] It can therefore be concluded that the investigated alternatives are likely to be as bioaccumulative as the PFAAs.

Contrary to this conclusion, two biomonitoring studies indicated lower bioaccumulative potential of the alternatives HFPO-DA and DONA than determined for PFOA.[10,16,114,135] This difference can only partly be explained by the somewhat lower sorption to the physiologically relevant matrices measured in this work and might be caused by different active transport mechanisms. However, such biomonitoring studies for alternatives are rare for humans, and exposures can only be estimated. Therefore, no clear conclusion can be drawn from the available monitoring data. Considering their high persistency and intrinsic toxicity similar to that of the classical PFAAs,[13,18,20,136] it becomes clear that these compounds need further research attention.

The experimental approach presented here could in future also be applied to other perfluoroalkyl acids of emerging concern such as perfluoroalkyl phosphonic acids or perfluoroalkyl phosphinic acids[137] and to more alternatives.[138] Along with eventually predicting the bioaccumulative potential of structurally diverse highly fluorinated compounds, these investigations can help to gain molecular insights into the sorption process of alkyl acids with different functional groups.

The determined equilibrium partition coefficients were used for physiologically based distribution calculations to predict the distribution of PFAAs and alternatives. Results showed that albumin, membrane lipids, and also structural proteins are of major relevance to estimate the accumulation of PFAAs in different organs. Future models should, therefore, include all three matrices. FABP in

liver and kidney was additionally considered and found to possess a high sorption capacity for short-chain PFAAs and also PFOA, if the highest reported $K_{FABP/w}$ for PFOA was used. For long-chain PFAAs, only minor relevance of FABP for the organ distribution was predicted. To further clarify the relevance of FABP for short-chain PFAAs and alternatives (and PFOA), it might be beneficial to re-measure $K_{FABP/w}$ with a consistent method.

Evaluation of the performed estimations revealed good consistency with measured PFAA concentrations in most organs of the rat and indicated that the distribution of PFAAs in kidney, liver and brain is affected by active transport. Quantitative knowledge on the effect of active transporters will thus be needed in order to parameterize more complex physiologically based toxicokinetic models. For now, the determined partition coefficients together with the reported anionic membrane permeabilities can already be used as valuable input data in such models.[133] Overall, the systematic investigation of the equilibrium distribution processes for these compounds made it possible to replace former uncertainties with a more consistent and comprehensive view on the biopartitioning of PFAAs.

6. Abstracts of original publications

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

Environmental Science Processes & Impacts

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Cite this: *Environ. Sci.: Processes Impacts*, 2019, **21**, 1852

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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Perfluoroalkyl acids (PFAAs) are persistent, ubiquitous environmental contaminants and their long-chain representatives are bioaccumulative. The phase-out of these compounds (*e.g.* PFOA and PFOS) shifted the production to alternatives. However, little is known about the bioaccumulative behaviour of the alternatives, which are still highly fluorinated. PFAAs are predominantly detected in blood, where they bind to the transport protein serum albumin. This sorption can be described by the albumin/water partition coefficient. It is unclear whether the partition coefficients of the alternatives are lower than or in the same range as those of classical PFAAs. We determined albumin/water partition coefficients for seven perfluoroalkyl carboxylates, three perfluoroalkane sulfonates and four alternatives by dialysis experiments in a physiologically representative system. Quantification was done by LC-MS/MS and a mass balance approach. Logarithmic albumin/water partition coefficients for PFAAs range from 2.8 to 4.8 [L_{water} $kg_{albumin}^{-1}$] and increase with increasing chain length. Perfluorinated sulfonates sorb more strongly than their carboxylate counterparts. The albumin/water partition coefficients for the alternatives (HFPO-DA, DONA, 9Cl-PF3ONS and PFECHS) are in the same range as for classical PFAAs. Structural modifications such as the introduction of ether groups into the chain do not reduce sorption to albumin, whereas the chlorine atom in 9Cl-PF3ONS seems to even increase the sorption to albumin. We further investigated whether the sorption strength could be affected in the presence of medium- or long-chain fatty acids. Binding competition between medium-chain fatty acids and PFAAs appeared to be possible. However, the presence of physiologically more relevant long-chain fatty acids should not alter the albumin/water partition coefficients of PFAAs.

Received 13th June 2019
Accepted 8th August 2019

DOI: 10.1039/c9em00290a

rsc.li/espi

6.2. Membrane/water partitioning and permeabilities of perfluoroalkyl acids and four of their alternatives and the effects on toxicokinetic behavior

Environmental Science & Technology

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Membrane/Water Partitioning and Permeabilities of Perfluoroalkyl Acids and Four of their Alternatives and the Effects on Toxicokinetic Behavior

Andrea Ebert,*,⊥ Flora Allendorf,⊥ Urs Berger, Kai-Uwe Goss, and Nadin Ulrich

Cite This: https://dx.doi.org/10.1021/acs.est.0c00175 Read Online

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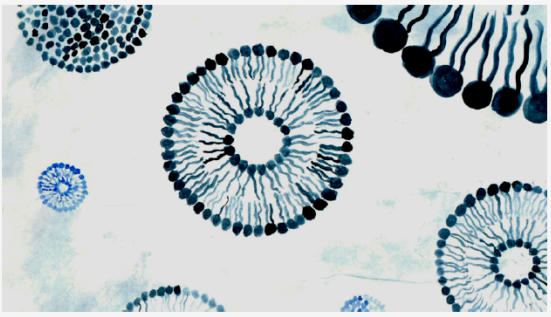
ABSTRACT: The search for alternatives to bioaccumulative perfluoroalkyl acids (PFAAs) is ongoing. New, still highly fluorinated alternatives are produced in hopes of reducing bioaccumulation. To better estimate this bioaccumulative behavior, we performed dialysis experiments and determined membrane/water partition coefficients, $K_{mem/w}$s, of six perfluoroalkyl carboxylic acids (PFCAs), three perfluoroalkanesulfonic acids, and four alternatives. We also investigated how passive permeation might influence the uptake kinetics into cells, measuring the passive anionic membrane permeability P_{ion} through planar lipid bilayers for six PFAAs and three alternatives. Experimental $K_{mem/w}$ and P_{ion} were both predicted well by the COSMO-RS theory (log RMSE 0.61 and 0.46, respectively). $K_{mem/w}$ values were consistent with the literature data, and alternatives showed similar sorption behavior as PFAAs. Experimental P_{ion} values were high enough to explain observed cellular uptake by passive diffusion with no need to postulate the existence of active uptake processes. However, predicted pK_a and neutral permeabilities suggest that also the permeation of the neutral species should be significant in case of PFCAs. This can have direct consequences on the steady-state distribution of PFAAs across cell membranes and thus toxicity. Consequently, we propose a model to predict pH-dependent baseline toxicity based on $K_{mem/w}$, which considers the permeation of both neutral and anionic species.



6.3. Estimating the equilibrium distribution of perfluoroalkyl acids and 4 of their alternatives in mammals

Environmental Toxicology and Chemistry—Volume 00, Number 00—pp. 1–11, 2021
Received: 7 July 2020 | Revised: 27 August 2020 | Accepted: 30 November 2020

Hazard/Risk Assessment

Estimating the Equilibrium Distribution of Perfluoroalkyl Acids and 4 of Their Alternatives in Mammals

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Abstract: Perfluoroalkyl acids (PFAAs) mostly exist as ionic compounds that are of major concern because of their accumulative behavior. The discussion about their risk is ongoing considering the increasing production of structurally similar alternatives. We conducted model calculations based on equilibrium distribution coefficients that allow studying the distribution of PFAAs and their alternatives in various mammalian organs through comparison to empirical measurements in humans and rats. The calculations rely on experimentally determined distribution coefficients of a series of PFAAs and 4 of their alternatives to physiological matrices such as structural proteins, storage lipids, membrane lipids, albumin, and fatty acid binding protein (FABP). The relative sorption capacities in each organ were calculated from the combination of distribution coefficients and physiological data. The calculated distribution of PFAAs and alternatives within the organs showed that albumin and membrane lipids and, to a lesser extent, structural proteins have the highest relative sorption capacities for the compounds. Sorption to FABP is only relevant in the distribution of short-chain PFAAs. Storage lipids play a minor role in the distribution of all studied compounds. Our calculated distribution of PFAAs was evaluated by comparison to reported PFAA concentrations in various organs. *Environ Toxicol Chem* 2021;00:1–11. © 2020 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Accumulation of PFAAs and alternatives; Structural protein–water distribution coefficients; Storage lipid–water distribution coefficients; Organ–water distribution coefficients; Predicting tissue distribution of PFAAs; Physiologically based distribution

Abbreviations

µg	microgram
3D	three dimensional
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonate
ADME	absorption, distribution, metabolism and elimination
alb	albumin
App	appendix
Bcrp	breast cancer resistance protein
BSA	bovine serum albumin
c	concentration
d	day
DONA	4,8-dioxa-3H-perfluorononanoate
f	fraction
FABP	fatty acid binding protein
f_{bound}	fraction bound
g	gram
h	hour
HFPO-DA	tetrafluoro-2-(heptafluoropropoxy)- propanoic acid
HBSS	Hank's balanced salt solution
HDA	heptadecanoic acid
HSA	human serum albumin
i	a given compound
K_a	association constant
kDa	kilo Daltons
kg	kilogram
$K_{matrix/w}$	partition coefficient between a given matrix and water
L	liter
log	logarithmic
m	mass
M	molar (mol/L)
ml	membrane lipids
mL	milliliter
Mrp	multidrug resistant-related protein

MW	molecular weight
Oat	organic anion transporters
Oatp	organic anion transporting polypeptides
PBTK	physiologically based toxicokinetic (models)
PFAA	perfluoroalkyl acid
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonic acid
PFCA	perfluoroalkyl carboxylic acid
PFDA	perfluorodecanoic acid
PFECHS	perfluoro-4-ethylcyclohexanesulfonate
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFPeA	perfluoropentanoic acid
PFSA	perfluoroalkane sulfonic acid
PFUnDA	perfluoroundecanoic acid
pH	negative log of hydrogen ion concentration in a water-based solution
pm	picometer
POPC	1-palmitoyl-2-oleoyl-glycero-3-phosphocholine
ref	reference
rpm	rounds per minute
SD	standard deviation
sl	storage lipids
sp	structural protein
TDA	tridecanoic acid
w	water
δ	density

Appendix

A. Experimental details

A.1. Milk composition

Components of milk are fat including storage and membrane lipids, along with carbohydrates and proteins (Table A.1. Milk composition). Proteins are composed of mainly casein but also whey proteins, and serum albumin.[139] Since casein makes up a major fraction of the milk additional dialysis experiments were conducted to determine casein/water partition coefficients ($K_{cas/w}$) for PFOA, PFNA and PFDA. Milk/water partition coefficient ($K_{i,milk/w}$) was calculated then with the following formula (with the assumption that neither carbohydrates nor salt represent a sorbing matrix):

$$K_{i,\mathrm{milk/w}} = f_{i,\mathrm{sl}}K_{i,\mathrm{sl/w}} + f_{i,\mathrm{ml}}K_{i,\mathrm{ml/w}} + f_{i,\mathrm{cas}}K_{i,\mathrm{cas/w}} + f_{i,\mathrm{alb}}K_{i,\mathrm{alb/w}} + f_{i,\mathrm{wprot}}K_{i,\mathrm{sp/w}} + f_{i,\mathrm{w}} \quad \text{(Eq. A1)}$$

with the fraction of storage lipid ($f_{i,\mathrm{sl}}$), membrane lipid ($f_{i,\mathrm{ml}}$), casein ($f_{i,\mathrm{cas}}$), serum albumin ($f_{i,\mathrm{alb}}$) and remaining whey protein ($f_{i,\mathrm{wprot}}$) and water ($f_{i,\mathrm{w}}$).

Table A.1. Components of 3.8 % fresh cow milk (homogenized, pasteurized).[139]

Milk composition	**Water**	**Storage lipids**	**Membrane lipids**	**Carbo-hydrates**	**Casein**	**Albumin**	**Remaining whey proteins**	**Salt**
(w/w) %	88.2	3.76	0 .04	4.70	2.60	0.04	0.56	0.01

A.2. Quantification method

Table A.2. LC gradient for the analysis of PFAAs and alternatives. 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.

Time/ [min]	Flow/ [mL/min]	% A	% B
0	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 A.3. MS/MS parameters for the analysis in ESI^- mode of PFAAs and alternatives and for the corresponding internal standards. Retention time (rt), source sampling cone (cone), collision energy (CE), multi reaction monitoring (MRM) transitions, internal standard (IS).

Compound	Rt/ [min]	Cone/ [V]	CE/ [V]	MRM transitions	Qualifier transit-ions	Internal standard	Cone/ [V]	CE/ [V]	IS MRM transit-ions
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	299 > 99	$^{13}C_2$PFHxA	2	12	315 > 270
PFHxS	4.57	30	32	399 > 80	399 > 99	$^{18}O_2$PFHxS	30	32	403 > 103
PFOS	5.05	30	38	499 > 80	499 > 99	$^{13}C_4$PFOS	30	38	503 > 99
DONA	5.63	4	14	377 > 251	377 > 85	$^{13}C_3$HFPO-DA	8	20	287 > 185
HFPO-DA	5.27	8	20	285 > 185	285 >169	$^{13}C_3$HFPO-DA	8	20	287 > 185
9Cl-PF3ONS	5.49	8	26	531 > 350	531 > 83	$^{13}C_8$PFOS	30	38	507 > 99
PFECHS	5.08	54	32	461 > 381	461 > 99	$^{13}C_8$PFOA	4	10	421 > 376

B. Determination of equilibrium partition coefficients

B.1. Precision experiments

Table B.1. Week-to-week precision test for the determination of albumin/water partition coefficients ($K_{alb/w}$, with K in L water/kg albumin). 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 of $K_{alb/w}$ was calculated for both sampling points (three x triplicates).

PFNA	**log ($K_{alb/w}$ /[L_w/kg_{alb}])**			
	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**

B.2. Batch A7906

Albumin/water partition coefficients were determined 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.

Some differences were found in the $K_{alb/w}$ measured with the different BSA batches (Table B.2.1 and Figure B.2.2, below). Especially short-chain PFAAs and PFHxS showed a lower sorption strength of at least 0.3 log units when A7906 was used 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 $K_{alb/w}$ 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.[68] 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.

It was decided to use essentially fatty acid free albumin to determine the $K_{alb/w}$ of the PFAAs and of the alternatives to avoid potential interferences by impurities.

Table B.2.1. Logarithmic albumin/water partition coefficients (log $K_{alb/w}$ (with K in L water/kg albumin)) of a series of perfluoroalkyl acids and four of their alternatives determined with BSA batch A7906.

Number of perfluorinated carbons	Compound	log ($K_{alb/w}$/ [L_w/kg_{alb}])[a]	f_{bound}[b]	Recovery (including extracts)	c_{BSA}/[g/L][c]
PFCAs					
3	PFBA	1.90 ± 0.07	29 % ± 4 %	104%	1.0E+01
5	PFHxA	3.02 ± 0.07	35 % ± 4 %	95%	1.0E+00
6	PFHpA	3.79 ± 0.05	62 % ± 3 %	98%	5.0E-01
7	PFOA	4.17 ± 0.05	43 % ± 3 %	94%	1.0E-01
8	PFNA	4.48 ± 0.05	45 % ± 3 %	101%	1.0E-01
9	PFDA[d]	4.84 ± 0.05	63 % ± 3 %	84% (119%)	5.0E-02
10	PFUnDA[d,e]	4.72 ± 0.10	44 % ± 6 %	59% (149%)	2.5E-02
	HFPO-DA[f]	2.71 ± 0.04	44 % ± 2 %	92%	3.0E+00
	DONA[f]	3.80 ± 0.04	61 % ± 2 %	91%	5.0E-01
PFSAs					
4	PFBS	2.65 ± 0.12	32 % ± 6 %	106%	2.0E+00
6	PFHxS	4.49 ± 0.13	44 % ± 7 %	96%	5.0E-02
8	PFOS[d]	4.83 ± 0.11	77 % ± 4 %	92%	1.0E-01
8	9Cl-PF3ONS[d]	5.02 ± 0.03	58 % ± 2 %	81% (110%)	2.5E-02
8	PFECHS	4.30 ± 0.03	50 % ± 2 %	≈ 90%	1.0E-01

[a] ± standard deviation of six measurements (three measurements on two days). [b] Fraction bound represents the mass of compound that is bound to albumin divided by the total available mass of compound ($m_{i,alb}/m_{i,ref}$). [c] Concentration of bovine serum albumin (BSA) used to determine $K_{alb/w}$. [d] Compounds sorbed substantially to the glass surface of the dialysis cells. For total mass balance, an extraction step with methanol was included and the partition coefficient was calculated with the mass that was not adsorbed to glass surface. [e] Mean and standard deviation of only four measurements. [f] HFPO-DA and DONA are not fully fluorinated, therefore no number of perfluorinated carbons is assigned. [a] not analyzable (n.a.). Partition coefficient could not be determined since a mass balance could not be applied. Determined fraction bound was around 0 %, indicating a very low binding affinity.

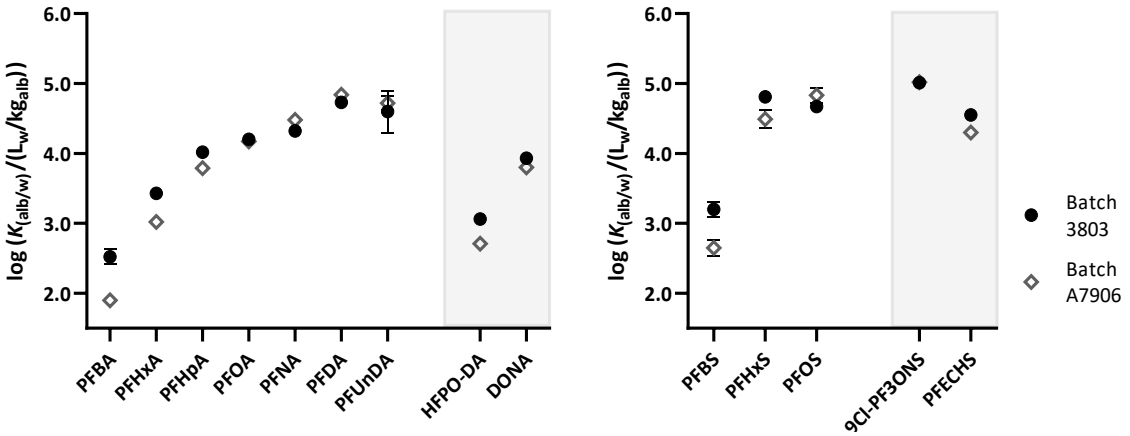


Figure B.2.2. Comparison of logarithmic albumin/water partition coefficients (log $K_{alb/w}$ (with K in L water/kg albumin)) for the series of PFCAs (left) and three PFSAs (right) and investigated alternatives determined with different batches of BSA (A3803 and A7906). When A7906 was used, lower partition coefficients could be noted for short-chain PFAAs and alternatives, except for PFECHS. Error bars of standard deviations are partly covered by symbols of data points.

B.3. Comparison of $K_{alb/w}$ with literature

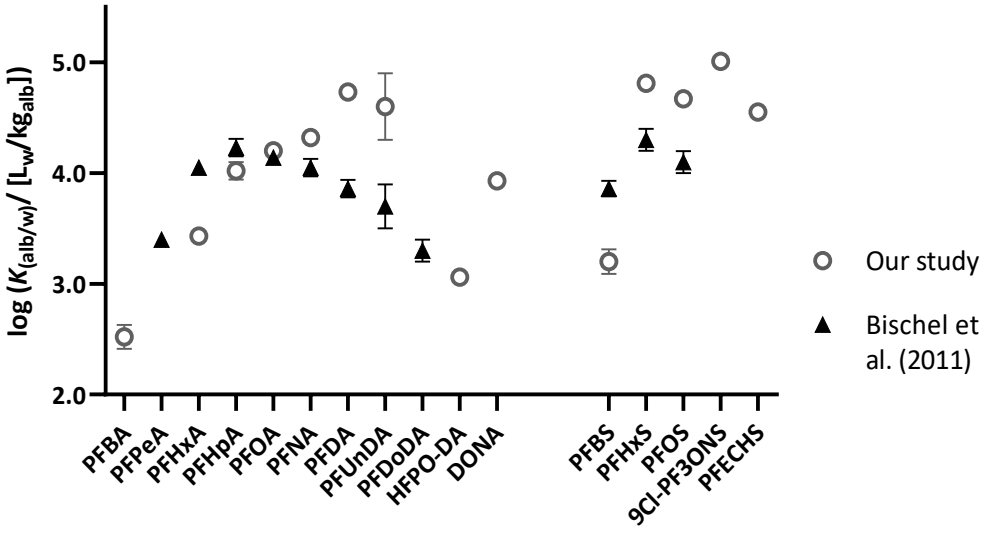


Figure B.3.1. Comparison of the logarithmic albumin/water partition coefficients (log $K_{alb/w}$ (with K in L water/kg albumin)) the present study to the log $K_{alb/w}$ reported by Bischel et al. (2011)[66] for a similar series of PFAAs. The other study measured additionally perfluoropentanoic acid (PFPeA with 5 perfluorinated carbons) and perfluorododecanoic acid (PFDoDA with 12 perfluorinated carbons) but not PFBA. They described $K_{alb/w}$ with an increasing and a decreasing trend with increasing number of perfluorinated carbons with a peak at PFHpA, whereas we see an increasing trend with increasing number of perfluorinated carbons. Error bars of standard deviations are partly covered by symbols of data points.

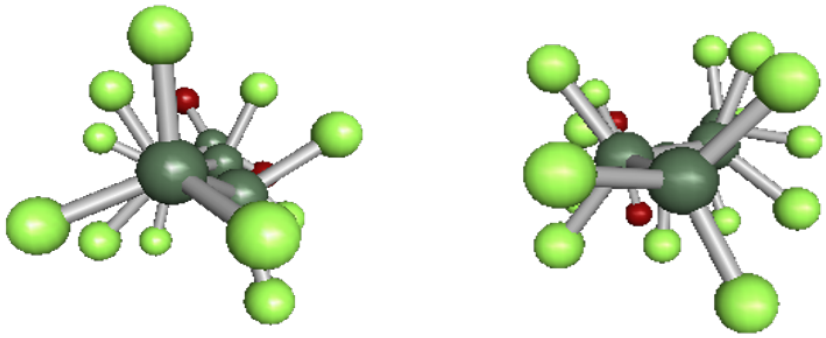


Figure B.3.2. Conformers of PFHxA (five perfluorinated carbons) on the left and PFOA (seven perfluorinated carbons) on the right, calculated by COSMOconf/TURBOMOLE[63,64] in cross-section. Literature discussed a conformeric change from a rather zig-zag form to a more helical structure occurring when the number of perfluorinated carbons exceeds six. This difference is not discernable here.

B.4. Influence of fatty acids

The concentration of free fatty acids in human blood ranges from 0.2 to 1 mM.[56,140] The blood concentration of the fatty acids is thus considerably higher compared to the concentrations of PFAAs in general population (1 – 50 nM).[77,78] The albumin concentration was subsequently adapted for the determination of the partition coefficient for PFHxA and PFNA, respectively. Fatty acid concentrations were adjusted to the physiological situation in the human blood ([fatty acid] : [alb] ≈ 2.5 : 1) and two smaller concentrations of the fatty acid to meet approximately equimolarity and below saturation (i.e. a ratio below 1). All other concentrations in the setup were kept constant (with [PFCA] : [alb] molar ratio < 0.1). First, albumin and TDA or HDA were equilibrated for 24 h before the respective PFCA was added. After 72 h and 96 h, samples were taken and $K_{alb/w}$ were determined. Results are displayed in Figure B.4.1 and in Table B.4.2, where $K_{alb/w}$ for PFHxA and PFNA are shown in dependence of different amounts of TDA or HDA. There is a slight decrease in the log $K_{alb/w}$ for PFHxA with increasing concentration of TDA from 3.4 (no TDA added) to 2.9 ([TDA] : [alb] ratio of 2.3). When applying a linear regression, the slope is -0.22. Considering our inter-week precision of 0.1 log units from former dialysis setups with PFNA, the set of TDA/PFHxA-experiments was repeated (except for the lowest [TDA] : [alb] ratio of 0.6 : 1) for confirmation of the trend. The results of a log $K_{alb/w}$ of 3.4 with no TDA added and a log $K_{alb/w}$ of 3.0 at the highest molar ratio confirm the described trend (slope: -0.14 of 3 values) and the slight influence of the present TDA concentration on the PFHxA partition coefficient. For PFNA the log $K_{alb/w}$ are 4.4 (no TDA added) and shift to 4.6 at the [TDA] : [alb] ratio of 0.3 and slightly decline (similar to PFHxA) to a log K of 4.3 at the highest [TDA] : [alb] ratio. The result without TDA for

PFNA was taken from the precision experiments (Section 3.2.1. Albumin/water partition coefficients $K_{alb/w}$). This shift is difficult to interpret. One assumption is that the binding site could be different for PFNA than for PFHxA and, in case of PFNA, the described shift could be caused by the effect of cooperative binding. Specific binding of fatty acid(s) to serum albumin can cause conformational changes in the tertiary structure of the protein. If this change occurs in binding sites, interaction possibilities could be created or facilitated resulting in an increased binding strength of the ligand. This cooperative binding effect was already found for other compounds.[141] While conformation of the binding site for PFHxA may not be affected when TDA is binding, the conformation of the binding site for PFNA may be exhibiting such a change in low molar concentrations of TDA. Increasing the amount of fatty acids, the competition to the binding sites may compensate/overtake the cooperative binding effect.

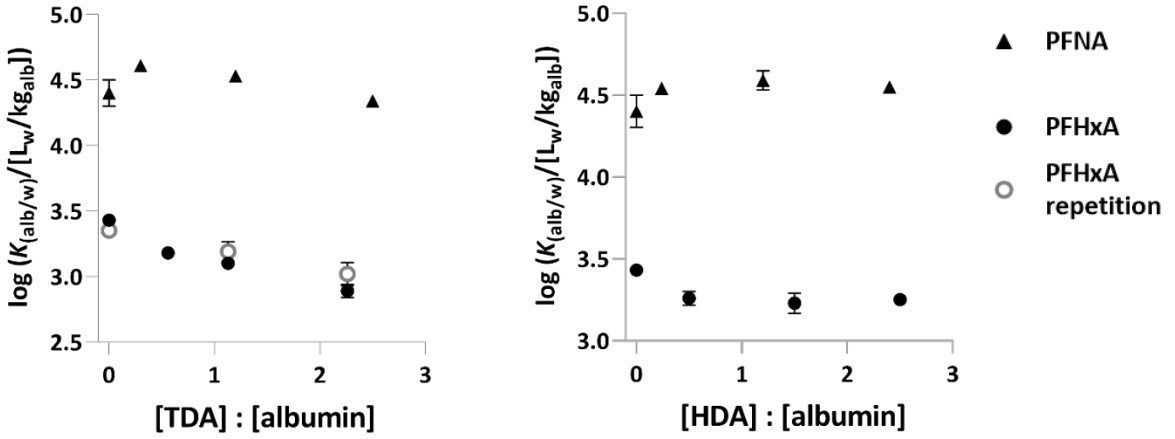


Figure B.4.1. Logarithmic albumin/water partition coefficients (log $K_{alb/w}$ (with K in L water/kg albumin)) of PFNA and PFHxA in presence of different molar ratios of the fatty acid TDA (C13-FA; left) or HDA (C17-FA; right) to albumin at equilibrium. Each data point comprises six measurements. Error bars of standard deviations are partly covered by symbols of data points. Log $K_{alb/w}$ without TDA (molar ratio: 0) for PFHxA and PFNA was taken from former experiments, the log $K_{alb/w}$ for PFNA from precision experiments. Experiments with TDA/PFHxA were repeated once, except for the molar ratio of 0.6.

For both compounds it appeared that the TDA concentration in the dialysis approach has a slight influence on the respective $K_{alb/w}$ of the PFCA. TDA belongs to the group of medium-chain fatty acids which bind preferentially to lower affinity fatty acid binding sites on albumin whereas long-chain fatty acids (> C16) can be found at high-affinity fatty acid binding sites.[142] In literature it is discussed that potential binding sites of PFCAs[39,66] are known drug-binding sites (Sudlow site I and II and additionally a third drug-binding site next to fatty acid binding site 6) that partly overlap with well-known fatty acid binding sites.[67,143,144] From the presented data a TDA specific binding

site cannot be deduced since one has to assume that TDA tends to sorb to all available binding sites at the given molar ratios.[56,145] A more general interpretation could be that the observed slight decline of the $K_{alb/w}$ of PFHxA and PFNA by increasing amount of TDA could propose either that both compounds bind to fatty acid binding sites and that TDA is displacing the PFCA at higher concentrations, or that the respective PFCA binds to one of the drug-binding sites and the binding of TDA alters the albumin conformation thereby disrupting PFCA-albumin interaction. Both scenarios have already been described for other organic anions.[141,146] In contrast to the slight trends observed in the experiments with TDA, almost no differences in the determined partition coefficients occurred in the presence of HDA at different adjusted [HDA] : [alb] ratios (Figure B.4.1, right panel). For PFHxA and PFNA, log $K_{alb/w}$ remain close to 3.25 and 4.55, respectively. Again, a slight difference was observed for the $K_{alb/w}$ determined in the absence of HDA. It should be noted that HDA experiments for PFNA and PFHxA were each conducted from one single batch/stock solution of albumin and PFNA or PFHxA, respectively, except for the setup without HDA. This could explain the slight difference. Both values are within the standard deviation of the precision tests (PFNA) and observed deviations could not be assigned to a specific effect in this case.

Table B.4.2. Logarithmic albumin/water partition coefficients (log $K_{alb/w}$ (with K in L water/kg albumin)) for PFHxA or PFNA determined in presence of fatty acids (tridecanoic acid, TDA or heptadecanoic acid, HDA) at different molar ratios ([fatty acid] : [alb]). Mean and standard deviations are taken by the displayed six measurements.

TDA Dialysis Experiments			
[TDA] : [alb]	2.5	1.2	0.3
	log $K_{alb/w}$ for PFNA		
72 h Vial1	4.34	4.54	4.63
72 h Vial2	4.32	4.52	4.67
72 h Vial3	4.40	4.50	4.58
96 h Vial1	4.28	4.52	4.60
96 h Vial2	4.32	4.55	4.61
96 h Vial3	4.37	4.57	4.58
Mean	**4.34**	**4.53**	**4.61**
SD	0.04	0.02	0.03
[TDA] : [alb]	2.3	1.1	0.6
	log $K_{alb/w}$ for PFHxA		
72 h Vial1	2.88	3.10	3.17
72 h Vial2	2.87	3.04	3.19
72 h Vial3	2.91	3.14	3.19
96 h Vial1	2.82	3.16	3.16
96 h Vial2	2.94	3.08	3.25
96 h Vial3	2.95	3.07	3.13
Mean	**2.89**	**3.10**	**3.18**
SD	0.05	0.05	0.04

HDA Dialysis Experiments			
[HDA] : [alb]	2.4	1.2	0.2
	log $K_{alb/w}$ for PFNA		
72 h Vial1	4.59	4.53	4.49
72 h Vial2	4.55	4.58	4.56
72 h Vial3	4.53	4.52	4.51
96 h Vial1	4.56	4.59	4.51
96 h Vial2	4.54	4.66	4.59
96 h Vial3	4.54	4.65	4.55
Mean	**4.55**	**4.59**	**4.54**
SD	0.02	0.06	0.04
[HDA] : [alb]	2.5	1.5	0.5
	log $K_{alb/w}$ for PFHxA		
72 h Vial1	3.26	3.27	3.31
72 h Vial2	3.24	3.16	3.26
72 h Vial3	3.25	3.23	3.24
96 h Vial1	3.23	3.32	3.32
96 h Vial2	3.25	3.16	3.23
96 h Vial3	3.26	3.23	3.22
Mean	**3.25**	**3.23**	**3.26**
SD	0.01	0.06	0.04

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

B.5. Comparison of $K_{ml/w}$ with literature

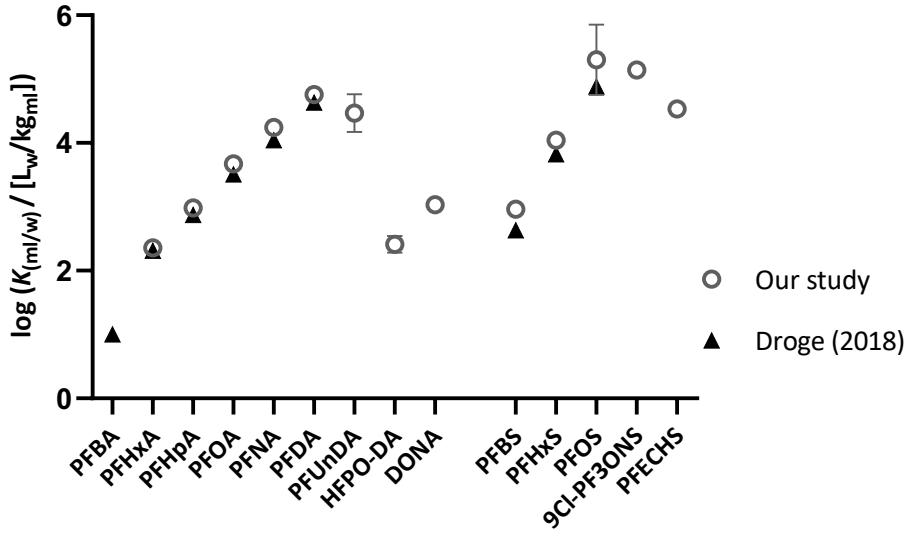


Figure B.5. Comparison of the logarithmic membrane/water partition coefficients of the present study to the log $K_{ml/w}$ reported by Droge[87] for a similar series of PFAAs. Droge conducted an assay with solid supported lipid membranes (SSLM). Error bars of standard deviations are partly covered by symbols of data points.

B.6. Sorption to storage lipids

Table B.6.1. Logarithmic partition coefficients between olive oil and different salt solutions (buffer HBSS) in bidestilled water (log $K_{sl/aq}$ (with K in L aqueous solution/L storage lipid)). Dilutions of buffer solutions were used to investigate ion pair formation, see text.

Compound	100 % HBSS		10 % HBSS		1 % HBSS		Water	
	log ($K_{sl/aq}$/ [L_{sl}/L_{aq}])	f_b	log ($K_{sl/aq}$/ [L_{sl}/L_{aq}])	f_b	log ($K_{sl/aq}$/ [L_{sl}/L_{aq}])	f_b	log ($K_{sl/aq}$/ [L_{sl}/L_{aq}])	f_b
PFCAs								
PFBA	n.a.[a]		n.a.		-1.42 ± 0	26 %	-1.36 ± 0.12	28 %
PFHxA	n.a.		-1.49 ± 0.07	23 %	-1.21 ± 0.06	36 %	-1.16 ± 0.05	38 %
PFHpA	n.a.		-1.12 ± 0.06	41 %	-0.61 ± 0.03	69 %	-0.55 ± 0.04	72 %
PFOA	-1.37 ± 0.01	28 %	-0.22 ± 0.02	84 %	-0.36 ± 0.03	31 %	-0.23 ± 0.03	37 %
PFNA	-0.73 ± 0.02	63 %	-0.15 ± 0.07	41 %	0.32 ± 0.02	68 %	0.41 ± 0.01	72 %
PFDA	-0.55 ± 0.04	72 %	0.14 ± 0.08	58 %	0.66 ± 0.08	66 %	0.79 ± 0.01	72 %
PFUnDA	n.a.		-0.30 ± 0.08	34 %	0.74 ± 0.05	70 %	0.87 ± 0.00	76 %
HFPO-DA	n.a.		-1.48 ± 0.15	23 %	-1.27 ± 0.09	33 %	-1.26 ± 0.05	33 %
DONA	n.a.		-0.77 ± 0.09	60 %	-0.33 ± 0.03	81 %	-0.27 ± 0.03	83 %
PFSAs								
PFBS	n.a.		n.a.		n.a.		n.a.	
PFHxS	-1.57 ± 0.17	20 %	-1.51 ± 0.09	22 %	-1.29 ± 0.06	32 %	-1.23 ± 0.02	35 %
PFOS	-0.54 ± 0.03	72 %	-0.27 ± 0.06	35 %	-0.16 ± 0.1	41 %	-0.26 ± 0.08	35 %
9Cl-PF3ONS	-0.86 ± 0.00	55 %	-0.55 ± 0.13	22 %	0.01 ± 0.05	51 %	-0.02 ± 0.11	49 %
PFECHS	-1.11 ± 0.05	41 %	-0.79 ± 0.04	60 %	-0.54 ± 0.04	72 %	-0.59 ± 0.01	70 %

[a] not analyzable (n.a.). Partition coefficient could not be determined since a mass balance could not be applied. Determined fraction bound was around 0 %, indicating a very low binding affinity.

Table B.6.2. Milk/water partition coefficients $K_{milk/w}$ determined either with cow milk by dialysis experiments or by calculation (Eq. A.*1. Milk composition*). For calculating $K_{milk/w}$ we used the determined partition coefficients to relevant matrices that are listed in the corresponding sections of Chapter 3. We additionally determined the casein/water partition coefficient ($K_{cas/w}$) by dialysis experiments.

Compound	$K_{milk/w}$ [L_w/kg_{milk}]	$K_{milk/w}$ [L_w/kg_{milk}]	log $K_{cas/w}$[a]	log $K_{sl/w}$[b]	log $K_{ml/w}$	log $K_{alb/w}$	log $K_{sp/w}$
	experimental	**calculated**	**with K in [L_w/kg_{matrix}]**				
PFOA	**184.5** ± 87.4	**9.8**	1.69 ± 0.13	-1.3	3.52	4.20	1.48
PFNA	**404.2** ± 189.9	**18.7**	1.99 ± 0.07	-0.7	4.25	4.32	2.04
PFDA	**1554.8** ± 519.8	**51.1**	1.85 ± 0.11	-0.5	4.82	4.73	2.83

[a] Mean and standard deviation of three measurements on two days. [b] Storage lipid/water partition coefficients measured with olive oil were converted into [L_w/kg_{sl}] using oil density of 0.918 kg/L.

Table B.6.3. Experimentally determined logarithmic milk/water partition coefficients (log $K_{milk/w}$ (with K in L water/kg milk)) for the series of PFAAs and four alternatives.

Number of perfluorinated carbons	Compound	log ($K_{milk/w}$/ [L_w/kg_{milk}])[a]	f_{bound}	Recovery	c_{milk}/ [g/L][b]
PFCAs					
3	PFBA	n.a.[c]			
5	PFHxA[d]	0.38 ± 0.16	13 % ± 5 %	104 %	122
6	PFHpA	1.02 ± 0.12	40 % ± 7 %	102 %	122
7	PFOA	1.98 ± 0.26	20 % ± 8 %	97 %	30.5
8	PFNA	2.33 ± 0.24	35 % ± 11 %	93 %	30.5
9	PFDA	2.95 ± 0.15	68 % ± 8 %	92 %	30.5
10	PFUnDA	3.50 ± 0.14	49 % ± 8 %	70 %	0.61
	HFPO-DA[e]	0.47 ± 0.17	7 % ± 19 %	97 %	122
	DONA[f]	1.02 ± 0.23	42 % ± 12 %	94 %	122
PFSAs					
4	PFBS	n.a.			
6	PFHxS[f]	1.47 ± 0.14	64 % ± 7 %	99 %	122
8	PFOS	2.87 ± 0.14	27 % ± 7 %	96 %	0.98
8	9Cl-PF3ONS	3.15 ± 0.12	41 % ± 6 %	87 %	0.98
8	PFECHS	1.97 ± 0.06	36 % ± 3 %	102 %	12.2

[a] ± standard deviation of at six to eight measurements (at least three measurements on two days). Kmilk/w is referred to the solid components in milk that represent possible sorbing matrices (storage lipids, membrane lipids, proteins). [b] Concentration of solid components in milk. 122 g/L corresponds to 100 % cow milk with 3.8 % fat content. [c] Not analyzable (n.a.). Partition coefficient of PFBA and PFBS to 100 % milk could not be determined since a mass balance could not be applied. Determined fraction bound was around 0 %, indicating a very low binding affinity. [d] $K_{milk/w}$ determination for PFHxA and HFPO-DA show an increased uncertainty than determinations for other compounds since the fraction bound is lower than 20 % (see method section). [e] Mean and standard deviation of only four measurements. [f] Mean and standard deviation of only five measurements.

C. Physiologically based distribution

C.1. Physiological data

Table C.1.1. Collected physiological data of a male human.

Human	Organ volume total [mL][a]	Volume fractions of tissue (v/v) in %[b]					
		Storage lipids	Membrane lipids[c]	Albumin in interstitial space[d]	Structural proteins	Water	FABP
Adipose[e]	12000	79.80	4.78	0.09	5.00	15.30	
Blood	5400	0.30	0.39	1.76[f]	16.24[g]	80.70	
Blood plasma	3240	0.30	0.50	2.94[h]	2.21[h]	92.80	
Brain	1350	4.50	5.53	0.00	8.00	77.10	
Gonads[i]	37	0.00	1.07[j]	0.08	12.00	81.00	
Gut[k]	1800	4.70[k]	1.26[j]	0.07	13.00	79.00	
Heart[e]	300	8.90	0.79	0.09	17.00	74.20	
Kidney	270	3.60	1.66	0.14	16.67	77.80[l]	0.33[l]
Liver	1470	3.70	1.15	0.12	17.69	73.70[l]	0.31[l]
Lung	775	0.30	0.56	0.27	18.00	79.60	
Muscle	23000	1.30	0.92	0.09	17.00	77.30	
Skin[e]	1800	3.60	5.02	0.18	29.00	66.70	
Spleen	145	1.40	1.03	0.13	19.00	78.30	

[a] From Freitas 1999, page 231.[147] [b] Adapted from Ruark et al. (2014).[100] [c] Only neutral phospholipids [d] According to Goss et al. (2018) for female body.[107] [e] Note, that the sum of volume fractions are over 100 %. Such inconsistencies are caused by the compilation of matrix fractions from different sources in Ruark et al. (2014). [f] Calculated with plasma albumin fraction assuming a plasma/blood volume ratio of 0.6. [g] Corrected for albumin fraction in blood. [h] Calculated with data provided in ICRP (1975),[148] an average albumin plasma concentration of 42 g/L[83], and a density of 1.36 kg/L. Protein fraction was corrected with the calculated albumin fraction. [i] Compositional data was collected from ICRP (1975).[148] [j] From Rodgers et al. (2005).[134] [k] Compositional data was taken from ICRP (1975). Since this reference lists 'fat' only as the total fat content without differentiating between storage lipids and membrane lipids, we adopted the neutral phospholipid fraction from Rodgers et al. (2005) given for rat. Storage lipid fraction was then calculated by subtracting the total fat fraction (ICRP, 1975) by the total phospholipid fraction (neutral + acidic) (Rodgers et al., 2004).[134,148] [l] FABP fraction in liver and kidney was calculated assuming highest concentration stated in literature (0.4 mM),[149,150] for depicting the 'worst case' with the model. Protein fractions in these tissues were corrected with the calculated FABP fraction.

Table C.1.2. Collected physiological data of a male rat.

Rat	Organ volume total [mL][a]	Volume fractions (v/v) in %[b]					
		Storage lipids	Membrane lipids[c]	Albumin in interstitial space[d]	Structural proteins	Water	FABP
Adipose[e]	10.00	85.30	0.16	0.09	6.00	17.50	
Blood	15.54[f]	0.10	0.16	1.43[g]	16.57[h]	82.30	
Plasma	9.32[i]	0.20	0.10	2.45[j]	1.02[k]	96.00	
Brain[l]	1.50	3.12[m]	3.94[m]	0.00	8.00	77.40	
Gonads[n]	3.40[o]	0.00	1.07	0.08	12.00[n]	86.00	
Gut[p]	11.20	3.80	1.26	0.07	5.63	76.00	
Heart	1.10	1.40	1.06	0.09	19.00	73.00	
Kidney	2.85	1.20	2.40	0.14	17.69	71.70[q]	0.31[q]
Liver	15.30	1.40	2.38	0.12	20.75	58.30[q]	0.25[q]
Lung	1.65	2.20	1.23	0.27	11.00	53.00	
Muscle	125.00[r]	1.00	0.72	0.09	19.00	75.00	
Skin[e]	41.90	2.40	0.44	0.18	41.00	64.50	
Spleen	1.30	0.80	1.07	0.13	23.00	52.00	

[a] Mean of Davies and Morris (1993) and Igari et al. (1983).[151,152] [b] Adapted from Ruark et al. (2014).[100] [c] Only neutral phospholipids [d] According to Goss et al. 2018 for female body.[107] [e] Note, that the sum of volume fractions are over 100 %. Such inconsistencies are caused by the compilation of matrix fractions from different sources in Ruark et al. (2014). [f] Mean of Davies and Morris (1993), Lee and Blaufox (1985), Šebestík and Brabec (1974), Bernareggi and Rowland (1991) for a 250 g rat.[151,153-155] [g] Calculated with plasma albumin fraction assuming a plasma/blood volume ratio of 0.6. [h] Corrected for the albumin fraction in blood. [i] Calculated with the plasma fraction of blood (0.6). [j] Mean albumin plasma fraction from Davies and Morris (1993), Bracht et al. (2016), Habgood et al. (1992) and Rose et al. (2015).[151,156-158] [k] Calculated with the albumin fraction of total plasma proteins. This albumin fraction was taken from Davies and Morris (1993) and Bracht et al. 2016.[151,156] [l] Mean of values from Davies and Morris (1993), Igari et al. (1983) and Reddy et al. (1986).[151,152,159] [m] From Reddy et al. (1983) from 9-week old rat. Storage lipids include cholesterol and neutral phospholipids (phosphatidylcholine, sphingomyelin and phosphatidylethanolamine).[159] Phospholipid fractions found in Ruark et al. (2014) are not consistent with the reported original source Rodgers et al. (2005) and were therefore replaced by primary data from Reddy et al. (1983). In this study, they conducted lipid measurements in rat brain. Lipid fractions from Reddy et al. were additionally checked to be in alignment with data from other sources.[160-163] [n] Compositional data from Rodgers et al. (2005) except for protein fraction (ICRP 1975, for human). [o] Based on Adkins (1982), calculated for a 250 g rat assuming a tissue density of 1 kg/L. [p] Compositional data from Rodgers et al. (2005) except for protein fraction (McNurlan et al., 1980).[134,164] Protein fraction was calculated based on the gut volume of 11.2 mL. [q] FABP fraction in liver and kidney was calculated assuming the highest concentration stated in literature (0.4 mM)[149] for depicting the 'worst case' with the model. Protein fractions in these tissues were corrected with the calculated FABP fraction. [r] From Igari et al. (1983). Data from Davies and Morris (1993) seem not to be reliable here: According to the publication a 0.250 kg rat would be composed of 98 % muscle (assuming a body density of 1 kg/L).[151,152]

C.2. Overview of the equilibrium partition coefficients

Table C.2.1. Overview of the experimentally determined partition coefficients between physiologically relevant matrices and water (*w*). Matrices include membrane lipid (*ml*), albumin (*alb*), structural protein (*sp*) and storage lipid (*sl*).

Compound	log ($K_{alb/w}$/ [L_w/L_{alb}])[a]	SD	log ($K_{ml/w}$/ [L_w/L_{ml}])[a]	SD	log ($K_{sp/w}$ / [L_w/L_{sp}])[a]	SD	log ($K_{sl/w}$/ [L_w/L_{sl}])[b]	SD
PFCAs								
PFBA	2.65	± 0.24	< 1.7		n.a.		< -1.4[c]	
PFHxA	3.56	± 0.17	2.32	± 0.08	0.64	± 0.31	< -1.5[d]	
PFHpA	4.16	± 0.22	2.91	± 0.06	1.30	± 0.24	< -1.1[d]	
PFOA	4.33	± 0.18	3.52	± 0.08	1.61	± 0.28	-1.37	± 0.01
PFNA	4.46	± 0.20	4.25	± 0.04	2.17	± 0.20	-0.73	± 0.02
PFDA	4.86	± 0.17	4.82	± 0.11	2.96	± 0.29	-0.55	± 0.04
PFUnDA	4.74	± 0.44	4.54	± 0.30	3.42	± 0.27	< -0.32[d]	
HFPO-DA	3.19	± 0.16	2.41	± 0.13	0.80	± 0.36	< -1.51[d]	
DONA	4.06	± 0.17	3.03	± 0.07	1.21	± 0.19	< -0.8[d]	
PFSAs								
PFBS	3.34	± 0.25	2.86	± 0.06	0.74	± 0.24	n.a.	
PFHxS	4.94	± 0.18	4.13	± 0.05	1.73	± 0.30	-1.58	± 0.17
PFOS	4.81	± 0.20	4.89	± 0.30	2.94	± 0.28	-0.56	± 0.03
9Cl-PF3ONS	5.14	± 0.17	5.14	± 0.03	3.49	± 0.30	-0.86	± 0.00
PFECHS	4.68	± 0.16	4.53	± 0.05	2.57	± 0.43	-1.09	± 0.05

[a] See Section 3.2.1. Albumin/water partition coefficients $Kalb_{/w}$ -3, $K_{alb/w}$ and $K_{sp/w}$ were converted using a protein density of 1.36 kg/L, respectively. $K_{ml/w}$ were converted using a density of 1 kg/L. [b] See Section 3.2.4. Storage lipid/water partition coefficients $Ksl_{/w}$. [c] $K_{sl/w}$ determined using 1 % HBSS solution. True $K_{sl/w}$ for a physiological relevant buffer solution is probably lower, see text for details. [d] $K_{sl/w}$ determined using 10 % HBSS solution. $K_{sl/w}$ for a physiological relevant buffer solution is probably lower, see text for details.

SD = standard deviation

Table C.2.2. Logarithmic partition coefficients between human or rat liver fatty acid binding protein (FABP) and water (log $K_{FABP/w}$ (with K in L water/L FABP)) based on literature data. Reported binding affinities to this protein were converted to partition coefficients.

References	**Zhang et al. (2013)**[a]	**Sheng et al. (2016)**[a]	**Sheng et al. (2018)**[a]	**Woodcroft et al. (2010)**[a]
	human FABP	human FABP	human FABP	rat FABP
Compound	**log ($K_{FABP/w}$ /[L_w/L_{FABP}])**[bc]			
PFCAs				
PFBA	not detected			
PFHxA	not detected			**2.3**
PFHpA	2.2			**2.9**
PFOA	3.0	**4.3**	3.8	3.6
PFNA	3.5	**4.6**		3.9
PFDA	**3.6**			
PFUnDA	**3.7**			
HFPO-DA			**3.5**	
DONA				
PFSAs				
PFBS	**1.7**			
PFHxS	2.8	**3.2**		
PFOS	3.4		**4.0**	
9Cl-PF3ONS			**4.6**	
PFECHS				

[a] Binding constant measured by fluorescence displacement. [b] $K_{FABP/w}$ values in bold print were used for the distribution calculations. [c] Converted from corresponding association/dissociation constants (K_a or K_d) with $K_{FABP/w}$ = Ka/MW_{FABP}/δ_{FABP}, with a molecular weight (MW_{FABP}) of 14 kg/mol [101], and a calculated density of 1.46 kg/L (δ_{FABP})[165], according to the formula adapted from Endo et al. (2011).[43] Same parameters were used for converting reported binding affinities to rat FABP.

C.3. Calculations of relative sorption capacities of physiological matrices in an organ

Following formulas were used to calculate relative sorption capacities of the matrices water (w), albumin (alb), structural proteins (sp), membrane lipids (ml), and storage lipids (sl) for each organ.

An additional term and equation was included to account for the sorption capacity of FABP in liver and kidney. For all other organs, V_{FABP} is set to 0.

$$f_{i,\mathrm{w}} = \frac{1}{1+ K_{i,\mathrm{alb/w}} \frac{\mathrm{V_{alb}}}{\mathrm{V_w}} + K_{i,\mathrm{sp/w}} \frac{\mathrm{V_{sp}}}{\mathrm{V_w}} + K_{i,\mathrm{ml/w}} \frac{\mathrm{V_{ml}}}{\mathrm{V_w}} + K_{i,\mathrm{sl/w}} \frac{\mathrm{V_{sl}}}{\mathrm{V_w}} + K_{i,\mathrm{FABP/w}} \frac{\mathrm{V_{FABP}}}{\mathrm{V_w}}} \quad \text{(A2)}$$

$$f_{i,\mathrm{alb}} = \frac{1}{1 + K_{i,\mathrm{w/alb}} \frac{\mathrm{V_w}}{\mathrm{V_{alb}}} + K_{i,\mathrm{sp/alb}} \frac{\mathrm{V_{sp}}}{\mathrm{V_{alb}}} + K_{i,\mathrm{ml/alb}} \frac{\mathrm{V_{ml}}}{\mathrm{V_{alb}}} + K_{i,\mathrm{sl/alb}} \frac{\mathrm{V_{sl}}}{\mathrm{V_{alb}}} + K_{i,\mathrm{FABP/alb}} \frac{\mathrm{V_{FABP}}}{\mathrm{V_{alb}}}} \quad \text{(A3)}$$

$$f_{i,\mathrm{sp}} = \frac{1}{1 + K_{i,\mathrm{w/sp}} \frac{\mathrm{V_w}}{\mathrm{V_{sp}}} + K_{i,\mathrm{alb/sp}} \frac{\mathrm{V_{alb}}}{\mathrm{V_{sp}}} + K_{i,\mathrm{ml/sp}} \frac{\mathrm{V_{ml}}}{\mathrm{V_{sp}}} + K_{i,\mathrm{sl/sp}} \frac{\mathrm{V_{sl}}}{\mathrm{V_{sp}}} + K_{i,\mathrm{FABP/sp}} \frac{\mathrm{V_{FABP}}}{\mathrm{V_{sp}}}} \quad \text{(A4)}$$

$$f_{i,\mathrm{ml}} = \frac{1}{1 + K_{i,\mathrm{w/ml}} \frac{\mathrm{V_w}}{\mathrm{V_{ml}}} + K_{i,\mathrm{alb/ml}} \frac{\mathrm{V_{alb}}}{\mathrm{V_{ml}}} + K_{i,\mathrm{sp/ml}} \frac{\mathrm{V_{sp}}}{\mathrm{V_{ml}}} + K_{i,\mathrm{sl/ml}} \frac{\mathrm{V_{sl}}}{\mathrm{V_{ml}}} + K_{i,\mathrm{FABP/ml}} \frac{\mathrm{V_{FABP}}}{\mathrm{V_{ml}}}} \quad \text{(A5)}$$

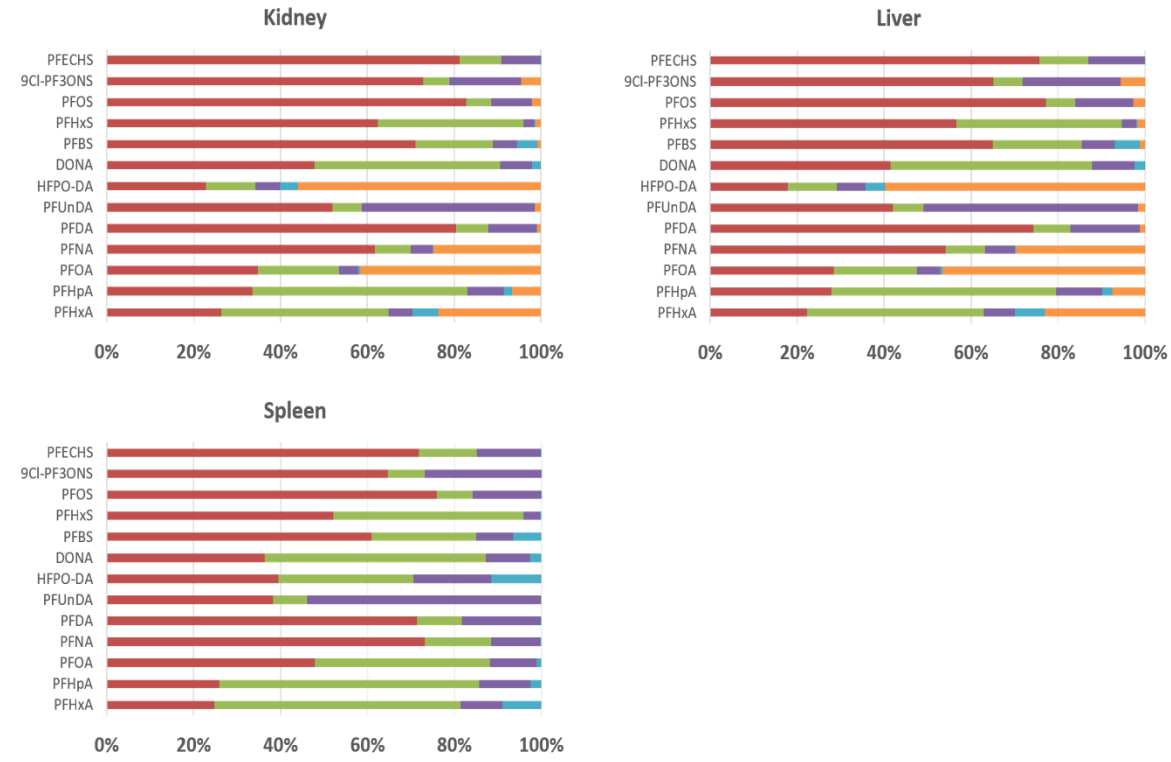
$$f_{i,\mathrm{sl}} = \frac{1}{1 + \mathrm{K}_{i,\mathrm{w/sl}} \frac{\mathrm{V_w}}{\mathrm{V_{sl}}} + \mathrm{K}_{i,\mathrm{alb/sl}} \frac{\mathrm{V_{alb}}}{\mathrm{V_{sl}}} + \mathrm{K}_{i,\mathrm{sp/sl}} \frac{\mathrm{V_{sp}}}{\mathrm{V_{sl}}} + \mathrm{K}_{i,\mathrm{ml/sl}} \frac{\mathrm{V_{ml}}}{\mathrm{V_{sl}}} + \mathrm{K}_{i,\mathrm{FABP/sl}} \frac{\mathrm{V_{FABP}}}{\mathrm{V_{sl}}}} \quad \text{(A6)}$$

$$f_{i,\mathrm{FABP}} = \frac{1}{1 + K_{i,\mathrm{w/FABP}} \frac{\mathrm{V_w}}{\mathrm{V_{FABP}}} + K_{i,\mathrm{alb/FABP}} \frac{\mathrm{V_{alb}}}{\mathrm{V_{FABP}}} + K_{i,\mathrm{sp/FABP}} \frac{\mathrm{V_{sp}}}{\mathrm{V_{FABP}}} + K_{i,\mathrm{ml/FABP}} \frac{\mathrm{V_{ml}}}{\mathrm{V_{FABP}}} + K_{i,\mathrm{sl/FABP}} \frac{\mathrm{V_{sl}}}{\mathrm{V_{FABP}}}} \quad \text{(A7)}$$

C.4. Relative sorption capacities of matrices

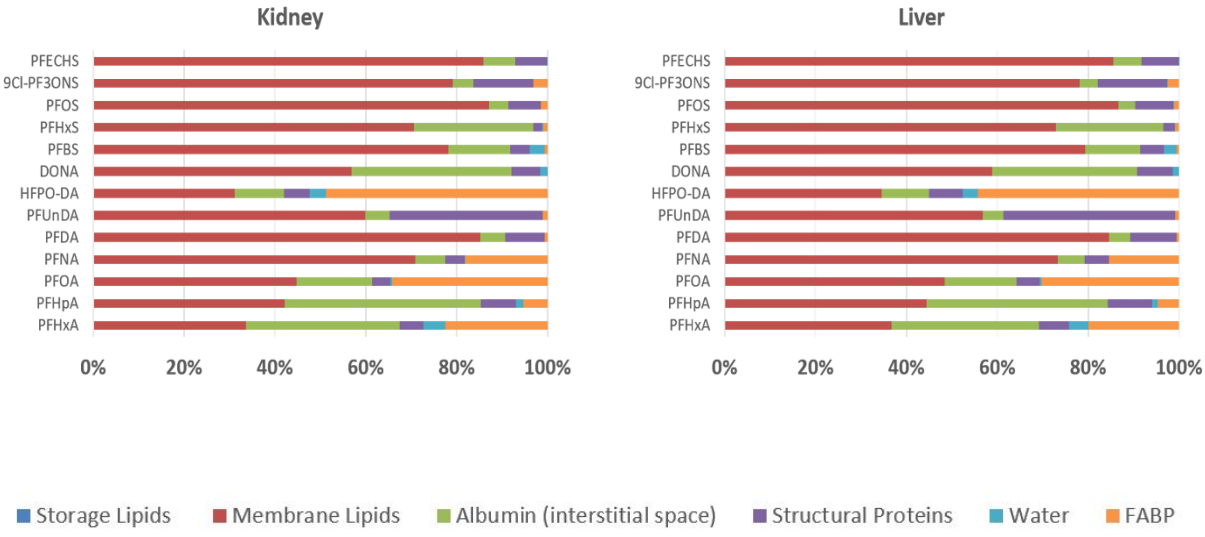
C.4.1. Relative sorption capacities - Human

In the following figures, relative sorption capacities for PFAAs and alternatives of the different matrices are displayed for each organ separately (relative sorption capacity in %). Calculations were based on the human model that accounted for albumin in interstitial space in all organs. The legend is depicted at the bottom of both pages.



C.4.2 Relative sorption capacities in liver and kidney - Rat

In the following two figures, the relative sorption capacity for PFAAs and alternatives of the different physiological matrices are displayed for kidney and liver in the rat. For these two organs, the matrix FABP was considered due to its proposed relevance for the distribution of PFAAs. Results are shown to allow a comparison to the results for human (see above). Calculations were based on the rat model that accounted for albumin in interstitial space in all organs.



C.5. Organ/water partition coefficients $K_{organ/w}$

Table C.5.1. Logarithmic organ/water partition coefficients (log $K_{organ/w}$ (with K in L water/L organ)) for a series of perfluoroalkyl acids and four of their alternatives according to Eq. 4. Calculations are based on human (male) physiology.

HUMAN log $K_{organ/w}$	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	HFPO-DA	DONA	PFBS	PFHxS	PFOS	9Cl-PF3ONS	PFECHS
Adipose	1.1	1.7	2.3	2.9	3.5	3.3	1.2	1.8	1.6	2.9	3.6	3.8	3.2
Blood	1.8	2.4	2.6	2.8	3.2	3.2	1.5	2.3	1.7	3.2	3.2	3.6	3.0
Plasma	1.3	1.9	2.2	2.4	2.6	2.6	1.3	1.8	1.1	2.6	2.6	3.0	2.4
Brain	1.1	1.7	2.3	3.0	3.6	3.3	1.2	1.8	1.6	2.9	3.6	3.9	3.3
Gonads	0.8	1.4	1.8	2.4	2.9	2.9	0.8	1.4	1.0	2.4	3.0	3.3	2.7
Gut	0.8	1.5	1.9	2.4	3.0	3.0	0.8	1.5	1.1	2.4	3.0	3.3	2.7
Heart	0.8	1.4	1.7	2.3	2.9	2.9	0.7	1.4	1.0	2.3	2.9	3.2	2.6
Kidney	1.0	1.6	2.2	2.7	3.1	3.0	1.3	1.6	1.2	2.6	3.2	3.5	2.8
Liver	0.9	1.5	2.1	2.6	3.0	3.0	1.2	1.5	1.1	2.4	3.1	3.4	2.7
Lung	1.1	1.7	1.9	2.3	2.9	2.9	0.9	1.6	1.1	2.5	2.9	3.2	2.6
Muscle	0.8	1.4	1.8	2.3	2.9	2.9	0.7	1.4	1.0	2.3	3.0	3.3	2.6
Skin	1.3	1.9	2.3	3.0	3.6	3.4	1.3	1.9	1.6	2.9	3.6	3.9	3.3
Spleen	0.9	1.5	1.9	2.4	3.0	3.0	0.8	1.5	1.1	2.4	3.0	3.3	2.7

Table C.5.2. Logarithmic organ/water partition coefficients (log $K_{organ/w}$ (with K in L water/L organ)) for a series of perfluoroalkyl acids and four of their alternatives according to Eq. 4. Calculations are based on rat (male) physiology.

RAT log $K_{organ/w}$	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	HFPO-DA	DONA	PFBS	PFHxS	PFOS	9Cl-PF3ONS	PFECHS
Adipose	0.6	1.2	1.5	1.8	2.4	2.4	0.4	1.1	0.6	2.0	2.4	2.7	2.1
Blood	0.8	1.4	1.7	2.3	2.9	2.9	0.7	1.3	0.9	2.3	2.9	3.2	2.6
Plasma	1.1	1.7	2.3	2.8	3.3	3.1	1.3	1.7	1.3	2.7	3.3	3.6	3.0
Brain	1.1	1.6	2.2	2.8	3.3	3.2	1.3	1.6	1.3	2.6	3.3	3.6	3.0
Gonads	1.0	1.5	2.1	2.9	3.4	3.2	1.1	1.6	1.5	2.7	3.5	3.8	3.1
Gut	0.9	1.4	1.8	2.4	3.0	3.0	0.8	1.4	1.1	2.4	3.0	3.3	2.7
Heart	0.9	1.5	1.9	2.4	3.0	3.0	0.8	1.5	1.1	2.4	3.0	3.4	2.7
Kidney	0.8	1.4	1.8	2.4	2.9	2.9	0.8	1.4	1.0	2.4	3.0	3.3	2.7
Liver	1.1	1.7	2.0	2.5	3.0	2.9	0.9	1.7	1.2	2.6	3.1	3.4	2.8
Lung	0.8	1.5	1.9	2.4	3.0	3.0	0.8	1.5	1.1	2.4	3.0	3.4	2.7
Muscle	1.0	1.6	1.9	2.3	2.9	3.1	0.9	1.5	1.0	2.4	2.9	3.3	2.6
Skin	1.7	2.3	2.5	2.7	3.1	3.1	1.4	2.2	1.5	3.1	3.1	3.4	2.9
Spleen	2.0	2.5	2.7	2.9	3.3	3.1	1.6	2.5	1.7	3.3	3.2	3.5	3.1

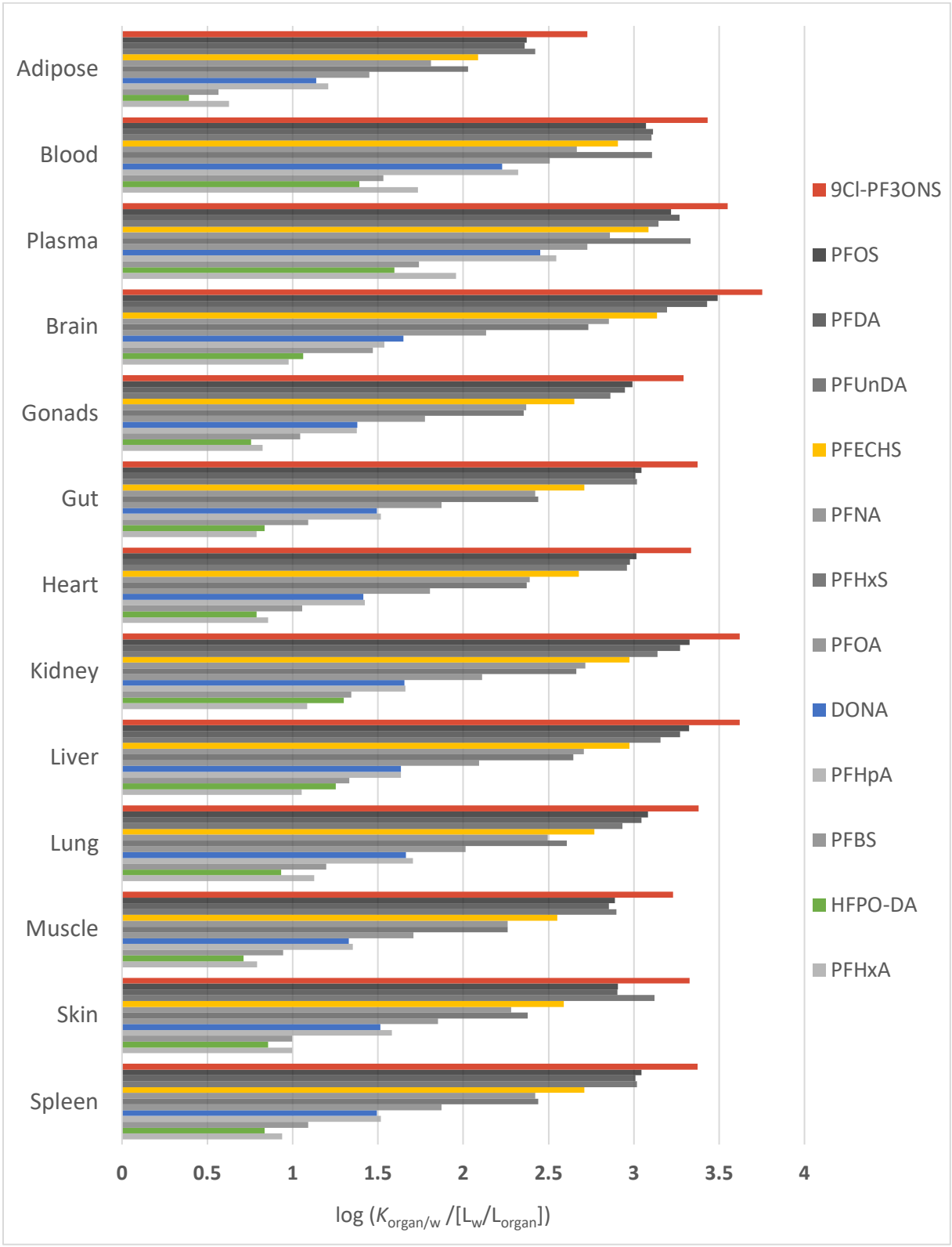


Figure C.5.3. Logarithmic organ/water partition coefficients (log $K_{organ/w}$ (with K in L water/L organ)) for a series of perfluoroalkyl acids and four of their alternatives. Calculations are based on rat (male) physiology. Compounds are intentionally arranged to facilitate comparison. For the same reason, alternatives to long-chain PFAAs are highlighted in bright colors.

D. Evaluation with experimental distribution data

D.1 Reported PFAA organ concentrations in literature - Human

Table D.1. Reported PFAA organ concentrations in human. Lung PFAA concentrations are highlighted because this organ was used as the reference organ for all calculations to allow a direct comparison between the two studies.

Compound	PFOA[a]	PFOA[b]	PFOS[a]	PFOS[b]	PFHxA[b]	PFHpA[b]	PFNA[b]	PFDA[b]	PFUnDA[b]	PFBS[b]	PFHxS[b]
Organ concentration	ng/g organ										
Adipose	1.4		1.7								
Blood	3.0		5.1								
Plasma											
Brain	0.5	bdl[c]	1.3	1.9	141.0	bdl[c]	13.5	12.4	bdl[c]	bdl[c]	2.3
Gonads	1.9		3.4								
Gut											
Heart											
Kidney	3.5	1.5	6.4	55.0	2.7	2.6	10.9	6.2[d]	1.5	1.7	18.0
Liver	3.1	4.0	13.6	41.9	68.3	1.5	1.0	bdl[c]	bdl[c]	0.7	1.8
Lung	3.8	12.1	7.9	28.4	207.0	1.5	3.5	1.5	1.4	1.1	5.7
Muscle	0.6		1.0								
Skin											
Spleen											

[a] Concentration of pooled samples from seven individuals.[111] [b] Median concentrations from 20 individuals.[112] [c] PFAA concentration were analyzed below detection limit (bdl) in the respective organ. [d] Mean concentration instead of the median was used, since median was measured below detection limit (bdl).

D.2 Comparison to measured organ concentrations in literature - Human

Table D.2. Calculated relative organ concentrations based on our modeled organ/water partition coefficients ($K_{i,organ/w}$ see Table C.5. *Organ/water partition coefficients* Korgan$_{/w}$1) and reported organ concentrations in human bodies (Table D.1 *Reported PFAA organ concentrations in literature - Human*) according to Eq. 5. Deviations between measured and calculated organ concentration are depicted by assigned colors (see legend below). We used one of the reported PFOA organ concentration (*ref*) to calculate the relative concentrations in all other organs with the respective organ/reference organ partition coefficients.

Compound	PFOA[a]	PFOA[b]	PFOS[a]	PFOS[b]	PFHxA[b]	PFHpA[b]	PFNA[b]	PFDA[b]	PFUnDA[b]	PFBS[b]	PFHxS[b]
Organ concentration						ng/g organ					
Adipose	8.2	26.2	39.5	141.8	228.8	1.7	15.2	6.7	3.2	3.5	13.1
Blood	18.1	57.6	16.4	58.9	1110.1	8.3	10.3	3.5	2.6	4.1	28.7
Plasma	24.0	76.5	17.2	62.0	1505	11.2	12.4	3.8	2.4	5.2	38.5
Brain	8.5	27.2[c]	45.1	162.3	211.8	1.5[c]	17.2	7.7	3.7[c]	3.9[c]	13.5
Gonads	2.7	8.6	10.3	36.9	109.8	0.8	4.0	1.8	1.3	1.0	4.0
Gut	3.2	10.3	10.9	39.1	107.3	1.0	4.3	2.0	1.6	1.2	4.8
Heart	2.4	7.8	8.5	30.6	108.5	0.8	3.3	1.5	1.3	0.9	3.5
Kidney	5.8	18.4	16.2	58.4	166.2	1.2	7.5	2.8	1.9	1.6	6.5
Liver	4.8	15.2	12.1	43.4	137.6	1.0	5.8	2.1[c]	1.6[c]	1.2	5.0
Lung[d]	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*
Muscle	2.6	8.2	9.5	34.1	108.5	0.8	3.7	1.7	1.4	1.0	3.7
Skin	9.9	31.5	44.1	158.6	315.0	2.3	17.0	7.6	4.5	4.0	15.3
Spleen	3.2	10.3	10.9	39.1	143.8	1.0	4.3	2.0	1.6	1.2	4.8

[a] Concentration of pooled samples from seven individuals.[111] [b] Median concentrations from 20 individuals.[112] [c] Reported PFAA concentration in the tissue was below the detection limited (see Table D.1 Reported PFAA organ concentrations in literature - Human). When a concentration was calculated that should have been higher than the experimental detection limit (> 1 ng/g organ), it was assumed that the concentration in that tissue was overestimate. [d] The reported concentration in the lung was used as the reference value ('reference organ') for the calculations in all data sets.

ref = reference organ

Legend to Table above. *Criteria for classifying the deviation of measured/calculated PFAA concentrations in organs.*

< 2x	good correlation
2-4x	acceptable
> 4x	underestimation
> 4x	overestimation

D.3 Reported PFAA organ concentrations in literature – Rat

In the following, all results are displayed for each compound and sex separately.

Table D.3.1. Reported PFOA organ concentrations in male rat. Highlighted values were used as reference PFOA organ concentrations in our distribution calculations.

MALE rat PFOA	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Kudo et al. 2007**	**Kudo et al. 2007**	**Benskin et al. 2009**	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**
Dose (mg/kg)	7.7E-05	7.7E-05	3.8E-04	3.8E-04	1.8E-03	1.8E-03	0.1	0.041	16.56	0.4	1	5	25	1
Exposure duration	**1 month**	**3 months**	**1 month**	**3 months**	**1 month**	**3 months**	**Single Dose**	**Single Dose**	**Single Dose**	**Single Dose**	**Single Dose**	**Single Dose**	**Single Dose**	**15 d**
Time post-dosing	0	0	0	0	0	0	Cmax	2 h	2 h	3 d	28 d	28 d	28 d	0
Exposure route	Oral	Oral	Oral	Oral	Oral	Oral	Oral	iv	iv	Oral	Oral	Oral	Oral	Oral
Organ concentration	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/g organ	µg/g organ	ng/g	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue
Adipose								0.009	3.430	173	0.014	0.052	0.291	0.009
Blood	3.24	4.55	17.80	20.00	87.90	97.60	265.00	0.132	57.640	1170	0.357	1.103	5.771	0.293
Plasma/Serum	6.51	8.90	35.40	40.80	160.00	184.00	759.00	0.254	105.350	2223	0.678	2.096	10.965	0.557
Brain	0.18	0.06	0.39	1.31	2.99	3.92	8.77	0.003	1.380	30	0.008	0.027	0.139	0.007
Gonads								0.018	7.670	245	0.092	0.264	1.227	0.052
Gut											0.051	0.165	1.166	0.038
Heart	1.07	1.65	5.27	7.59	28.40	30.80	108.00	0.029	13.010	440	0.121	0.349	1.863	0.084
Kidney	3.61	7.66	19.60	23.90	109.00	123.00	624.00	0.267	79.490	1170	0.357	0.935	6.229	0.272
Liver	20.20	33.30	106.00	151.00	557.00	447.00	1,270.00	0.558	87.450	2820	1.987	5.331	16.609	0.940
Lung								0.039	17.960	784	0.171	0.492	2.658	0.121
Muscle										138	0.035	0.102	0.476	0.025
Skin											0.115	0.345	1.790	0.083
Spleen	0.38	1.41	2.24	3.75	13.00	15.70	49.20	0.015	8.080	193	0.054	0.159	1.248	0.036

equiv. = equivalents; iv = intravenous

MALE rat PFOA *(continued)*	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**	**Ylinen et al 1990**	**Ylinen et al. 1990**	**Ylinen et al. 1990**	**Hundley et al. 2006**	**Cui et al. 2009**	**Cui et al. 2009**	**Kim et al. 2016**	**Kim et al. 2016**	**Vanden Heuvel et al. 1991**	**Vanden Heuvel et al. 1991**
Dose (mg/kg)	1	5	25	3	10	30	10	5	20	1	1	4	4
Exposure duration	**Single Dose**	**Single Dose**	**Single Dose**	**28 d**	**28 d**	**28 d**	**Single Dose**	**28 d**	**28 d**	**Single Dose**	**Single Dose**	**Single Dose**	**Single Dose**
Time post-dosing	171 h[a]	171 h[a]	171 h[a]	0	0	0	120 h	0	0	12 d	12 d	2 h	1 d
Exposure route	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	iv	ip	ip
Organ concentration	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg/mL	µg/mL	µg/mL	µg ^{14}C equiv./ g tissue	µg/g tissue	µg/g tissue	µg/g tissue[b]	µg/g tissue[b]	µmol/g tissue	µmol/g tissue
Adipose	0.074	0.616	2.238				1.7					3.01	2.54
Blood	0.976	7.085	26.647				23.5	39.2[c]	58.8[c]				
Plasma/Serum				48.60	87.27	51.65	44.7					18.71	15.32
Brain	0.021	0.155	0.536	0.40	1.46	0.71	0.6	10.5	7.2				
Gonads	0.231	1.244	4.022	6.24	9.35	7.22	3.2	16.7	16.8			3.10	2.54
Gut	0.140	0.963	3.546										
Heart	0.339	2.442	8.192				6.4	35.5	34.6	0.70	0.78	3.95	3.67
Kidney	0.773	4.459	19.153	1.55	40.56	39.81	24.0	228.0	209.0	3.72	2.76	8.93	6.96
Liver	4.180	18.242	54.704	39.90	51.71	49.77	40.0	218.0	196.0	5.76	5.04	19.08	19.55
Lung	0.416	3.101	10.614	2.95	22.58	23.71	8.7	63.0	64.3	0.95	1.06		
Muscle	0.108	0.749	2.626				1.9					2.44	1.97
Skin	0.280	1.792	7.114										
Spleen	0.125	0.868	3.007	4.75	7.59	4.10		13.6	6.9	0.36	0.49		

[a] Concentrations measured at Tmax/2 [b] Concentrations were extracted from Fig. 6 using WebPlotDigitizer V4.2 (https://automeris.io/WebPlotDigitizer). [c] Blood concentration was given in the unit µg/mL blood.

equiv. = equivalents; iv = intravenous; ip = intraperitoneal

Table D.3.2. Reported PFOS organ concentrations measured in male rat. Highlighted values were used as reference PFOA organ concentrations in our distribution calculations.

MALE rat PFOS	Curran et al. 2008	Curran et al. 2008	Curran et al. 2008	Curran et al. 2008	Iwabuchi et al. 2017	Iwabuchi et al. 2017	Iwabuchi et al. 2017	Iwabuchi et al. 2017	Iwabuchi et al. 2017	Iwabuchi et al. 2017	Iwabuchi et al. 2017	Benskin et al. 2009
Dose (mg/kg)	2	20	50	100	7.7E-05	7.7E-05	3.8E-04	3.8E-04	1.8E-03	1.8E-03	0.1	0.27
Exposure duration	**28 d**	**28 d**	**28 d**	**28 d**	**1 month**	**3 months**	**1 month**	**3 months**	**1 month**	**3 months**	**Single Dose**	**Single Dose**
Time post-dosing	0	0	0	0	0	0	0	0	0	0	Cmax	3 d
Exposure route	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral
Organ concentration	µg/g tissue	µg/g tissue	µg/g tissue	µg/g tissue	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	ng/g
Adipose												
Blood					0.69	1.54	3.78	6.96	10.80	50.20	52.60	296
Plasma/Serum	0.95	13.45	20.93	29.88	1.09	2.71	6.51	12.40	17.20	73.70	127.0	
Brain					0.10	0.35	0.67	1.48	1.91	9.97	9.17	38
Gonads												
Gut												
Heart	4.67	33	90.28	154.13	0.17	0.60	1.13	2.80	2.78	16.40	27.70	110
Kidney					1.13	3.65	5.46	12.60	13.30	81.20	197.0	343
Liver	48.28	560.23	856.9	1030.4	46.30	116.00	223.00	500.00	585.00	2410.00	2730.0	4200
Lung												386
Muscle												
Skin												
Spleen	6.07	45.27	122.51	230.73	0.36	0.96	1.76	4.52	4.85	29.60	46.90	145

MALE rat PFOS *(continued)*	Johnson et al. 1980	Cui et al. 2009	Cui et al. 2009	Kim et al. 2016	Kim et al. 2016	Huang et al. 2019	Huang et al. 2019	Huang et al. 2019	Huang et al. 2019	Huang et al. 2019	Huang et al. 2019	Chang et al. 2012
Dose (mg/kg)		5	20	2	2	20	2	2	20	2	2	4.2
Exposure duration	**Single Dose**	**28 d**	**28 d**	**Single Dose**	**Single Dose**	**Single Dose**	**5 d**	**Single Dose**	**Single Dose**	**5 d**	**Single Dose**	**Single Dose**
Time post-dosing	89 d	0	0	70 d	70 d	1 d	1 d	1 d	34 d	34 d	34 d	89 d
Exposure route	iv	Oral	Oral	Oral	iv	Oral	Oral	Oral	Oral	Oral	Oral	iv
Organ concentration	µg/g	µg/g tissue	µg/g tissue[c]	µg/g[a]	µg/g[a]	µM/g tissue[a]	µM/g tissue[a]	µM/g tissue[a]	µM/g tissue[a]	µM/g tissue[a]	µM/g tissue[a]	µg/g
Adipose	0.2[b]											0.2[b]
Blood		72.0[c]	[d]									
Plasma/Serum		2.2				169.4[c]		10.0[c]	55.1[c]	21.15[c]	3.3[c]	2.21[c]
Brain	< 0.05	13.6	146			19.6	8.4	1.6	5.3	2.54		≤0.05
Gonads	0.36	39.5	127									0.36
Gut												
Heart		168.0	497	6.4	6.4							
Kidney	1.09	93.9	248	43.2	33.6	139.1	52.6	10.7	38.2	16.6	3.1	1
Liver	20.56	345.0	648	425.6	345.6	313.5	165.2	54.6	192.1	105.3	26.6	20.56
Lung	1.06	46.6	228.0	27.2	24							1.06
Muscle	0.29											0.29
Skin	0.35											0.35
Spleen	0.51	38.5	167	11.2	9.6							0.51

[a] Concentrations were extracted from plots using WebPlotDigitizer V4.2 (https://automeris.io/WebPlotDigitizer). Extracted from Fig. 6 in Kim et al. 2016 and Fig. 2 and Fig. 5 in Huang et al. 2017. In the latter reference, plasma samples and tissue concentrations were extracted from the same time points in the different plots. [b] Concentration measured in subcutaneous fat. In Chang et al. 2012, abdominal fat PFOS concentration was < 0.08 µg/g. [c] Concentration was given in µg per mL blood or plasma, respectively. [d] No data available because all 10 rats died during the experiment.

iv = intravenous

Table D.3.3. Reported PFAA organ concentrations measured in male rat. Highlighted values were used as reference PFOA organ concentrations in our distribution calculations.

	PFHxA	PFHxA	PFHxA	PFHxA	PFHxA	PFNA	PFNA	PFNA	PFNA	PFNA	PFNA	PFNA	PFBS	PFHxS	PFHxS	PFHxS
MALE rat PFAAs	**Gannon 2011**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Benskin 2009**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Huang et al. 2019**	**Kim et al. 2016**	**Kim et al. 2016**	**Huang et al. 2019**
Dose (mg/kg)	100	3.8E-04	3.8E-04	1.8E-03	1.8E-03	203.09	7.7E-05	7.7E-05	3.8E-04	3.8E-04	1.8E-03	1.8E-03	20	4	4	16
Exposure duration	**Single Dose**	**1 month**	**3 months**	**1 month**	**3 months**	**Single Dose**	**1 month**	**3 months**	**1 month**	**3 months**	**1 month**	**3 months**	**Single Dose**	**Single Dose**	**Single Dose**	**Single Dose**
Time post-dosing	1 d	0	0	0	0	3 Days	0	0	0	0	0	0	6 h	72 d	72 d	13 h
Exposure route	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	iv	Oral
Organ concentration	µg equiv./g	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	ng/g	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µM/g tissue[a]	ng/g[a]	ng/g[a]	µM/g tissue[a]
Adipose	<LOQ					42.3										
Blood	<LOQ	0.114	0.118	0.453	0.452	433.9	2.49	4.27	10.70	17.90	46.70	107.00				
Plasma/Serum		0.253	0.196	0.704	0.831		4.57	8.48	19.40	36.30	89.50	189.00	115.6[b]			322.3
Brain	<LOQ		0.078	0.047	0.062	10.8	0.29	0.41	0.56	1.32	2.26	5.20	0.6			4.3
Gonads	0.11					73.8										
Gut						34.6										
Heart	0.22			0.203	0.196	116.4	0.94	1.68	3.08	6.04	12.70	30.20		94	105	
Kidney	0.3	0.252	0.073	0.6	0.8	354.4	4.95	7.57	15.00	25.40	54.20	133.00	42.7	162	192	88.2
Liver	1.56	0.199		0.366	0.357	2550.3	54.00	94.10	202.00	426.00	967.0	1770.0	142.3	225	263	172.3
Lung	0.17					261.6								87	97	
Muscle	<LOQ					49.2										
Skin	19.03															
Spleen	<LOQ			0.06		81.7	0.65	1.17	2.11	4.02	9.64	22.40		49	59	

[a] Concentrations were extracted from plots using WebPlotDigitizer V4.2 (https://automeris.io/WebPlotDigitizer). Extracted from Fig. 6 in Kim et al. (2016) and Fig. 2 – 4 in Huang et al. (2017). In the latter reference, plasma samples and tissue concentrations were extracted from the same time points in the different plots. [b] Concentration was given in µM/mL plasma. equiv. = equivalents; LOQ = limit of detection; iv = intravenous

Table D.3.4. Reported PFOA organ concentrations measured in female rat. Highlighted values were used as reference PFOA organ concentrations in our distribution calculations.

FEMALE rat PFOA	Kemper 2003	Kemper 2003	Kemper 2003	Kemper 2003	Kemper 2003	Kemper 2003	Kemper 2003	Ylinen et al. 1990	Ylinen et al. 1990	Ylinen et al. 1990	Hundley et al. 2006	Kim et al. 2016	Kim et al. 2016	Vanden Heuvel et al. 1991	Vanden Heuvel et al. 1991
Dose (mg/kg)	1	5	25	1	1	5	25	3	10	30	10	1	1	4	4
Exposure duration	**Single Dose**	**Single Dose**	**Single Dose**	**14 d**	**Single Dose**	**Single Dose**	**Single Dose**	**28 d**	**28 d**	**28 d**	**Single Dose**	**Single Dose**	**Single Dose**	**Single Dose**	**Single Dose**
Time post-dosing	7 days	7 days	7 days	15 d	4 h	4 h	4 h	0	0	0	120 h	24 h	24 h	2 h	1 d
Exposure route	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral		Oral	iv	ip	ip
Organ concentration	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./g tissue	µg ^{14}C equiv./ g tissue[a]	µg ^{14}C equiv./ g tissue[a]	µg ^{14}C equiv./ g tissue[a]	µg/mL	µg/mL	µg/mL	µg/g wet weight	µg/g[b]	µg/g[b]	µM/g tissue[c]	µM/g tissue[c]
Adipose	NA	NA	NA	NA	0.16	0.87	6.16				< 0.1				
Blood	0.001	NA	NA	0.001	1.75	9.07	60.15				< 0.1				
Plasma/Serum								2.4	12.47	13.92	< 0.1			22.5	0.2
Brain	NA	NA	NA	NA	0.04	0.24	1.55	< LOQ	0.03	0.04	< 0.1				
Gonads	NA	NA	NA	NA	0.47	2.88	18.72	< LOQ	0.41	1.16				5.0	0.5
Gut	NA	NA	NA	NA	1.57	9.90	25.27								
Heart	NA	NA	NA	NA	0.53	2.89	18.17				< 0.1	0.70	0.99		
Kidney	0.002	0.01	0.074	0.006	2.90	15.74	134.32	0.06	7.36	12.54	< 0.1	1.94	3.63	18.8	0.564
Liver	0.002	0.016	0.078	0.008	2.06	11.30	72.21	1.81	3	6.64	< 0.1	1.62	2.30	14.382	0.564
Lung	NA	NA	NA	0.001	0.85	4.82	32.62	0.24	0.22	0.75	< 0.1	0.87	1.56		
Muscle	NA	NA	NA	NA	0.19	0.77	5.30				< 0.1				
Skin	0.001	NA	0.024	0.002	0.42	2.53	16.65				< 0.01				
Spleen	NA	NA	NA	NA	0.23	1.20	8.39	0.15	0.38	1.59		0.30	0.53		

[a] Concentrations measured at Tmax/2. [b] Concentrations were extracted from Fig. 6 using WebPlotDigitizer V4.2 (https://automeris.io/WebPlotDigitizer). [c] Concentrations were converted by multiplying the dose of 9.4 µM/kg.

equiv. = equivalents; NA = not applicable; LOQ = limit of detection; iv = intravenous; ip = intraperitoneal

Table D.3.5. Reported PFOS organ concentrations measured in female rat. Highlighted values were used as reference PFOA organ concentrations in our distribution calculations.

FEMALE rat PFOS	Curran et al. 2008	Curran et al. 2008	Curran et al. 2008	Curran et al. 2008	Kim et al. 2016	Kim et al. 2016	Huang et al. 2019	Huang et al. 2019	Huang et al. 2019	Huang et al. 2019	Huang et al. 2019	Huang et al. 2019	Austin et al. 2003	Austin et al. 2003
Dose (mg/kg)	2	20	50	100	2	2	20	2	2	20	2	2	1	10
Exposure duration	**28 d**	**28 d**	**28 d**	**28 d**	**Single Dose**	**Single Dose**	**Single Dose**	**5**	**Single Dose**	**Single Dose**	**5**	**Single Dose**	**14 d**	**14 d**
Time post-dosing	0	0	0	0	70 d	70 d	1 d	1 d	1 d	34 d	34 d	34 d	0	0
Exposure route	Oral	Oral	Oral	Oral	Oral	iv	Oral	Oral	Oral	Oral	Oral	Oral	ip	ip
Organ concentration	µg/g tissue	µg/g tissue	µg/g tissue	µg/g tissue	µg/g[a]	µg/g[a]	µM/g tissue[a]	µM/g tissue[a]	µM/g tissue[a]	µM/g tissue[a]	µM/g tissue[a]	µM/g tissue[a]	µg/g	µg/g
Adipose														
Blood														
Plasma/Serum	1.50	15.40	31.93	43.20			204.8		13.9[b]	75.5[b]	34.4[b]	5.8[b]	10.48[b]	45.45[b]
Brain							21.5	7.0	1.4	5.5	2.8		1.46	44.30
Gonads													3.03	15.49
Gut														
Heart	6.54	54.65	107.53	214.45	8.0	4.8							1.28	23.49
Kidney					48.0	24.0	256.08	89.7	21.9	86.5	36.3	6.4	9.58	47.80
Liver	43.44	716.55	596.75	1008.59	433.6	388.8	298.41	165.1	45.6	138.7	72.9	20.1	26.62	97.36
Lung					28.8	24.0								
Muscle														
Skin														
Spleen	7.94	70.03	139.45	294.96	16.0	11.2							0.08	15.87

[a] Concentrations were extracted from plots using WebPlotDigitizer V4.2 (https://automeris.io/WebPlotDigitizer). Extracted from Fig. 6 in Kim et al. 2016 and Fig.2 and Fig. 5 in Huang et al. 2017. In the latter reference, plasma samples and tissue concentrations were extracted from the same time points in the different plots. [b] Concentration was given in per mL plasma, respectively.

iv = intravenous; ip = intraperitoneal

Table D.3.6. Reported PFAA organ concentrations measured in female rat. Highlighted values were used as reference PFOA organ concentrations in our distribution calculations.

FEMALE rat	PFHxA Gannon 2011	PFBS Huang et al. 2019	PFHxS Kim et al. 2016	PFHxS Kim et al. 2016	PFHxS Huang et al. 2019
Dose (mg/kg)	100	20	4	4	16
Exposure duration	**Single Dose**	**Single Dose**	**Single Dose**	**Single Dose**	**Single Dose**
Time post-dosing	1 d	6 h	14 d	14 d	13 h
Exposure route	Oral	Oral	Oral	iv	Oral
Organ concentration	µg equiv./g tissue	µM/g tissue[a]	ng/g[a]	ng/g[a]	µM/g tissue[a]
Adipose	< LOQ				
Blood					
Plasma/Serum	< LOQ	5.13[b]			198.4[b]
Brain	< LOQ				3.2
Gonads	< LOQ				
Gut					
Heart	0.17		101.3	108.8	
Kidney	0.32	3.9	165.3	197.3	76.7
Liver	1.12	5.9	266.7	272.0	95.9
Lung	0.17		110.9	98.1	
Muscle	< LOQ				
Skin	< LOQ				
Spleen	< LOQ		80.0	69.3	

[a] Concentrations were extracted from plots using WebPlotDigitizer V4.2 (https://automeris.io/WebPlotDigitizer). Extracted from Fig. 6 in Kim et al. (2016) and Fig. 2 – 4 in Huang et al. (2017). In the latter reference, plasma samples and tissue concentrations were extracted from the same time points in the different plots.
[b] Concentration was given in µM/mL plasma, respectively.

equiv. = equivalents; LOQ = limit of detection; iv = intravenous

D.4 Comparison to measured organ concentrations in literature – Rat

D.4.1 Relevance of interstitial albumin

Relevance of interstitial volume of albumin in organs was tested by including or excluding the albumin volumes in the physiological composition of the rat (Table C.1. Physiological data). The included albumin fractions were relatively small (< 1 %) for all organs. Albumin in the interstitial space improves the accuracy of the calculated concentrations for PFOA (compare D.4.1. and D.4.3 for males and females, respectively). No effect was observed for PFOS for the same organs (compare D.4.2. and D.4.4. for males and females, respectively).

The calculated PFAA organ concentrations are directly dependent on their $K_{\text{organ/w}}$, i.e. the sorption of the compound to different organ matrices ($K_{\text{matrix/w}}$) and the relative volumes of these matrices in the organs (Eq. 4). The observed differences between the compounds resulted from their relatively different sorption behavior. For PFOA, determined sorption to albumin (log $K_{\text{alb/w}}$ of 4.3 (with K in L_w/L_{alb})) is distinctively higher than sorption to other matrices such as membrane lipids (log $K_{\text{ml/w}}$ 3.5 [L_w/L_{ml}], see Table App. C.2. Overview of the equilibrium partition coefficients1). For PFOS, $K_{\text{alb/w}}$ and $K_{\text{ml/w}}$ are similar (4.8 and 3.9 log units, respectively). The accumulation of PFOA in an organ (the calculated $K_{\text{organ/w}}$) is therefore still influenced by sorption to albumin even if only small fractions of albumin are present, while the accumulation of PFOS is – at the same composition – dominated by sorption to membrane lipids.

This correlation becomes clear considering a simplified version of Equation 4 for the composition of the heart, assuming the heart organ consists only of membrane lipids (1.06 %), interstitial albumin (0.09 %) and water (73 %).

$$K_{i,\text{heart/w}} = f_{i,\text{alb}}\, K_{i,\text{alb/w}} + f_{i,\text{ml}}\, K_{i,\text{ml/w}} + f_{i,\text{w}}$$

$$K_{i,\text{heart/w}} = 0.09\ \% * K_{i,\text{alb/w}} + 1.06\ \% * K_{i,\text{ml/w}} + 73\ \%$$

Albumin would become only relevant for $K_{i,\text{organ/w}}$ when $K_{i,\text{alb/w}}$ is significantly higher than the sorption to the other present matrix (here: membrane lipids). Otherwise, the albumin volume is too small compared to the volume of other existing matrices to noticeably affect the $K_{i,\text{organ/w}}$.

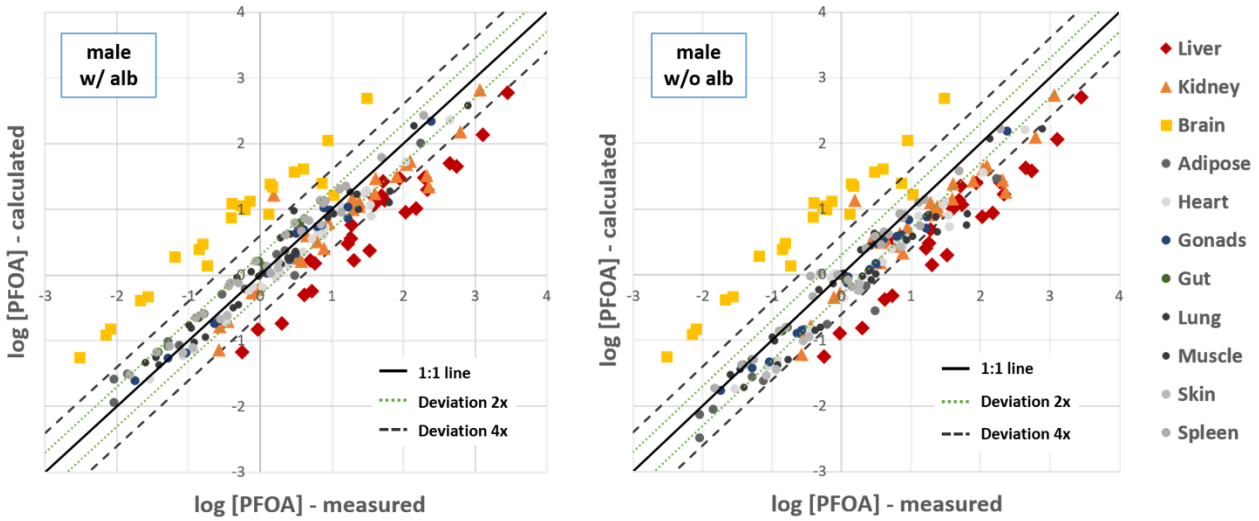


Figure D.4.1. Comparison between measured and calculated relative PFOA concentrations in various organs for male rat. Concentrations were calculated using the rat model which included (w/ alb, left) or excluded albumin in interstitial space of organs other than blood (w/o alb, right). Two limits were defined to evaluate deviations between the measured to calculated concentrations, a twofold (green dotted line) and a fourfold deviation (dashed line). Concentration units are dependent on the units of the measured concentrations and were not standardized.

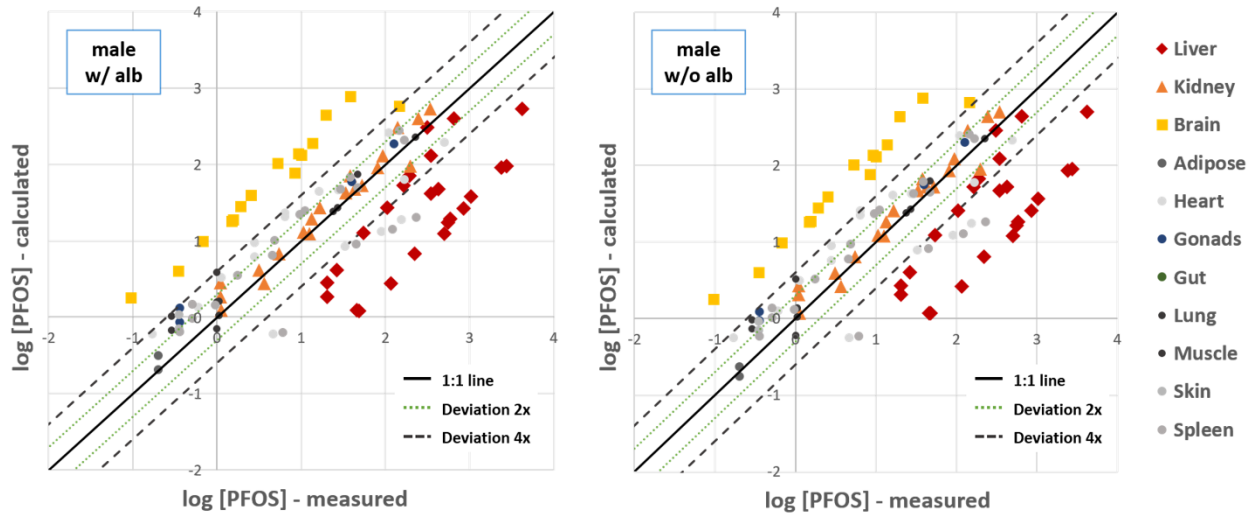


Figure D.4.2. Comparison between measured and calculated relative PFOS concentrations in various organs for male rat. Concentrations were calculated using the rat model which included (w/ alb, left) or excluded albumin in interstitial space of organs other than blood (w/o alb, right). We defined two limits to evaluate deviations between the measured to calculated concentrations, a twofold (green dotted line) and a fourfold deviation (dashed line). Concentration units are dependent on the units of the measured concentrations and were not standardized.

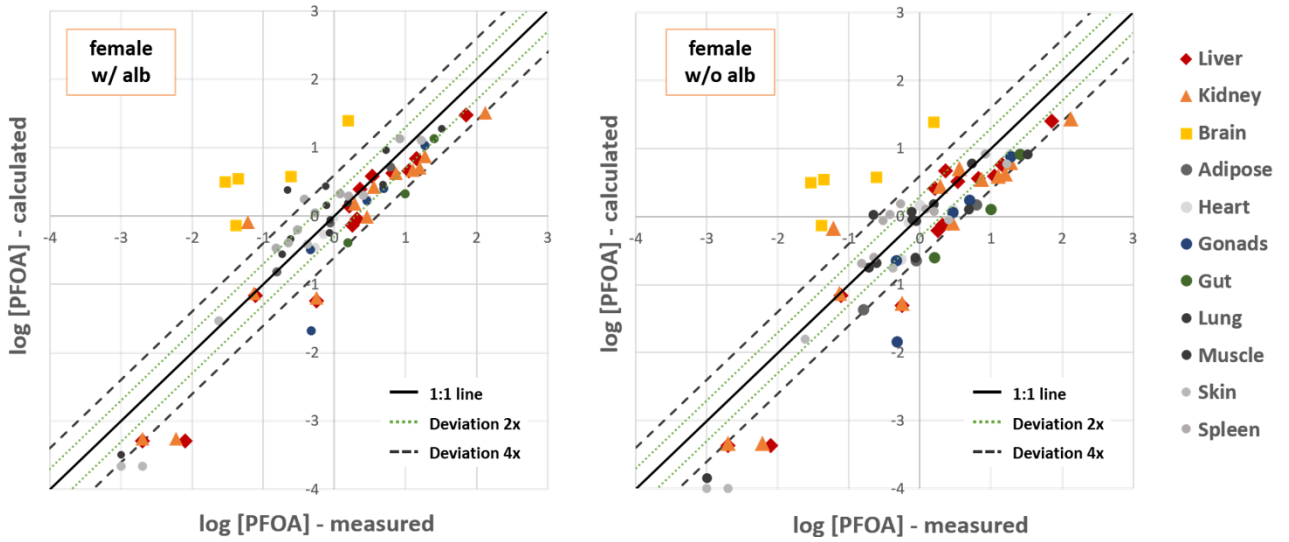


Figure D.4.3. Comparison between measured and calculated relative PFOA concentrations in various organs for female rat. Concentrations were calculated using the rat model which included (w/ alb, left) or excluded albumin in interstitial space of organs other than blood (w/o alb, right). We defined two limits to evaluate deviations between the measured to calculated concentrations, a twofold (green dotted line) and a fourfold deviation (dashed line). Concentration units are dependent on the units of the measured concentrations and were not standardized.

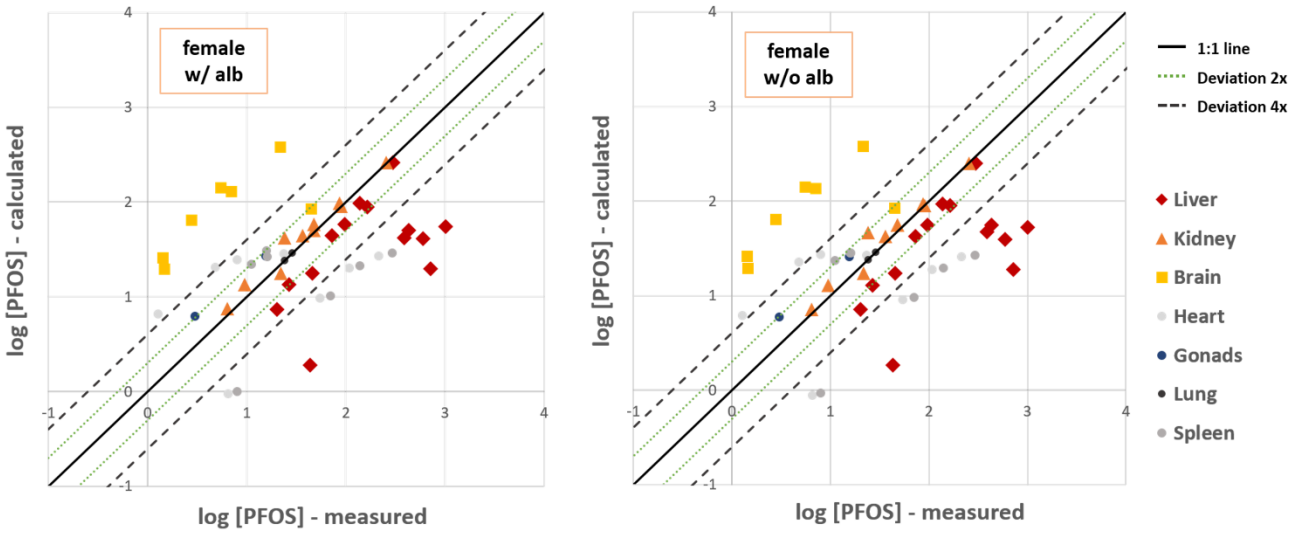


Figure D.4.4. Comparison between measured and calculated relative PFOS concentrations in various organs for female rat. . Concentrations were calculated using the rat model which included (w/ alb, left) or excluded albumin in interstitial space of organs other than blood (w/o alb, right). We defined two limits to evaluate deviations between the measured to calculated concentrations, a twofold (green dotted line) and a fourfold deviation (dashed line). Concentration units are dependent on the units of the measured concentrations and were not standardized.

Table D.4.5. Calculated relative PFOA organ concentrations in male rat (see also Figure 8 in main text). One of the reported PFOA organ concentration (marked by *ref*) was used to calculate the relative concentrations in all other organs with the respective organ/reference organ partition coefficients (Eq. 5). Deviations between measured and calculated organ concentration are depicted by assigned colors (see legend below). For reported PFOA concentrations and the used reference organ concentration, see Table D.3 *Reported PFAA organ concentrations in literature – Rat*1. For calculations we assumed a density of 1 kg/L for all organs.

MALE rat PFOA	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Kudo et al. 2007**	**Kudo et al. 2007**	**Benskin et al. 2009**	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**
Calculated organ concentration	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/g organ	µg/g organ	ng/g	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue
Adipose	0.28	0.40	1.56	1.76	7.72	8.57	23.27	0.01	5.06	102.73	0.03	0.10	0.51	0.03
Blood	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*
Plasma/Serum	5.37	7.54	29.50	33.15	145.68	161.76	439.21	0.22	95.53	1939.14	0.59	1.83	9.56	0.49
Brain	1.38	1.93	7.56	8.49	37.31	41.43	112.49	0.06	24.47	496.65	0.15	0.47	2.45	0.12
Gonads	0.60	0.84	3.30	3.71	16.32	18.12	49.20	0.02	10.70	217.22	0.07	0.20	1.07	0.05
Gut	0.75	1.06	4.14	4.65	20.43	22.68	61.58	0.03	13.39	271.88	0.08	0.26	1.34	0.07
Heart	0.65	0.91	3.55	3.99	17.53	19.46	52.85	0.03	11.49	233.32	0.07	0.22	1.15	0.06
Kidney	1.81	2.54	9.93	11.16	49.03	54.44	147.81	0.07	32.15	652.60	0.20	0.62	3.22	0.16
Liver	1.66	2.33	9.10	10.23	44.94	49.90	135.50	0.07	29.47	598.23	0.18	0.56	2.95	0.15
Lung	1.04	1.47	5.74	6.44	28.33	31.45	85.39	0.04	18.57	377.03	0.12	0.36	1.86	0.09
Muscle	0.51	0.72	2.82	3.17	13.94	15.48	42.03	0.02	9.14	185.55	0.06	0.17	0.92	0.05
Skin	0.72	1.01	3.93	4.42	19.42	21.57	58.56	0.03	12.74	258.54	0.08	0.24	1.28	0.06
Spleen	0.75	1.06	4.14	4.65	20.43	22.68	61.58	0.03	13.39	271.88	0.08	0.26	1.34	0.07

equiv. = equivalents; *ref* = reference organ

Legend. *Criteria for classifying the deviation of measured/calculated PFAA concentrations in tissues.*

< 2x	good correlation
2-4x	acceptable
> 4x	underestimation
> 4x	overestimation

MALE rat PFOA *(continued)*	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**	**Ylinen et al 1990**	**Ylinen et al. 1990**	**Ylinen et al. 1990**	**Hundley et al. 2006**	**Cui et al. 2009**	**Cui et al. 2009**	**Kim et al. 2016**	**Kim et al. 2016**	**Vanden Heuvel et al. 1991**	**Vanden Heuvel et al. 1991**
Calculated organ concentration	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg/mL	µg/mL	µg/mL	µg ^{14}C equiv./ g tissue	µg/g tissue	µg/g tissue	µg/g tissue	µg/g tissue	µmol/g tissue	µmol/g tissue
Adipose	0.09	0.62	2.34	2.57	4.62	2.74	2.06	3.44	5.16	0.26	0.29	0.99	0.81
Blood	*ref*	*ref*	*ref*	29.32	52.66	31.16	*ref*	*ref*	*ref*	2.95	3.30	11.29	9.24
Plasma/Serum	1.62	11.74	44.16	*ref*	*ref*	*ref*	38.95	64.97	97.45	4.89	5.47	*ref*	*ref*
Brain	0.41	3.01	11.31	12.45	22.35	13.23	9.98	16.64	24.96	1.25	1.40	4.79	3.92
Gonads	0.18	1.32	4.95	5.44	9.78	5.79	4.36	7.28	10.92	0.55	0.61	2.10	1.72
Gut	0.23	1.65	6.19	6.81	12.24	7.24	5.46	9.11	13.66	0.69	0.77	2.62	2.15
Heart	0.19	1.41	5.31	5.85	10.50	6.21	4.69	7.82	11.73	0.59	0.66	2.25	1.84
Kidney	0.54	3.95	14.86	16.36	29.37	17.38	13.11	21.86	32.80	1.64	1.84	6.30	5.16
Liver	0.50	3.62	13.62	14.99	26.92	15.93	12.02	20.04	30.06	1.51	1.69	5.77	4.73
Lung	0.31	2.28	8.59	9.45	16.97	10.04	7.57	12.63	18.95	*ref*	*ref*	3.64	2.98
Muscle	0.15	1.12	4.23	4.65	8.35	4.94	3.73	6.22	9.33	0.47	0.52	1.79	1.47
Skin	0.22	1.57	5.89	6.48	11.64	6.89	5.19	8.66	12.99	0.65	0.73	2.49	2.04
Spleen	0.23	1.65	6.19	6.81	12.24	7.24	5.46	9.11	13.66	0.69	0.77	2.62	2.15

equiv. = equivalents; *ref* = reference organ

Table D.4.6. Calculated relative PFOS organ concentrations in male rat (see also left panel in D.4.2). One of the reported PFOS organ concentration (marked by *ref*) was used to calculate the relative concentrations in all other organs with the respective organ/reference organ partition coefficients (Eq. 5). Differences between measured and calculated organ concentration are depicted by assigned colors (see legend below). For reported PFOS concentrations and the used reference organ concentration, see Table D.3 *Reported PFAA organ concentrations in literature – Rat*2. For calculations we assumed a density of 1 kg/L for all organs.

MALE rat PFOS	**Curran et al. 2008**	**Curran et al. 2008**	**Curran et al. 2008**	**Curran et al. 2008**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Iwabuchi et al. 2017**	**Benskin et al. 2009**
Calculated organ concentration	µg/g tissue	µg/g tissue	µg/g tissue	µg/g tissue	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	ng/g
Adipose	0.14	1.91	2.98	4.25	0.14	0.31	0.75	1.39	2.15	9.99	10.47	58.92
Blood	0.68	9.62	14.97	21.37	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*
Plasma/Serum	*ref*	*ref*	*ref*	*ref*	0.96	2.15	5.29	9.73	15.10	70.20	73.56	413.94
Brain	1.78	25.26	39.31	56.12	1.81	4.04	9.93	18.28	28.37	131.85	138.16	777.47
Gonads	0.57	8.01	12.47	17.80	0.57	1.28	3.15	5.80	9.00	41.81	43.81	246.54
Gut	0.64	9.02	14.04	20.04	0.65	1.44	3.55	6.53	10.13	47.08	49.33	277.62
Heart	0.60	8.47	13.17	18.81	0.61	1.36	3.33	6.13	9.51	44.19	46.30	260.54
Kidney	1.22	17.25	26.84	38.31	1.23	2.76	6.78	12.48	19.36	90.01	94.31	530.73
Liver	1.21	17.19	26.75	38.19	1.23	2.75	6.76	12.44	19.31	89.74	94.03	529.12
Lung	0.70	9.87	15.35	21.92	0.71	1.58	3.88	7.14	11.08	51.50	53.96	303.66
Muscle	0.44	6.29	9.79	13.98	0.45	1.01	2.47	4.55	7.07	32.85	34.42	193.69
Skin	0.46	6.58	10.24	14.61	0.47	1.05	2.59	4.76	7.39	34.34	35.98	202.47
Spleen	0.64	9.02	14.04	20.04	0.65	1.44	3.55	6.53	10.13	47.08	49.33	277.62

ref = reference organ

Legend: *Criteria for classifying the deviation of measured/calculated PFAA concentrations in organs.*

< 2x	good correlation
2-4x	acceptable
> 4x	underestimation
> 4x	overestimation

MALE rat PFOS *(continued)*	**Johnson et al. 1980**	**Cui et al. 2009**	**Cui et al. 2009**	**Kim et al. 2016**	**Kim et al. 2016**	**Huang et al. 2019**	**Huang et al. 2019**	**Huang et al. 2019**	**Huang et al. 2019**	**Huang et al. 2019**	**Huang et al. 2019**	**Chang et al. 2012**
Calculated organ concentration	µg/g	µg/g tissue	µg/g tissue	µg/g	µg/g	µM/g tissue	µM/g tissue	µM/g tissue	µM/g tissue	µM/g tissue	µM/g tissue	µg/g
Adipose	0.21	14.33	44.24	5.28	4.66	24.11	5.90	1.42	7.84	3.01	0.46	0.31
Blood	1.03	*ref*	222.25	26.51	23.39	121.14	29.62	7.15	39.38	15.12	2.32	1.58
Plasma/Serum	1.44	100.69	310.80	37.08	32.72	*ref*	41.42	*ref*	*ref*	*ref*	*ref*	*ref*
Brain	2.71	189.11	583.76	69.64	61.45	318.17	77.79	18.79	103.45	39.72	6.11	4.15
Gonads	0.86	59.97	185.12	22.08	19.49	100.90	24.67	5.96	32.80	12.60	1.94	1.32
Gut	0.97	67.53	208.45	24.87	21.94	113.61	27.78	6.71	36.94	14.18	2.18	1.48
Heart	0.91	63.37	195.62	23.34	20.59	106.62	26.07	6.30	34.67	13.31	2.05	1.39
Kidney	1.85	129.10	398.50	47.54	41.95	303.74	*ref*	12.83	70.62	27.12	4.17	2.83
Liver	1.85	128.70	397.28	47.40	41.82	302.81	52.47	12.79	70.40	27.03	4.16	2.82
Lung	*ref*	73.86	*ref*	*ref*	*ref*	124.27	30.38	7.34	40.40	15.52	2.39	1.62
Muscle	0.68	47.11	145.43	17.35	15.31	79.26	19.38	4.68	25.77	9.90	1.52	1.03
Skin	0.71	49.25	152.02	18.14	16.00	82.86	20.26	4.89	26.94	10.34	1.59	1.08
Spleen	0.97	67.53	208.45	24.87	21.94	113.61	27.78	6.71	36.94	14.18	2.18	1.48

ref = reference organ

Table D.4.7. Calculated relative PFAA organ concentrations in male rat. One of the reported PFAA organ concentration (marked by *ref*) was used to calculate the relative concentrations in all other organs with the respective organ/reference organ partition coefficients (Eq. 5). Differences between measured and calculated organ concentration are depicted by assigned colors (see legend below). For reported PFAA concentrations and the used reference organ concentration, see Table D.3 *Reported PFAA organ concentrations in literature – Rat*3. For calculations we assumed a density of 1 kg/L for all organs.

MALE rat PFAAs	**PFHxA** **Ganno n 2011**	**PFHxA** **Iwabuchi et al. 2017**	**PFHxA** **Iwabuchi et al. 2017**	**PFHxA** **Iwabuchi et al. 2017**	**PFHxA** **Iwabuchi et al. 2017**	**PFNA** **Benskin 2009**	**PFNA** **Iwabuchi et al. 2017**	**PFNA** **Iwabuchi et al. 2017**	**PFNA** **Iwabuchi et al. 2017**	**PFNA** **Iwabuchi et al. 2017**	**PFNA** **Iwabuchi et al. 2017**	**PFNA** **Iwabuchi et al. 2017**	**PFBS** **Huang et al. 2019**	**PFHxS** **Kim et al. 2016**	**PFHxS** **Kim et al. 2016**	**PFHxS** **Huang et al. 2019**
Calculated organ concentration	µg equiv./ g	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	ng/g	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µg/kg tissue volume	µM/g tissue	ng/g	ng/g	µM/g tissue
Adipose	0.04	0.01	0.01	0.04	0.04	60.47	0.35	0.60	1.49	2.49	6.51	14.91	5.74	23.05	25.58	16.01
Blood	0.54	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*	68.73	276.0	306.2	191.6
Plasma/Serum	0.90	0.19	0.20	0.76	0.76	675.25	3.88	6.65	16.65	27.86	72.68	166.53	*ref*	464.1	515.0	*ref*
Brain	0.23	0.02	0.02	0.08	0.08	670.47	3.85	6.60	16.53	27.66	72.17	165.35	29.11	116.8	129.7	81.18
Gonads	0.09	0.01	0.01	0.06	0.06	219.14	1.26	2.16	5.40	9.04	23.59	54.04	12.12	48.68	54.02	33.81
Gut	0.12	0.01	0.01	0.05	0.05	247.08	1.42	2.43	6.09	10.19	26.59	60.93	14.78	59.34	65.86	41.21
Heart	0.10	0.02	0.02	0.06	0.06	229.08	1.31	2.25	5.65	9.45	24.66	56.49	12.68	50.93	56.52	35.37
Kidney	0.36	0.03	0.03	0.10	0.10	565.42	3.24	5.56	13.94	23.33	60.86	139.44	24.75	99.39	110.3	69.02
Liver	0.35	0.02	0.02	0.09	0.09	542.16	3.11	5.34	13.37	22.37	58.36	133.71	23.78	95.51	105.9	66.33
Lung	*ref*	0.03	0.03	0.11	0.11	292.49	1.68	2.88	7.21	12.07	31.48	72.13	21.78	*ref*	*ref*	60.74
Muscle	0.08	0.01	0.01	0.05	0.05	170.12	0.98	1.67	4.20	7.02	18.31	41.96	9.82	39.44	43.77	27.39
Skin	0.10	0.02	0.02	0.08	0.08	179.31	1.03	1.76	4.42	7.40	19.30	44.22	12.86	51.66	57.33	35.87
Spleen	0.12	0.02	0.02	0.07	0.07	247.08	1.42	2.43	6.09	10.19	26.59	60.93	14.78	59.34	65.86	41.21

equiv. = equivalents; *ref* = reference organ

Legend: *Criteria for classifying the deviation of measured/calculated PFAA concentrations in organs.*

< 2x	good correlation
2-4x	acceptable
> 4x	underestimation
> 4x	overestimation

Table D.4.8. Calculated relative PFOA organ concentrations in female rat (see also left panel in D.4.3). One of the reported PFOA organ concentration (marked by *ref*) was used to calculate the relative concentrations in all other organs with the respective organ/reference organ partition coefficients (Eq. 5). Differences between measured and calculated organ concentration are depicted by assigned colors (see legend below). For reported PFOA concentrations and the used reference organ concentration, see Table D.3 *Reported PFAA organ concentrations in literature – Rat*4. For calculations we assumed a density of 1 kg/L for all organs.

FEMALE rat PFOA	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**	**Kemper 2003**	**Ylinen et al. 1990**	**Ylinen et al. 1990**	**Ylinen et al. 1990**	**Hundley et al. 2006**	**Kim et al. 2016**	**Kim et al. 2016**	**Vanden Heuvel et al. 1991**	**Vanden Heuvel et al. 1991**
Calculated organ concentration	µg ^{14}C equiv./ g tissue	Not calculated[a]	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./g tissue	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg ^{14}C equiv./ g tissue	µg/mL	µg/mL	µg/mL	Not calculated[a]	µg/g	µg/g	µmol/g tissue	µmol/g tissue
Adipose	8.8E-05		0.01	8.8E-05	0.15	0.80	5.28	0.13	0.66	0.74		0.24	0.42	1.19	0.01
Blood	*ref*		0.13	*ref*	*ref*	*ref*	*ref*	1.45	7.52	8.40		2.71	4.84	13.56	0.11
Plasma/Serum	1.7E-03		0.22	1.7E-03	2.91	15.02	99.68	*ref*	*ref*	*ref*		4.50	8.01	*ref*	*ref*
Brain	4.2E-04		0.06	4.2E-04	0.74	3.85	25.53	0.61	3.19	3.57		1.15	2.05	5.75	0.05
Gonads	1.9E-04		0.02	1.9E-04	0.33	1.68	11.17	0.27	1.40	1.56		0.50	0.90	2.52	0.02
Gut	2.3E-04		0.03	2.3E-04	0.41	2.11	13.98	0.34	1.75	1.95		0.63	1.12	3.15	0.03
Heart	2.0E-04		0.03	2.0E-04	0.35	1.81	11.99	0.29	1.50	1.67		0.54	0.96	2.70	0.02
Kidney	5.6E-04		*ref*	0.001	0.98	5.06	33.55	0.81	4.20	4.68		1.51	2.70	7.56	0.06
Liver	5.1E-04		0.07	0.001	0.90	4.63	30.75	0.74	3.85	4.29		1.39	2.47	6.93	0.06
Lung	3.2E-04		0.04	3.2E-04	0.56	2.92	19.38	0.47	2.42	2.71		*ref*	*ref*	4.37	0.04
Muscle	1.6E-04		0.02	1.6E-04	0.28	1.44	9.54	0.23	1.19	1.33		0.43	0.77	2.15	0.02
Skin	2.2E-04		0.03	2.2E-04	0.39	2.00	13.29	0.32	1.66	1.86		0.60	1.07	3.00	0.03
Spleen	2.3E-04		0.03	2.3E-04	0.41	2.11	13.98	0.34	1.75	1.95		0.63	1.12	3.15	0.03

[a] Data set was excluded because either less than three organ concentrations were reported or concentrations were estimated (actual concentrations should be smaller than limit of detection). See Table D.3 Reported PFAA organ concentrations in literature – Rat4.

equiv. = equivalents; *ref* = reference organ

Legend: *Criteria for classifying the deviation of measured/calculated PFAA concentrations in organs.*

< 2x	good correlation
2-4x	acceptable
> 4x	underestimation
> 4x	overestimation

Table D.4.9. Calculated relative PFOS organ concentrations in female rat (see also left panel in D.4.4). One of the reported PFOS organ concentration (marked by *ref*) was used to calculate the relative concentrations in all other organs with the respective organ/reference organ partition coefficients (Eq. 5). Differences between measured and calculated organ concentration are depicted by assigned colors (see legend below). For reported PFOS concentrations and the used reference organ concentrations, see Table D.3 *Reported PFAA organ concentrations in literature – Rat*5. For calculations we assumed a density of 1 kg/L for all organs.

FEMALE rat PFOS	**Curran et al. 2008**	**Curran et al. 2008**	**Curran et al. 2008**	**Curran et al. 2008**	**Kim et al. 2016**	**Kim et al. 2016**	**Huang et al. 2019**	**Huang et al. 2019**	**Huang et al. 2019**	**Huang et al. 2019**	**Huang et al. 2019**	**Huang et al. 2019**	**Austin et al. 2003**	**Austin et al. 2003**
Calculated organ concentration	µg/g tissue	µg/g tissue	µg/g tissue	µg/g tissue	µg/g	µg/g	µM/g tissue	µM/g tissue	µM/g tissue	µM/g tissue	µM/g tissue	µM/g tissue	µg/g	µg/g
Adipose	0.21	2.19	4.54	6.15	5.59	4.66	29.15	10.05	1.98	10.74	4.89	0.83	1.49	6.47
Blood	1.07	11.01	22.83	30.89	28.07	23.39	146.47	50.48	9.96	53.97	24.59	4.15	7.49	32.50
Plasma/Serum	*ref*	*ref*	*ref*	*ref*	39.26	32.72	*ref*	70.59	*ref*	*ref*	*ref*	*ref*	*ref*	*ref*
Brain	2.82	28.92	59.97	81.14	73.74	61.45	384.70	132.59	26.15	141.76	64.58	10.91	19.68	85.36
Gonads/Testes	0.89	9.17	19.02	25.73	23.38	19.49	121.99	42.05	8.29	44.95	20.48	3.46	6.24	27.07
Gut	1.01	10.33	21.41	28.97	26.33	21.94	137.37	47.35	9.34	50.62	23.06	3.89	7.03	30.48
Heart	0.94	9.69	20.10	27.19	24.71	20.59	128.92	44.43	8.76	47.50	21.64	3.65	6.60	28.60
Kidney	1.92	19.75	40.94	55.39	50.34	41.95	262.61	*ref*	17.85	96.77	44.08	7.45	13.44	58.27
Liver	1.92	19.69	40.81	55.22	50.18	41.82	261.82	89.45	17.80	96.47	43.95	7.42	13.40	58.09
Lung	1.10	11.30	23.42	31.69	*ref*	*ref*	150.26	51.79	10.21	55.37	25.22	4.26	7.69	33.34
Muscle	0.70	7.21	14.94	20.21	18.37	15.31	95.84	33.03	6.51	35.31	16.09	2.72	4.90	21.26
Skin	0.73	7.53	15.62	21.13	19.20	16.00	100.18	34.53	6.81	36.92	16.82	2.84	5.13	22.23
Spleen	1.01	10.33	21.41	28.97	26.33	21.94	137.37	47.35	9.34	50.62	23.06	3.89	7.03	30.48

ref = reference organ

Legend: *Criteria for classifying the deviation of measured/calculated PFAA concentrations in organs.*

< 2x	good correlation
2-4x	Acceptable
> 4x	underestimation
> 4x	overestimation

Table D.4.10. Calculated relative PFAA organ concentrations in female rat. One of the reported PFAA organ concentration (marked by *ref*) was used to calculate the relative concentrations in all other organs with the respective organ/reference organ partition coefficients (Eq. 5). Differences between measured and calculated organ concentration are depicted by assigned colors (see legend below). For reported PFAA concentrations and the used reference organ concentrations, see Table D.3 *Reported PFAA organ concentrations in literature – Rat*6. For calculations we assumed a density of 1 kg/L for all organs.

	PFHxA	**PFBS**	**PFHxS**	**PFHxS**	**PFHxS**
FEMALE rat PFAAs	**Gannon 2011**	**Huang et al. 2019**	**Kim et al. 2016**	**Kim et al. 2016**	**Huang et al. 2019**
Calculated organ concentration	µg equiv./g tissue	µM/g tissue	ng/g	ng/g	µM/g tissue
Adipose	0.05	0.34	29.23	25.86	9.85
Blood	0.69	3.17	350.04	309.65	117.98
Plasma/Serum	1.16	*ref*	588.61	520.69	*ref*
Brain	0.12	2.76	148.25	131.15	49.97
Gonads	0.09	1.03	61.74	54.62	20.81
Gut	0.08	1.16	75.26	66.58	25.37
Heart	0.09	1.07	64.59	57.14	21.77
Kidney	0.15	2.06	126.06	111.51	42.49
Liver	0.14	2.01	121.13	107.15	40.83
Lung	*ref*	1.47	*ref*	*ref*	37.39
Muscle	0.08	0.82	50.02	44.25	16.86
Skin	0.13	0.93	65.51	57.95	22.08
Spleen	0.11	1.16	75.26	66.58	25.37

equiv. = equivalents; *ref* = reference organ

< 2x	good correlation
2-4x	acceptable
> 4x	underestimation
> 4x	overestimation

Legend: *Criteria for classifying the deviation of measured/calculated PFAA concentrations in organs.*

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Danksagung

An dieser Stelle möchte ich allen beteiligten Personen danken, die mich bei der Erstellung meiner Dissertation unterstützt haben.

Mein besonderer Dank gilt Prof. Dr. Kai-Uwe Goss und Dr. Nadin Ulrich, die mir ermöglicht haben, die Doktorarbeit in ihrer Forschungsgruppe auszuarbeiten und die mich bei ihrer Umsetzung der Arbeit intensiv angeleitet haben. Frau Dr. Ulrich half mir mit stets hohen Qualitätsansprüchen an die Laborarbeit schnelle und kreative Lösungen für vermeintlich unüberbrückbare Hindernisse zu finden. Ich danke ihr für die sorgfältigen Korrekturen meiner Manuskripte und die Freude, die wir bei Erfolgen miteinander geteilt haben. Herr Prof. Dr. Goss hatte immer den kritischen Blick für das große Ganze und gab mir wichtige Denkanstöße für das Projekt. Er zeigte mir ein ums andere Mal, dass wissenschaftliches Hinterfragen nicht außerhalb des eigenen Labors aufhören darf. Ferner möchte ich mich bei Dr. Urs Berger bedanken, der mit seiner reichen Erfahrung und Expertise auf dem Gebiet der PFAAs das Projekt initiiert und mich auf meinem Weg unterstützt hat.

Danken möchte ich auch all den anderen AUC-Teammitgliedern: allen voran meiner Bürogenossin in der Kommando-/Detektiv-/Kreativzentrale und dabei erste Anlaufstelle bei Problemen und Fragen, Sophia Krause. Bei Andrea Ebert für ihre geduldigen Erklärungen der Weiten des Cosmo-Universe's und den Tiefen der Permeabilitäten; an ihrer akribischen Arbeitsweise konnte ich mir selbst bei der Obsternte noch ein Beispiel nehmen. Bei Wolfgang Larisch, Estella Garessus, Martin Gerhards und Adaptiv-AUC'ler Andreas Baumer für die offenen Ohren, den Erfahrungsaustausch und für die hilfreichen Gespräche. Bei Andrea Pfenningsdorff und Heidrun Paschke, die mir im Labor mit Rat und Tat zur Seite standen.

Außerdem möchte ich vielen Personen außerhalb des Instituts danken, die mich in dieser Zeit bestärkt haben, mein Ziel nicht aus den Augen zu verlieren. Da ist vor allem meine Familie und mein Freund, auf deren Rat ich zu jeder Zeit und über jede Ferne zählen und aus denen ich Kraft schöpfen konnte. Abschließend möchte ich auch den verschiedenen Freundesgruppen in Darmstadt, Bonn, Kaiserslautern, Mannheim und Leipzig danken, die mich aus ihren vielen unterschiedlichen Erfahrungen und Perspektiven in diesen drei Jahren motiviert und bereichert haben.

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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich diese Arbeit selbstständig und ohne fremde Hilfe verfasst habe, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe und die den benutzten Werken inhaltlich oder wörtlich entnommenen Stellen als solche kenntliche gemacht habe.

Außerdem erkläre ich, dass ich noch keine vergeblichen Promotionsversuche unternommen habe und die vorliegende Dissertationsschrift weder in der gegenwärtigen noch in einer anderen Fassung bei einer anderen Fakultät vorgelegen hat.

Leipzig, 14.04.21 _____

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