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;40:910–920. © 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

INTRODUCTION

Perfluoroalkyl acids (PFAAs) are surface-active and persistent compounds that have been widely used as processing aids in the fluorochemical industry or as flame suppressants. Their persistence along with their environmental mobility led to their worldwide occurrence. All PFAAs—especially long-chain perfluoroalkyl carboxylic acids (PFCAs) and perfluoralkane sulfonic acids (PFSAs)—have been found to accumulate in various organisms and are suspected to cause developmental and organ toxic effects in humans (Conder et al. 2008). Regulatory and voluntary restrictions on the manufacture and use of long-chain PFAAs such as perfluoroocanoic acid (PFOA) led to the increased production of replacement compounds.

These alternative compounds include short-chain PFAAs and compounds that often exhibit ether-based structures that are still highly fluorinated. For simplification we use the term “alternatives” in this work only for these ether-based compounds.

Apart from the structural similarities between alternatives and conventional PFAAs, their observed persistence has raised concerns about their accumulative potential for organisms (Scheringer et al. 2014). However, quantitatively estimating the uptake and distribution has been challenging for all highly fluorinated alkyl acids. Standard approaches such as evaluation of the octanol–water distribution coefficient are not appropriate (US Environmental Protection Agency 2016). Furthermore, read-across between individual compounds often does not work. For example, one could have expected that incorporation of an ether group into a perfluorinated structure would lead to higher polarity and thus make it less sorptive. This is not the case, though, as we could show for their sorption behavior to proteins and membrane lipids (Allendorf et al. 2019; Ebert et al. 2020). Substantial differences in the accumulation in different species and even different sexes...
within one species increase the already high complexity of the behavior of these compounds (Han et al. 2012).

To be able to predict the accumulative potential of PFAAs, aspects of their physicochemical behavior, which have long been established and generally taken for granted with other compounds, need to be reconsidered and examined. One aspect is to understand the equilibrium partitioning behavior of these compounds. Even this simple partitioning behavior has not yet been fully understood for the PFAAs and alternatives. To improve this, we have investigated the sorption to all physiological sorption matrices that are potentially relevant such as various lipids and proteins. These matrices can become relevant either by their high fractional volume in the body or by particularly high distribution coefficients for the investigated compound.

Several biological matrices with high sorption capacities and the corresponding distribution coefficients or binding affinities have been reported for some PFAAs. These include serum albumin as a transport protein in blood, phospholipids as the major component of cellular membranes, alpha globulins, and liver fatty acid binding proteins (FABPs) that belong to the intracellular lipid-binding protein superfamily (Luebker et al. 2002; Han et al. 2003; Weaver et al. 2010; Woodcroft et al. 2010; Bischel et al. 2011; Armitage et al. 2012; Zhang et al. 2013; Droge 2018; Sheng et al. 2016, 2018; Allendorf et al. 2019). But not all potentially relevant distribution coefficients have been measured so far, and some other conceivable sorption mechanisms, such as interfacial sorption or ion-pair formation and subsequent sorption of ion pairs, have not even been discussed at all to date. Published models for accumulation in rats or fish used some of the available distribution coefficients but disregarded the possible partitioning of PFAAs to other biological matrices such as structural proteins or storage lipids or used summarily estimated values (Armitage et al. 2013; Cheng and Ng 2017). The model by Cheng and Ng (2017) included active transport processes to predict the accumulation behavior in rats. However, before increasing the complexity of the model by including active transport processes, it is crucial to understand all equilibrium partitioning processes to identify knowledge gaps in accumulative behavior.

Previously, we presented a consistent set of distribution coefficients for PFAAs and 4 of their alternatives to serum albumin and membrane lipids (Allendorf et al. 2019; Ebert et al. 2020). In these 2 publications, we reevaluated distribution coefficients of PFAAs to albumin determined by others. Also, we extended our data set to more PFAAs and alternatives to estimate their sorption behavior in organisms. However, for a comprehensive overview of the distribution of PFAAs and alternatives in organisms, one also has to consider the still unknown sorption behavior to other biological matrices such as structural proteins and storage lipids. Even if the compounds would have lower affinities to these matrices than to albumin or to membrane lipids, they could still have a relevant sorption capacity because they account for a high-volume fraction in organisms.

The present study investigated the partitioning for PFAAs and alternatives to all physiological matrices that are potentially relevant in mammals based on equilibrium distribution calculations for various organs, as has been done before for other compounds (Goss et al. 2018). In this approach, relative volumes of the different biological matrices in an organ (physiological composition) were combined with the respective matrix–water distribution coefficients of a compound to calculate the relative sorption capacity for that compound in individual organs. This enabled us to generate a comprehensive picture of the expected equilibrium partitioning of the studied compounds in mammals.

To this end, we first determined missing structural protein–water distribution coefficients ($D_{sp/w}$) by dialysis experiments and storage lipid–water distribution coefficients ($D_{sl/w}$) in batch experiments for 6 PFCAs, 3 PFSAs, and 4 of their alternatives. We further tested whether ion pair formation or surface-activity would affect $D_{sl/w}$.

Finally, we evaluated our approach by comparing estimated to measured organ concentrations in rats and humans. We used various studies that reported organ concentrations of PFAAs. Such validation is only meaningful to a limited extent because of the variability and uncertainties in these experimental studies. Nevertheless, this validation allowed us to assess whether we had covered all relevant sorption processes in our distribution calculations and to what extent there are indications of other processes such as active transport across cell membranes because several different experimental studies were available.

**MATERIALS AND METHODS**

**Chemicals**

The PFAAs and alternatives investigated in the present study included perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorooctanamide (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonic acid (PFHxS), perfluoroctane sulfonic acid (PFOS), dodecafluoro-3H-4, 8-dioxononanoate (DONA), tetrafluoro-2-(heptafluoropropoxy) propanoic acid (HFPO-DA), 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS), and perfluoro-4-ethylcyclohexanesulfonate (PFECHS). All other chemicals used, together with their abbreviations and suppliers, and the chemical structures of the alternatives are given in the Supplemental Data (SI-1.1–1.2).

Structural proteins were extracted from chicken breast fillet. Such muscle tissue mainly consists of the proteins actin and myosin (60–95%; Endo et al. 2012). In brief, structural proteins were extracted, freeze-dried, ground, and thoroughly defatted (Endo et al. 2012; Henneberger et al. 2016). Small fibers or fiber-like structures could not be completely ground and remained in the prepared protein sample, which was stored at −25 °C until use.

Olive oil with a high fraction of unsaturated fatty acids (cold-pressed, extra native; Bioplanet) and fresh milk with 3.8% fat content (homogenized, pasteurized) were bought from a local grocery store.
Determination of distribution coefficients

Dialysis experiments for determination of $D_{sp/w}$. The determination of $D_{sp/w}$ by dialysis experiments was conducted as described in Henneberger et al. (2016). Phosphate buffer (30 mM) was prepared ($\mathrm{pH}$ 7.7; see Supplemental Data, SI-1.3). Dialysis cells were composed of 2 glass chambers (custom-made) separated by a semipermeable membrane (molecular cutoff 3.5 kDa; Spectrum Laboratories; Supplemental Data, SI-1.4). It was shown that the membrane prevented proteins from permeating to 99.99% (see leaking tests in Henneberger et al. [2016] for more details). A total of 3 test and 3 reference cells were prepared for each compound individually. Buffer solution containing the respective compound (20 µg/L) was added to 1 of the 2 chambers of both test and reference cells (5 mL, respectively). For the test cells, a fixed amount of the protein extract was weighed directly into the second chamber of the dialysis cell assembly, and 5 mL of buffer solution was added. For the reference cells, this second chamber contained only buffer solution (5 mL). Dialysis cells were incubated at 37 °C under constant shaking (470 rpm) in darkness. Equilibration time was dependent on the investigated compound and was 72 or 96 h (Supplemental Data, SI-1.4; Allendorf et al. 2019).

When equilibrium was assumed, samples were taken (200 µL) from the protein-free chamber of the test cells and both chambers of the reference cells. Dialysis cells were incubated for 1 d more, and sampling was repeated to ensure that no significant difference was observed for the determined distribution coefficient between the 2 time points. This indicated whether equilibrium was reached (Allendorf et al. 2019).

For each experiment, the compound fraction that was sorbed to the proteins was kept between 20 and 80% to reduce measuring uncertainty (Supplemental Data, SI-1.5). Thus, the concentration of proteins was adapted to the sorption behavior of the investigated compound and ranged from 2 to 100 g/L (based on a chamber volume of 5 mL).

Batch experiments for determination of $D_{dl/w}$. Experiments for the determination of $D_{dl/w}$ were conducted with solutions consisting of olive oil and an aqueous phase. For the aqueous phase, buffer solution in 3 dilutions (100, 10, or 1% Hanks’ Balanced Salt Solution [HBSS]; Supplemental Data, SI-1.3) 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 were added to a total volume of 1 mL in polypropylene vials (Labosolute). Vials with oil and aqueous phase (test vials) and vials without oil (reference vials) were prepared in triplicate each, similar to the setup 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 lipid–water system is dominated by partitioning into the interface between the storage lipids and the surrounding aqueous phase, we compared calculated and experimentally determined milk–water distribution coefficients ($D_{milk/w}$). 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 (Geisler et al. 2011). We determined $D_{milk/w}$ for 3 PFCAs (PFOA, PFNA, PFDA) directly with fresh milk by conducting dialysis experiments similar to the protocol described in the previous section. For comparison, we calculated $D_{milk/w}$ for these PFCAs based on the bulk-phase distribution coefficients to all milk components and the milk composition. If the experimental overall distribution coefficient would be higher than the one calculated based on all known absorption processes, this would indicate that an additional sorption process—likely adsorption at the fat–water interface—played a role. Experimental details, milk composition, along with calculations can be found in Supplemental Data, SI-1.6.

Instrumental analysis. Compounds were quantified using ultra performance liquid chromatography with tandem mass spectrometry (Xevo TQ-S; Waters) in negative electrospray ionization mode (for a detailed description, see Supplemental Data, SI-1.7 and references Berger et al. [2009] and Allendorf et al. [2019]).

Determination of matrix–water distribution coefficients. Matrix–water distribution coefficients $D_{matrix/w}$ ($D_{sp/w}$ in liters of water per kilogram of structural protein and $D_{dl/w}$ in liters of water per liter of storage lipid) are defined as

$$D_{i,matrix/w} = \frac{c_{i,matrix}^*}{c_{i,w}^*}$$

where $i$ refers to the compound, $*$ states the equilibrium condition, $c_{i,matrix}$ is the compound concentration in the biological matrix (grams per kilogram of structural protein or grams per liter of oil, respectively), and $c_{i,w}$ is the concentration in the aqueous phase (grams per liter of water). Concentrations were determined based on a mass balance approach (Equation 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 ($m_{i,ref}$).

$$m_{i,matrix} = m_{i,ref} - m_{i,w}$$

For experiments using the dialysis cells, reference cells could not be used for PFDA, PFUnDA, PFOS, and 9Cl-PF3ONS because the chemicals sorbed to a different extent to the individually shaped glass surfaces of the chambers. As a result, the $m_{i,ref}$ in each dialysis cell was reduced differently and could not be used for a correct mass balance. (The polypropylene vials were identically shaped. The adsorption to the surface of the vials could therefore be compensated by using the reference cells).

For compounds with a fraction >20% sorbed to the glass surface, an extraction step was added. After the last sampling,
all fluids were discarded, and methanol was added (2 mL). For the chambers that had contained the proteins, several short washing steps with water were included to remove the remaining matrix before the extraction. After the cells were shaken for 1 h, the methanol was sampled. The compound mass was analyzed, and \( m_{\text{matrix}} \) was then calculated, thus taking into account the loss by adsorption to the glass surface.

**Physiologically based distribution calculations**

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

We used the physiological composition for rat and human based on Ruark et al. (2014), which considers fractional volumes of relevant matrices (storage lipids, membrane lipids, structural proteins, and water) in each organ (Supplemental Data, SI-1.8). We included the volume of liver FABP in the composition of liver and kidney because of the proposed relevance (Luebker et al. 2002; Cheng and Ng 2017). We further added albumin fractions in the interstitial space of each organ. Albumin as a component of the interstitium is usually not considered in physiologically based models for other compounds (Schmitt 2008; Ruark et al. 2014) but could be of relevance because of the high sorption capacity of albumin for PFAAs (Cheng and Ng 2017).

Distribution coefficients to the biological matrices for PFAAs and alternatives originated from the present study or were reported previously and are displayed in Table 1 (Allendorf et al. 2019; Ebert et al. 2020). The binding affinities to liver FABP were reported elsewhere (Weaver et al. 2010; Woodcroft et al. 2010; Zhang et al. 2013; Sheng et al. 2018) and converted to distribution coefficients (Supplemental Data, SI-1.9). Our distribution calculations assume that distribution coefficients to biological matrices resulting from in vitro experiments can be directly transferred to the in vivo situation and that all components of the organism are at equilibrium with each other. Once a compound enters the circulatory system, this equilibrium should indeed be established very quickly, provided that no substantial biotransformation or active transport occurs. The PFAAs and alternatives are not metabolically degraded, and the possibility of involved active transport processes is discussed within the evaluation of our approach.

The organ composition and the corresponding distribution coefficients allow calculation of the relative sorption of a compound in an organ to a specific matrix within this organ, which is called the “sorption capacity.” Equation 3 represents the calculation of the sorption capacity of albumin relative to all other organ components as an example (water = w, albumin = alb, structural proteins = sp, biological matrices = \( \text{matrix} \)).

### Table 1: Determined distribution coefficients

| Compoundb | \( \log (D_{\text{linv}}/L_{\text{linv}}) \) | SD | \( \log (D_{\text{albw}}/L_{\text{albw}}) \) | SD | \( \log (D_{\text{spbw}}/L_{\text{spbw}}) \) | SD | \( \log (D_{\text{alb}}/L_{\text{alb}}) \) | SD |
|-----------|---------------------------------|----|---------------------------------|----|---------------------------------|----|---------------------------------|----|
| PFCAs     |                                 |    |                                 |    |                                 |    |                                 |    |
| PFBA      | <1.7g                           | ±0.08 | 2.65                             | ±0.11 | n.a.                            | ±0.08 | n.a.                            | ±0.08 |
| PFHxS     | 3.22 ±0.08                      | 4.16 | ±0.04                           | 0.64  | ±0.18                           | 1.30 | ±0.11                           | 1.1h  |
| PFHpA     | 3.91 ±0.06                      | 4.33 | ±0.05                           | 1.61  | ±0.15                           | 1.51 | ±0.04                           | 0.04  |
| PFNa      | 4.25 ±0.04                      | 4.46 | ±0.07                           | 1.27  | ±0.06                           | 1.27 | ±0.06                           | 0.01  |
| PFDA      | 4.82 ±0.11                      | 4.86 | ±0.04                           | 2.96  | ±0.15                           | 1.51 | ±0.04                           | 0.04  |
| PFUnDA    | 4.54 ±0.30                      | 4.74 | ±0.3                            | 3.42  | ±0.14                           | 0.01 | ±0.01                           | 0.01  |
| HFPO-DA   | 2.41 ±0.13                      | 3.19 | ±0.03                           | 0.80  | ±0.23                           | 1.21 | ±0.05                           | 0.01  |
| DONA      | 3.03 ±0.07                      | 4.06 | ±0.03                           | 1.21  | ±0.05                           | 0.01 | ±0.01                           | 0.01  |
| PFBS      | 2.86 ±0.06                      | 3.34 | ±0.11                           | 0.74  | ±0.1                           | n.a. | ±0.08                           | n.a.  |
| PFHxS     | 4.13 ±0.05                      | 4.94 | ±0.05                           | 1.73  | ±0.17                           | 1.51 | ±0.04                           | 0.01  |
| PFOS      | 4.89 ±0.30                      | 4.81 | ±0.07                           | 2.94  | ±0.15                           | 0.01 | ±0.01                           | 0.01  |
| 9Cl-PF3ONS| 5.14 ±0.03                      | 5.14 | ±0.04                           | 3.49  | ±0.16                           | 0.86 | ±0.00                           | 0.00  |
| PFechS    | 4.53 ±0.05                      | 4.68 | ±0.03                           | 2.52  | ±0.3                            | 1.09 | ±0.05                           | 0.05  |

*a Experimentally determined distribution coefficients from the literature and from the present study between physiologically relevant matrices and water.

*b See methods and methods section for acronyms.

*Values for \( D_{\text{linv}} \) were converted assuming a density of 1 kg/L.

*Values for \( D_{\text{albw}} \) were converted assuming a density of 1.36 kg/L.

*Values for \( D_{\text{spbw}} \) were determined in batch experiments with olive oil and Hanks’ Balanced Salt Solution (HBSS) buffer solution; only results that showed a fraction bound between 20 and 80% are depicted.

*Values for \( D_{\text{alb}} \) could not be measured because of too weak sorption; \( D_{\text{alb}} \) of 1.7 log units corresponds to a fraction bound of 20% (see Ebert et al. 2020).

*Distribution coefficient could not be determined because a mass balance could not be applied. Determined fraction bound was approximately 0%, indicating a very low sorption.

*Values for \( D_{\text{linv}} \) were determined using 1% HBSS solution. True \( D_{\text{linv}} \) for a physiologically relevant buffer solution is probably lower; see text for details.

*Values for \( D_{\text{albw}} \) were determined using 10% HBSS solution; \( D_{\text{albw}} \) for a physiologically relevant buffer solution is probably lower; see text for details.

9Cl-9Cl3ONS = 9-chlorohexadecafluoro-3-oxygenane-1-sulfonate; \( D_{\text{albw}} \) = albumin-water distribution coefficient; \( D_{\text{spbw}} \) = membrane lipid-water distribution coefficient; \( D_{\text{alb}} \) = storage lipid-water distribution coefficient; \( D_{\text{ linv}} \) = storage matrix-water distribution coefficient; \( D_{\text{alb}} \) = storage lipid-water distribution coefficient; HFPO-DA = tetrafluoro-2-(heptfluoropropoxy)propanoic acid; n.a. = not analyzed; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFCA = perfluoroolyalk carbonic acid; PFDA = perfluorodecanoic acid; PFCSH = perfluoro-4-ethylcyclohexanesulfonate; PFHpA = perfluorooheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononoic acid; PFOS = perfluorooctanoic acid; FFSA = perfluorooctane sulfonic acid; PFUnDA = perfluoroundecanoic acid; SD = standard deviation.

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membrane lipid = ml, storage lipid = sl, or FABP). Analogous to this, formulas were compiled for each matrix separately (Supplemental Data, SI-1.10),

\[
f_{i, \text{alb}} = \frac{1}{1 + D_{i, \text{w/\text{alb}}} V_{\text{alb}} + D_{i, \text{sp/\text{alb}}} V_{\text{alb}} + D_{i, \text{ml/\text{alb}}} V_{\text{alb}}} \\
V_{\text{alb}} \left( D_{i, \text{FABP/\text{alb}}} V_{\text{FABP}} \right)
\]

where \( f_{i, \text{alb}} \) 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 (Supplemental Data, SI-1.8), and \( D_{i, \text{matrix/\text{alb}}} \) is the distribution coefficient of a compound between another matrix and albumin (in liters of albumin per liters of matrix). For kidney and liver, a term was inserted in the equations to account for the partitioning of storage lipids and to albumin (Allendorf et al. 2019; Ebert et al. 2020).

For each organ, organ–water distribution coefficients were calculated for a compound \( i \) (\( D_{i, \text{organ/w}} \)) with the following formula:

\[
D_{i, \text{organ/w}} = f_{i, \text{alb}} D_{i, \text{alb/w}} + f_{i, \text{sp}} D_{i, \text{sp/w}} + f_{i, \text{ml}} D_{i, \text{ml/w}} + f_{i, \text{sl}} D_{i, \text{sl/w}} + f_{i, \text{FABP}} D_{i, \text{FABP/w}} + f_{i, \text{w}}
\]

**Evaluation with experimental distribution data**

Our calculated organ distribution coefficients were evaluated by comparison with measured PFAN concentrations in organs of human and rat reported in the literature. Because of the time-variant exposure conditions, the organisms were not in a steady-state situation at any time. Nevertheless, we expect that the organisms had reached an internal equilibrium distribution after a few hours (in case of intravenous dosing) or after 171 h in male rats and 4 h in female rats after oral exposure. This is supported by the membrane permeabilities of these chemicals, which we published earlier and which are in the range of what is typically found for pharmaceuticals. Hence, at least the relative partitioning between various organs could be evaluated with our equilibrium distribution model. To this end, one reported organ concentration from each study was used as the reference value ("reference organ") 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 that have not been associated with active transport.

**RESULTS AND DISCUSSION**

**Equilibrium distribution coefficients**

**Structural protein–water distribution coefficients, \( D_{\text{sp/w}} \)** Values of \( D_{\text{sp/w}} \) determined by dialysis experiments for PFANs and alternatives ranged from 0.6 to 3.5 log units (Table 1; Supplemental Data, SI-2.1). Values of \( D_{\text{sp/w}} \) increased with increasing number of perfluorinated carbons for PFCAs and PFSAs, respectively, whereas PFSAs showed higher \( D_{\text{sp/w}} \) than their corresponding carboxylic counterparts (same number of perfluorinated carbons). Alternatives sorb to a similar extent to structural proteins compared to the PFANs.

As expected, PFANs and alternatives show in general lower sorption affinities to structural proteins than to membrane lipids and to albumin (Allendorf et al. 2019; Ebert et al. 2020). The trend between these compounds is, however, always similar. Structural modifications of the alternatives such as the ether bonds do not substantially affect the sorption behavior to proteins. Our present results for structural proteins support our previous assumption that alternatives are likely to be as accumulative as the classical PFANs (Allendorf et al. 2019; Ebert et al. 2020). Using quantum-chemical calculations, we have suggested that this is caused by the high electronegativity of the fluorine atoms in the direct neighborhood of the ether bond. A more detailed discussion can be found in the references. The variability in the experimental \( D_{\text{sp/w}} \) for all compounds was relatively high with a standard deviation of approximately 0.3 log units compared to the results from our other dialysis cell experiments, which generally deviated by <0.1 log units (Allendorf et al. 2019; Ebert et al. 2020). This could be due to the more heterogeneous biological extract.

**Storage lipid–water distribution coefficients, \( D_{\text{sl/w}} \)** In the past, storage lipids have only been considered for neutral compounds as a possible sorbing matrix, and are usually neglected for ions. Yet, in 2 cases, storage lipids could become relevant for the accumulation of PFANs. 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 interface between the storage lipids and the surrounding aqueous phase. To investigate this, we determined $D_{slw}$ by 2 different experiments.

In the batch experiments, $D_{slw}$ values for PFAAs and alternatives were determined in a setup containing olive oil and either different dilutions of a physiological buffer solution (100, 10, and 1% HBSS; Supplemental Data, SI-2.2) 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 (Geisler et al. 2012).

In general, the determined $D_{slw}$ values are low ($<0.5$ log units) for all compounds. In some cases (e.g., PFBS; see Supplemental Data, Table SI-2.2.1), the sorption to oil was not detectable at all.

The batch experiments with different dilutions of the buffer solution (10 and 1% HBSS) and pure water were supposed to answer the question of whether PFAAs form and partition as 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, will show stronger partitioning into olive oil than the negatively charged PFAAs without cationic counterparts. For decreasing salt concentration (i.e., fewer cations available for ion pair formation), we would expect decreasing sorption of PFAAs and their alternatives to oil. In the case of no ion pair formation, the determined $D_{slw}$ 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: $D_{slw}$ of the tested compounds increased with decreasing salt concentration. Highest $D_{slw}$ values were measured with pure water or 1% HBSS for all compounds (Supplemental Data, SI-2.2.2). We could not explain this observation mechanistically. However, the data show that ion pair formation does not play a relevant role under realistic conditions and that the overall sorption to storage lipids is very small for PFAAs and alternatives.

Possible partitioning of PFAAs into the interface between the storage lipids and the surrounding aqueous phase was investigated with the dialysis experiments. Experimentally determined $D_{milkw}$ values for PFOA, PFNA, and PFDA were compared to the respective $D_{milkw}$ values that were calculated without the consideration of possible interface effects (Supplemental Data, SI-2.2.3). The experimentally determined $D_{milkw}$ values for the 3 PFCAs are 1 order of magnitude higher compared to their respective calculated $D_{milkw}$ values. This indicates that the partitioning of PFAAs and alternatives to the storage lipids of milk is indeed increased approximately 1 order of magnitude 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, therefore, assume that sorption to the interface should be of low relevance regarding the overall accumulation of PFAAs and alternatives, and we did not further consider this process in our distribution model. This is supported by the observed low enrichment of PFAAs in adipose tissue in rat (Ng and Hungerbühler 2014). However, this additional—and so far overlooked—sorption process can be essential in the investigation of accumulation 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.

### Physiologically based distribution

The estimated distribution is based on matrix–water distribution coefficients (Table 1) and on physiological data for an adult male human or rat (Supplemental Data, SI-1.8). For the physiological data, we used data by Ruark et al. (2014), which listed matrix fractions for human and rat organs separately. The membrane lipid fraction in rat brain had to be revised in the values of Ruark et al. because it was not consistent with the data in the original references (see Supplemental Data, SI-1.8.2). More details about the difficulties and limitations encountered frequently in compiling such physiological data can be found in Supplemental Data, SI-1.8.

Although we specifically distinguish between human and rat organ compositions, we apply the same $D_{matrix/w}$ for PFAAs in both species (Table 1). This is the typical approach in physiologically based modeling (Schmitt 2008; Ruark et al. 2014) and is supported by experimental findings (Rouser et al. 1969). Proteins such as albumin show a close homology between human, bovine, and rat in their primary and tertiary structure (Peters 1995). Furthermore, only small differences were found for $D_{matrix/w}$ determined with albumin structural proteins or FABP of different species for various compounds, including PFOA and PFNA (Han et al. 2003; van der Vusse 2009; MacManus-Spencer et al. 2010; Woodcroft et al. 2010; Endo et al. 2012; Zhang et al. 2013; Sheng et al. 2016, 2018).

Equilibrium binding affinities to human and to rat FABP were reported for 9 of the investigated PFAAs and 2 alternatives (Supplemental Data, SI-1.9) and converted to $D_{FABP/w}$ (Woodcroft et al. 2010; Zhang et al. 2013; Sheng et al. 2016, 2018). If more than one binding affinity for a compound was specified, we used the highest value for the respective compound to depict the “worst” case with our distribution calculations. For the same reason, we included FABP at the highest concentration in the liver and kidney composition reported in the literature (0.4 mM [Zhang et al. 2013]).

Relative sorption capacities of the matrices were calculated for each organ in human and rat (Supplemental Data, Figure SI-2.3) to investigate which sorption process to which matrix is relevant for the distribution of PFAAs. The sorption capacity of a matrix is the product of the volume of the matrix in the organ combined with the volume-based distribution coefficient (Equation 3). For PFBA, the calculations were not conducted because no $D_{matrix/w}$ for this compound could be determined except for $D_{slw}$ (Table 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 either albumin or membrane lipids has already been reported (Armitage et al. 2012; Ng and Hungerbühler 2014). Previously published physiologically based models focused mainly on 1 of these 2 matrices, whereas other sorption processes were summarily estimated or not considered (Armitage et al. 2013; Cheng and Ng 2017). Our results suggest that both matrices should be considered explicitly to adequately predict 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 organs such as heart, lung, muscles, and spleen (Supplemental Data, SI-2.3). Storage lipids and water represent rather low sorption capacities, even in adipose tissue which is composed of approximately 80% storage lipids.

The sorption to FABP was proposed to play a key role in the accumulation of PFOA in the (rat) liver (Cheng and Ng 2017). According to our 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 in humans and rat (Supplemental Data, SI-2.3.1-2).

The proposed relevance of the sorption to FABP for PFOA (Cheng and Ng 2017) was not clearly seen in our data. However, our analysis is limited by substantial uncertainties in the input data. For FABP, we have to take into account that not only physiological data vary between species but also reported \(D_{\text{FABP}}\), from the literature vary by 1 log unit (Supplemental Data, SI-1.9; Zhang et al. 2013; Sheng et al. 2016). With the lowest reported \(D_{\text{FABP}}\), only approximately 4% of PFOA would be sorbed to these proteins according to our calculations. And even for the highest \(D_{\text{FABP}}\), combined with the highest FABP content, the sorption to FABP would still contribute less than the membrane lipids to the accumulation in the liver.

Organ–water distribution coefficients (\(D_{\text{organ/w}}\)) were calculated based on the organ composition and the corresponding distribution coefficient \(D_{\text{matrix/w}}\) (Figure 1; Supplemental Data, SI-2.4). Organs with the highest \(D_{\text{organ/w}}\) values were different for human and rat. In human, adipose, brain, and skin show the highest \(D_{\text{organ/w}}\), directly followed by blood, liver, and kidney. In rat, the highest \(D_{\text{organ/w}}\) values were calculated for brain, kidney, liver, and blood in that order.

Oberved differences in \(D_{\text{organ/w}}\) between human and rat are mainly caused by the higher fractions of membrane lipids in human organs compared to rat organs (Supplemental Data, SI-1.8). Overall, the range of \(D_{\text{organ/w}}\) values in different organs is quite narrow for any investigated compound (e.g., the \(D_{\text{organ/w}}\) values of 9CI-PF3ONS range between 3.2 and 3.9 log units). 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 the highest \(D_{\text{organ/w}}\) values for 9Cl-PF3ONS, PFOS, and PFDA and the lowest for PFHxA or HFPO-DA for both species.

Evaluation with experimental distribution data

Comparison to measured organ concentrations: Human. Organ concentrations for PFAAs in human were available from 2 references (Maestri et al. 2006; Perez et al. 2013). Maestri et al. (2006) measured PFOA and PFOS in adipose, blood, brain, gonads, kidney, liver, lung, and muscle (2 data sets) in 7 subjects (pooled samples of 5 males and 2 females with an age range of 12–83 yr), whereas Perez et al. (2013) measured additional PFAAs (PFHxA–PFUnDA and PFBS, PFHxS; in total 9 data sets) in only 5 organs (brain, kidney, liver, lung, and bone) in 20 subjects (sex not specified, with an age range of 28–83 yr). Organ concentrations and the corresponding reference organ of each data set are displayed in Supplemental Data, SI-1.11.

The number and overlap of available data sets in human were limited, so only the results for PFOA and PFOS could be validated (Supplemental Data, SI-3.1). 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 we will discuss in detail for the rat (see the following section). 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 (Supplemental Data, SI-3.2).

For long-chain PFCAs (PFNA–PFUnDA), but not for PFHxS and PFOS, the liver concentrations were overestimated. Kidney concentrations were in some cases slightly underestimated, except for PFHxA (>4x overestimation).

Because of the limited availability of comparable data sets in humans, we also applied our calculations on PFAA concentrations measured in 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 (15 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 (Supplemental Data, SI-1.12).

To check the postulated importance of interstitial albumin (Cheng and Ng 2017) for PFOA and PFOS, we compared calculations with and without interstitial albumin with reported experimental data. When calculations excluded interstitial albumin, 113 out of 239 total calculated concentrations were in good agreement with reported concentrations, and 126 out of 239 calculated concentrations deviated >2x from reported concentrations (out of that 54 calculated concentrations were >4x). In comparison, when calculations included interstitial albumin, 164 out of 239 calculated concentrations were in good agreement with reported concentrations, and only 75 out of 239 calculated concentrations deviated >2x from reported concentrations (out of that 46 calculated concentrations were >4x).
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 detail in Supplemental Data, SI-3.2.1. In the following only the calculated concentrations which considered interstitial albumin are presented and discussed.

Our calculations had to be based on available physiological composition, which might deviate from the actual individual case in each study. To somewhat mitigate this source of uncertainty, the discussion focuses on the results for PFOA (Supplemental Data, SI-3.2.5 and SI-3.2.8). The larger number of available studies allows seeing trends beyond the influence of the individual variability in physiological parameters.

For other PFAAs, evaluation of our estimated distributions in rat was limited by the small number of available data sets and more importantly by the small number of involved labs (Supplemental Data, SI-3.2). We often observed systematically high deviations between measured and calculated concentrations when data sets originated from the same source (e.g., PFOS in female rats; Supplemental Data, SI-3.2.9). This indicated that the studies were of different quality, which increases the uncertainty of our validation. Despite all these uncertainties that arise from a quality issue in the exposure studies as well as the use of generic physiological information that may not fit well for the specific individuals from the reported studies, we expect that the trends are still valid when based on a number of studies rather than a single one.

For PFOA, our estimations were generally quite accurate for adipose, heart, gonads, gut, lung, muscle, skin, and spleen because calculated PFOA concentrations matched with a deviation <2-fold to measured concentrations in the majority of investigated organs (Figure 2; Supplemental Data, SI-3.2.5). This good agreement indicates that all significant sorption processes were considered and quantified correctly by adding up the sorption capacities for all matrices in an organ.
For liver, there was a distinct underestimation in males and only small differences in females between our calculated and measured PFOA concentrations. Because there is no evidence that there are sex-specific differences in lipid and protein volume fractions in liver, this observation suggested a sex-dependent influence of active uptake in this organ. In the 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, PFSAs, and PFNA; Han et al. 2008; Zhao et al. 2015, 2017). Transporters included Oatps and sodium-dependent taurocholate cotransporting polypeptide. According to our observations, hepatocytes in males seem to have a higher PFOA uptake than female hepatocytes because deviations between measured and calculated concentrations are more apparent in males. This might indicate a sex-specific expression of transporters similar to the kidney. Indeed, sex-specific distribution in liver for PFOA and PFOS was reported in one rat study (Kim et al. 2016). There, females showed significantly lower PFOA and PFOS liver/plasma ratios than males. The study compared organ concentrations in females which were sampled 24 h after administration (orally and intravenously) to those in males which were sampled 12 d after administration (orally and intravenously). Given the assumption of equilibrium between all organs at the time of sampling, this would support our findings of a sex-specific distribution of PFOA and PFOS in liver (Kim et al. 2016).

For brain, our approach overestimated the concentrations for PFOA consistently in both sexes by a factor of 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 (Supplemental Data, SI-3.2.5-10). The high calculated PFOA concentrations were mostly due to high membrane lipid content in brain and the high affinity of PFOA to this matrix (Table 1; Supplemental Data, SI-1.8.2).

Two explanations could cause the significantly lower PFOA concentrations that were measured in brain: either the sorption affinity of PFOA to brain is smaller than expected (e.g., because of an effect of [negatively charged] 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 transporter candidates can be found in the literature (Morris et al. 2017). These include efflux transporters in the blood-facing membrane such as multidrug resistance–associated protein 2 (Mrp2) and breast cancer resistance protein, for which interaction with PFSAs was already described for human transporter protein (Zhao et al. 2015; Morris et al. 2017) 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 (Han et al. 2012). The influence of acidic phospholipids was suggested as another explanation for the overestimation of PFOA concentrations in brain because our estimated distribution into brain was based on experimental membrane lipid–water distribution coefficient values for neutral phospholipids only (Droge 2018; Ebert et al. 2020). In organs such as the brain,
however, also acidic phospholipids are present, although generally in much lower fractions (Rodgers et al. 2005). 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. However, the brain has a similarly high fraction of acidic phospholipids to neutral phospholipids as the kidney, liver, and heart (Rodgers et al. 2005); and when applying our approach, no differences were observed similar to those found for the brain concentrations. 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, PFOS brain concentrations were overestimated by our calculations. We assume similar transport processes to explain these deviations as for PFOA. 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 our calculations. Because the half-lives of PFOS are higher than for PFOA (Wang et al. 2013), we would assume that also reabsorption in kidney occurs. For this, more research regarding potential transporters needs to be done.

Overall, our physiologically based distribution calculations were able to estimate the PFOA concentrations in the majority of investigated organs with much less computational effort than a dynamic toxicokinetic model, thereby providing a helpful tool to identify organs that need more attention in future research.

CONCLUSIONS

Distribution calculations such as those presented can help to assess the accumulative potential of PFAAs and that of their increasingly used structurally similar alternatives. To estimate the distribution of PFAAs, we have provided—herein and in former work—a consistent set of distribution coefficients for a series of PFAAs and 4 of their alternatives to physiologically relevant matrices including albumin, membrane lipids, structural proteins, and storage lipids. The investigated alternatives sorb similarly strongly to each of these matrices as the classical PFAAs. These results indicate that ether-based polyfluorinated alternatives and cyclic perfluorinated compounds have a similar sorption behavior and therefore a similar accumulative potential in an organism.

The results of our physiologically based distribution calculations showed that albumin, membrane lipids, and structural proteins are of major relevance in estimating the accumulation of PFAAs in different organs. Future models should, therefore, include all 3 matrices. In liver and kidney FABP was also considered and found to possess a high sorption capacity for short-chain PFAAs and PFOA, if the highest reported $D_{\text{FABP/aw}}$ for PFOA was used. To further clarify the relevance of FABP for short-chain PFAAs and alternatives (and PFOA), it might be beneficial to remeasure $D_{\text{FABP/aw}}$ with a consistent method. For long-chain PFAAs, only minor relevance of FABP for the organ distribution was predicted.

Evaluation of our estimations revealed good consistency with measured PFAA distribution in most organs of the rat and indicated that the distribution of PFAAs in kidney, liver, and brain is affected by active transport. These active transport mechanisms may cause nonequilibrium conditions in vivo, which should be considered when modeling and assessing PFAAs exposure. In the future, quantitative knowledge on the effect of active transporters will be needed to parameterize more complex physiologically based toxicokinetic models. Overall, the systematic investigation of the equilibrium distribution processes for these compounds has allowed us to replace former uncertainties with a more consistent and comprehensive view on the biopartitioning of PFAAs.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4954.

Acknowledgment—The authors thank M. Haußecker, H. Paschke, and A. Pfenningsdorff for laboratory assistance. The presented study contributes to the program topic Chemicals in the Environment funded by the Helmholtz Research Program. Open Access funding enabled and organized by ProjektDEAL.

Disclaimer—The authors declare no competing financial interest.

Author Contributions Statement—F. Allendorf, K.U. Goss, and N. Ulrich: experimental conception and design; F. Allendorf: performance of experiments and chemical analysis; F. Allendorf: writing—manuscript; K.U. Goss and N. Ulrich: writing—assistance.

Data Availability Statement—All data generated or analyzed during the study are included in the present study (and its Supplemental Data files).

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