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Bioaccumulation of Perfluorinated Alkyl Acids: Observations and Models

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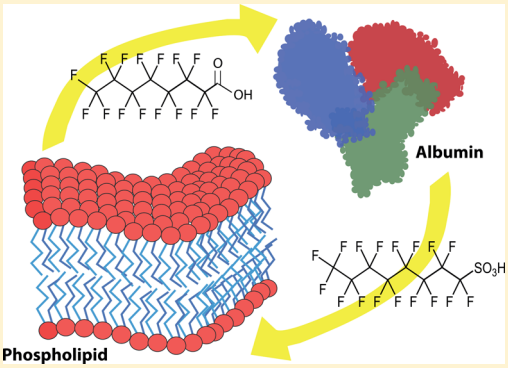
Supporting Information

ABSTRACT: In this review, we consider the two prevailing hypotheses for the mechanisms that control the bioaccumulation of perfluorinated alkyl acids (PFAAs). The first assumes that partitioning to membrane phospholipids, which have a higher affinity for charged species than neutral storage lipids, can explain the high bioaccumulation potential of these compounds. The second assumes that interactions with proteins—including serum albumin, liver fatty acid binding proteins (L-FABP), and organic anion transporters—determine the distribution, accumulation and half-lives of PFAAs. We consider three unique phenomena to evaluate the two models: (1) observed patterns of tissue distribution in the laboratory and field, (2) the relationship between perfluorinated chain length and bioaccumulation, and (3) species- and gender-specific variation in elimination half-lives. Through investigation of these three characteristics of PFAA bioaccumulation, we show the strengths and weaknesses of the two modeling approaches. We conclude that the models need not be mutually exclusive, but that protein interactions are needed to explain some important features of PFAA bioaccumulation. Although open questions remain, further research should include perfluorinated alkyl substances (PFASs) beyond the long-chain PFAAs, as these substances are being phased out and replaced by a wide variety of PFASs with largely unknown properties and bioaccumulation behavior.



1. INTRODUCTION

Perfluorinated alkyl acids (PFAAs), including perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs), are highly persistent and globally distributed pollutants. Long-chain PFAAs (C8−C12 especially) are also bioaccumulative.[1,2] Unlike neutral hydrophobic organic chemicals, they do not accumulate preferentially in storage lipids, but tend to concentrate primarily in blood and liver, among other tissues.[3−6] They also show a wide variability in whole-body half-life among species and between genders,[7−9] and in vitro studies have shown that they associate strongly with proteins such as serum albumin and liver fatty acid binding protein (L-FABP).[10−14]

Attempts to model the bioaccumulation of PFAAs using the equilibrium partitioning approach based on the octanol−water partition coefficient (K_{OW}) of the neutral fraction alone have largely failed due to the fact that PFAAs have very low pK_a values, and are thus almost completely ionized at environmentally relevant pH. Early models were based on an assumed pK_a of about 3.8[15] but more recent analyses point to a pK_a of between 0 and 1 for the PFCAs and even lower for the PFSAs.[16−21] Therefore, models were needed that could explain the substantial bioaccumulation that has been observed, especially for long-chain PFAAs, given their high degree of ionization.

There are currently two prevailing theories to explain PFAA bioaccumulation, from which two mechanistic models have emerged. The first is based on the relatively higher affinity of charged molecules for phospholipids than for neutral storage lipids.[22−24] Phospholipids are a major component of biological membranes and as such constitute a substantial sink for nonspecific association with charged or partially charged species. Based on this, Armitage et al. (2013)[25] proposed a mechanistic model for the bioaccumulation of ionogenic compounds that considers both neutral (storage) and membrane lipids. The general structure of this first-order one-compartment model is based on the fish bioaccumulation model of Arnot and Gobas (2004),[26] but replaces the octanol−water partition coefficient, K_{OW}, with octanol−water and membrane−water distribution coefficients (D_{OW} and D_{MW}, respectively). The distribution coefficients consider both the neutral and ionized fractions of the chemical. This model successfully predicts the whole-body bioconcentration factors for a number of acids and bases, including perfluorinated carboxylic acids (PFCAs) with 7−13 perfluorinated carbons.[25]

The second proposed mechanism for PFAA bioaccumulation is the interaction with proteins within the organism. A number of authors have suggested that the behavior of PFAAs within organisms is due to their similarity to endogenous fatty

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acids.[2,12,13,27−29] PFAAs activate many of the same genes, proteins, and metabolic pathways as fatty acids in mice, rats, and fish.[30−37] In vitro studies have shown that PFAAs associate strongly with serum albumin from several species.[10−12,38−44] Given that blood is known to be a major compartment of accumulation for PFAAs, albmin binding has been posited as the mechanism behind PFAA bioaccumulation and tissue distribution. PFAAs also to bind to liver fatty acid binding protein (L-FABP),[13,27,29] which may explain their high concentration in liver tissues and in kidneys, were L-FABP is also expressed. In addition, cellular uptake of PFAAs was shown to be mediated by organic anion transporter (OAT) proteins.[45−50] These proteins, which are highly expressed in the kidneys, may be responsible for the high variability observed in renal clearance rates and whole body half-lives for PFAAs.[9] Based on these observations, we developed a mechanistic model for the bioaccumulation of PFAAs in rainbow trout that included interactions with these three protein types.[51] Like the phospholipid-based model, our model could successfully predict bioconcentration factors for a number of PFCAs, as well as for PFOS. In addition, model results were in good agreement with observed patterns of tissue distribution in the rainbow trout.[52]

These two proposed models—which we will refer to from now on as the phospholipid (PL) or protein binding (PB) models—have so far been tested in a relatively limited way. The phospholipid model was developed not exclusively for predicting PFAA bioaccumulation, but rather as a general approach to modeling ionogenic substances. So far it has not been evaluated for its ability to explain or predict aspects of PFAA bioaccumulation beyond whole-body BCFs. The protein-binding model was developed to predict the tissue distribution and BCFs primarily in rainbow trout,[52] although the predictions were also successfully tested against common carp data.[53] However, many of its parameters had to be based on mammalian studies due to the paucity of data available for fish, and it remains unclear how much similarity actually exists across species, particularly as concerns protein interactions.

In this critical review, we survey what has been observed in the laboratory and field, for both mammals and nonmammals, for three important aspects of PFAA bioaccumulation: (1) their tissue distribution, (2) the relationship between bioaccumulation and perfluorinated carbon chain length, and (3) their widely variable species- and gender-specific half-lives. We consider whether and how the PL and PB models treat each of these aspects, and assess where the models are particularly useful and where they fail. Finally we discuss the most important open questions that remain and propose further studies that could help fill these gaps.

2. OBSERVED BIOACCUMULATION PATTERNS AND MODEL TREATMENT

2.1. Nomenclature. In Table 1, we list the acronyms, number of perfluorinated carbons, and names of the PFCAs and PFSAs that will be discussed in this review. Figures depicting the influence of chain length on bioaccumulation patterns will refer to perfluorinated carbon chain length as listed in Table 1. However, references to series of PFCAs or PFSAs (e.g., C7−C14 PFCAs) typically use the total number of carbons in the molecule (PFOA has seven perfluorinated carbons but eight carbons in total, and will be referred to as a C8 PFCA).

Table 1. Nomenclature for Perfluoroalkyl Carboxylic Acids (PFCAs) and Perfluoroalkane Sulfonic Acids (PFSAs) Discussed in This Review

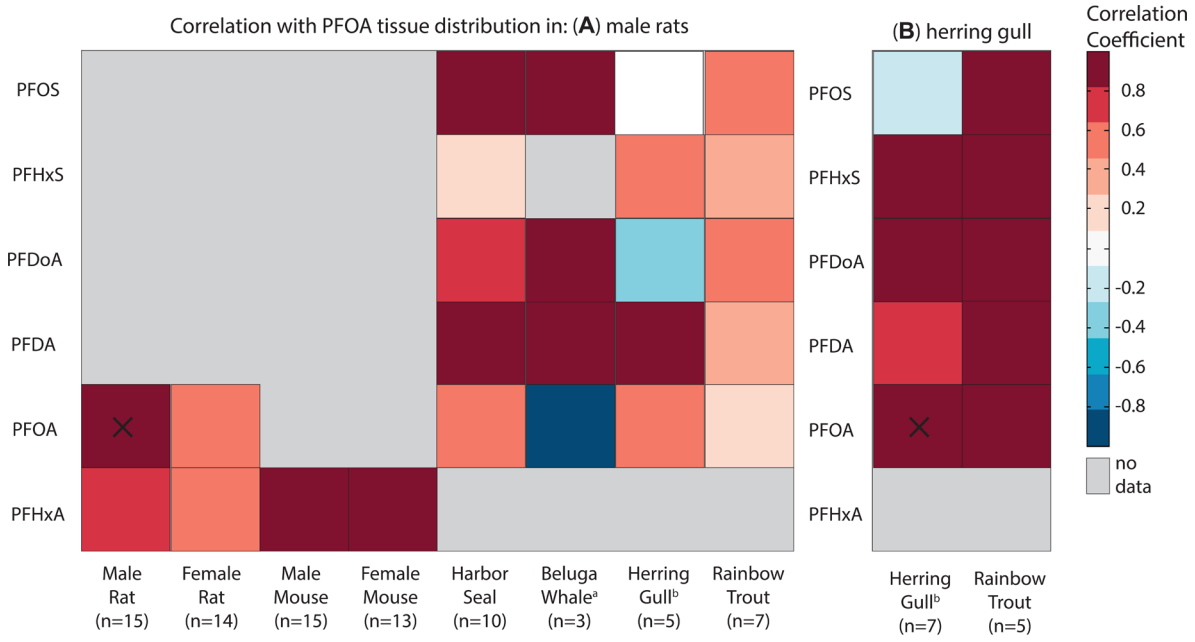
		perfluorinated carbons	name
PFCAs	PFBA	3	perfluorobutanoic acid
$F_3C-(CF_2)_n-COOH$	PFPeA	4	perfluoropentanoic acid
	PFHxA	5	perfluorohexanoic acid
	PFHpA	6	perfluoroheptanoic acid
	PFOA	7	perfluorooctanoic acid
	PFNA	8	perfluorononanoic acid
	PFDA	9	perfluorodecanoic acid
	PFUnA	10	perfluoroundecanoic acid
	PFDoA	11	perfluorododecanoic acid
	PFTrA	12	perfluorotridecanoic acid
	PFTeA	13	perfluorotetradecanoic acid
	PFHxA	15	perfluorohexadecanoic acid
	PFODA	17	perfluorooctadecanoic acid
PFSAs	PFBS	4	perfluorobutane sulfonic acid
$F_3C-(CF_2)_n-SO_3H$	PFHxS	6	perfluorohexane sulfonic acid
	PFOS	8	perfluorooctane sulfonic acid
	PFDS	10	perfluorodecane sulfonic acid

2.2. Tissue Distribution. A number of authors (ourselves included) have stated that PFAAs accumulate "primarily in blood and liver."[3,4,51] How consistent is this observation across species and PFAA structures? Field and laboratory studies that specifically address tissue distribution (and report concentrations or body burdens in more than two tissues) are available for rats (PFHxA, PFOA, and PFOS),[54−56] mice (PFBS, PFHxA),[54,57] rainbow trout (C8−C14 PFCAs, PFHxS, and PFOS),[52] harbor seals (C8−C14 PFCAs, PFBS, C4−C10 PFSAs),[5] beluga whales (C7−C14 PFCAs, C5−C10 PFSAs),[58] and herring gulls (C7−C14 PFCAs).[59]

In these eight studies, there are 58 total data series (for different substances in different tissues). In 45 out of 58 (or about 78%) blood or liver is the compartment with the highest concentration. Thus, the statement that PFAAs accumulate "primarily in blood and liver" appears to be a reasonable one. However, other tissues are also important, and there are 13 instances where a different compartment dominates. In the Supporting Information (SI), Figures S1−S8, we show the tissue distributions extracted from these eight studies.

In rats, PFAA levels were highest in blood (plasma) and liver,[54,56] but also in kidneys[55] and lowest in brain and fat. In two instances blood and plasma were not the primary compartments: for PFOA in the female rat[55] concentration was highest in the kidneys, and for PFHx in the female rat from a different study[54] the highest concentration was in the bladder. It should be noted that in the female rat both of these substances are rapidly eliminated via the urine.

In mice, PFAA levels were always highest in liver or blood (plasma)[54,57] and lowest in brain, muscle and fat. For PFBS, levels in stomach, small intestine and cartilage were comparable to blood and kidneys.[57]



[a]Negative correlation due to highest PFOA found in blubber (Kelly et al. 2009).
[b]Negative correlation for PFOS due to highest concentration found in fat.
Negative correlation for PFDoA due to highest concentration fount in brain (Gebbink et al. 2012).
× Indicates autocorrelation (male rat PFOA with male rat PFOA or herring gull PFOA with herring gull PFOA).

Figure 1. Correlation between tissue distribution of six PFAAs and six species to the distribution of (**A**) PFOA in male rats and (**B**) PFOA in herring gulls. For each comparison, the number of tissues (n) in common with male rat or herring gull is shown.

In harbor seals,[5] concentrations were highest in liver and blood for 11 out of 13 tested substances. However, for two—PFHpS and PFOA—concentrations were highest in the lung, and for PFHxS levels were highest in thymus (levels in thymus were also relatively high for PFHpS, PFOA, and PFTeA). Relative PFAA concentration was lowest in muscle and blubber. As PFCA chain length increased, relative levels in brain tissue increased but were never more than 40% of the level in liver.

In beluga whales (tissue distribution data extracted from the Supporting Information of Kelly et al. 2009[58]), levels were highest in liver and blood save for two instances: PFHxS was highest in fetal tissue (for pregnant female beluga) and PFOA was highest in male beluga whale blubber. For other PFAAs, levels were lowest in muscle and male blubber (no values in blubber were reported for female whales).[58]

Similarly in herring gulls,[59] levels are highest in plasma and liver and lowest in muscle and adipose except for PFOS, where levels were highest in fat. In addition, for herring gull, PFAA concentrations in the brain are high for long-chain PFAAs and brain becomes the compartment with the highest concentration for three PFCAs—PFDoA, PFTrA, and PFTeA—and one PFSA, PFDS.

Finally, for rainbow trout,[52] PFAA concentrations are highest in blood for six out of seven substances. For PFOA, relative concentration was highest in the gall bladder. Concentrations were also high in kidney and liver for most PFAAs, and lowest in muscle, adipose and blood cells.

Clearly, liver and blood are important compartments for the accumulation of PFAAs, but there also exists some variability among different species and different PFAAs. In Figure 1, we illustrate this variability in tissue distribution by comparing correlation coefficients for six PFAAs (PFHxA, PFOA, PFDA, PFDoA, PFHxS, and PFOS) and the six species discussed above. We first compare distributions of the six PFAAs in all six species to the distribution of PFOA in rats (Figure 1A). We then make the same comparison for nonmammals by comparing tissue distributions of the six PFAAs in herring gull and in rainbow trout to the distribution of PFOA in herring gull (Figure 1B).

PFOA distribution in rats correlates to a relatively high degree ($r^2 > 0.6$) with PFHxA in male rats and male and female mice, and with PFOS, PFDoA and PFDA in harbor seal and beluga whale (Figure 1A). For herring gull, this degree of correlation occurs only for PFDA. For rainbow trout, the correlation is <0.6 for all PFAAs. In addition, there are three instances where the correlation with rat PFOA tissue distribution is either zero or negative: for PFOS in herring gull, PFDoA in herring gull and for PFOA in beluga whale. For herring gull this is due to fat being the tissue with the highest concentration of PFOS and for brain being the tissue with highest concentration of PFDoA (in the male rat, these are two compartments with the lowest concentration of PFOA). For male beluga whales, PFOA was found in highest concentration in blubber, resulting in the negative correlation shown.

Clearly, PFAA tissue distributions in the two nonmammalian species (herring gull and rainbow trout) are more highly correlated with one another than with PFOA in the male rat (Figure 1B). The exception, PFOS in the herring gull, is due to the high concentration in fat, as was also the case for correlation with PFOA tissue distribution in rats.

We now investigate how well these tissue distributions can be explained by the PL and PB models. To do so, we test what we consider a "central hypothesis" of each model against these tissue distribution data: that bioaccumulation in the PL model depends in large part on phospholipid content and in the PB model on the concentration of albumin.

In the PL model, the fraction of phospholipids in the organism, the (storage) lipid fraction, and the octanol–water

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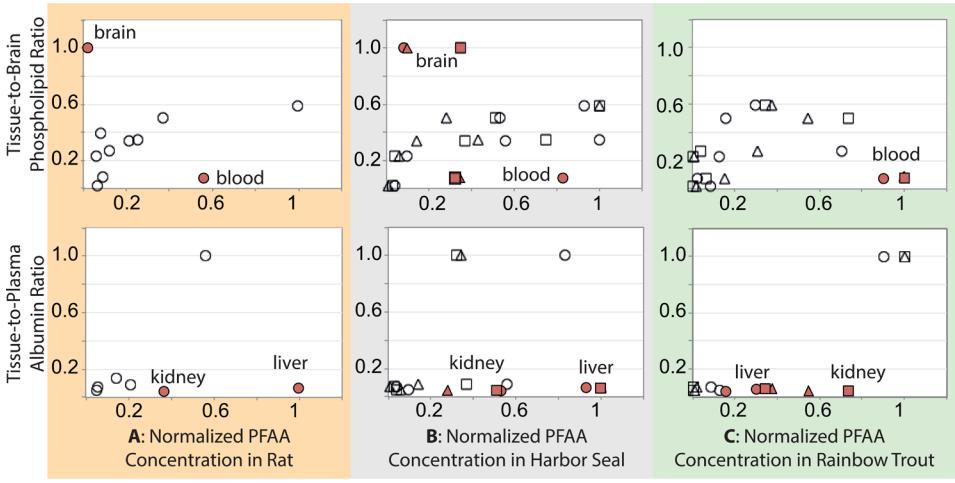

		Phospholipid r^2	Phospholipid r^{2*}	Albumin r^2	Albumin r^{2*}
rat	○ PFOA	0.10	0.75	0.27	0.96
seal	○ PFOA	-0.07	0.76	0.46	0.81
	□ PFDoA	0.42	0.85	-0.07	0.52
	△ PFOS	0.21	0.79	0.06	0.94
trout	○ PFOA	-0.11	0.31	0.97	0.99[a]
	□ PFDoA	0.16	0.76	0.74	0.99[a]
	△ PFOS	0.08	0.81	0.83	0.99[a]

r^2: correlation coefficient between tissue PFAA concentration and phospholipid or albumin content

r^{2*}: correlation coefficient with tissues marked in red removed (brain and blood or liver and kidney)

[a]Note that when liver and kidney are not included in the trout data set, the correlation between albumin and PFAA contents is based only on three data points and is therefore not a good measure of correlation.

Figure 2. Correlation between PFAA tissue distribution and tissue-specific phospholipid content (top row) or tissue-to-plasma albumin ratios (bottom row). Correlation coefficients are calculated both with (r^2) and without (r^2*) tissues identified as outliers for each model.

and membrane−water distribution coefficients determine the overall sorption capacity of the organism, D_{BW}:[25]

$$D_{BW} = f_{SL} D_{OW} + f_{PL} D_{MW} + f_{NLOM} \rho_{NLOM} D_{OW} + f_W$$

where f_{SL} and f_{PL} are the volume fractions of neutral lipids and phospholipids, respectively, f_{NLOM} is the fraction of nonlipid organic matter, ρ_{NLOM} is its density and f_W is the fraction of water. D_{OW} is the octanol−water distribution coefficient and D_{MW} is the membrane−water distribution coefficient. Because the D_{OW} can be several orders of magnitude smaller than the D_{MW} for chemicals with low pK_a, the D_{MW} will dominate this expression and, in theory, the distribution of phospholipids in an organism should determine the tissue distribution for substances like PFAAs.

In their paper, Armitage and co-workers assumed that phospholipids accounted for 1% of an organism on a whole-body basis.[25] This is not sufficient to assess how the model would predict tissue distributions. We therefore compiled tissue-specific phospholipid contents for three species (rats,[60,61] humans,[61] and rabbits[62]) from the available literature (see SI Figure S10 and Table S1). These phospholipid contents were well correlated to one another ($r^2 > 0.9$). We normalized the phospholipid distribution data by calculating tissue-to-brain phospholipid ratios (brain having the highest concentration of phospholipids) and then took the average for each tissue across the three studies. Since the phospholipid composition of fresh- and salt-water fish are comparable to that in mammals, we use this single average distribution to compare with PFAA tissue distributions in mammals, birds and fish.[63]

In the PB model, association with albumin is one of the main drivers of PFAA fate.[64] Between 30 and 40% of the total albumin pool in an organism is generally believed to be present in the plasma, with the remainder distributed in the extravascular fraction.[65,66] This extravascular distribution is not extensively documented for many tissues, and studies can disagree based on the methods used, or whether the authors considered only the extravascular extracellular fraction or also considered intracellular albumin.[65−69] We have compiled tissue-to-plasma albumin ratios from three studies,[65,66,70] along with those used in our fish bioconcentration model[51] (SI Table S2) in order to test the correlation with PFAA distribution. Because these studies were in general agreement, we averaged the available values for the tissue-to-plasma ratios.

We tested the correlation of tissue-to-brain phospholipid ratios and tissue-to-plasma albumin ratios against observed tissue distribution patterns in the laboratory and field. We chose three representative PFAAs−PFOA, PFOS, and PFDoA− and three species: rat,[55] harbor seal,[5] and rainbow trout[52] (Figure 2).

When all tissues are considered for which both relative PFAA concentration and phospholipid or albumin ratios are available, neither model fares well for all PFAAs in all species. For phospholipids, the highest correlation is for PFDoA in seals, but all correlations are poor ($r^2 < 0.5$). For the albumin ratios, the best correlations are for PFOA in trout followed by PFOS in trout, and worst for PFDoA in harbor seal. This is clearly because the correlation with albumin is driven by the blood compartment, and in the seal the blood compartment, particularly for PFDoA, had a lower PFAA concentration relative to the liver. However, these "face value" assessments are not necessarily fair tests of the performance of either of the two models.

For the PL model, there is no mechanism that would explain the high levels of PFAAs found in blood. Indeed, within the

blood compartment the red blood cells have a higher phospholipid content than does plasma (see SI figure S10), but plasma consistently has higher concentrations of PFAA than do blood cells.[52,54,59] However, the PL model makes no claim to explain this, and rather is meant to explain the sorption capacity of tissues. Therefore, it may be reasonable to omit the blood compartment from the assessment of the PL model. In addition, one of the major reasons for the poor correlation between PFAA distribution and tissue-specific phospholipid content is the high concentration of phospholipids in the brain. However, it is well-known that the blood brain barrier (BBB) is highly impermeable to anions, and charged molecules rely on active transport mechanisms to cross the BBB.[71] Moreover, Yang et al. (2010) showed PFOA was not a substrate of OATP1A2, a key influx transporter in the BBB.[49] This does not appear to be true, however, for long-chain PFAAs. Here, it can be seen that the relative concentration of PFDoA in harbor seal brain is substantially higher than that of PFOA or PFOS, and the PL model performs better for this species-PFAA combination.

When brain and blood are omitted from the data sets, the correlation (r^2*) between PL content and relative PFAA concentrations is much higher, between 0.75 and 0.85 for all species and PFAAs, except for PFOA in the rainbow trout, where there was a very low concentration in the spleen despite the relatively high phospholipid content of this organ (similar to kidney or heart). Overall, the correlations are best for harbor seal (particularly for PFDoA, where the concentration in harbor seal brain is also higher than for other species or PFAAs). The PL−PFAA correlation is driven in large part by the liver compartment, which has both high phospholipid content and high PFAA concentration, and by muscle and adipose, which have both low phospholipid contents and low PFAA concentrations.

A similar assessment can be made for the correlation between tissue-to-plasma albumin ratios and relative PFAA concentrations. When considering all tissues for which both albumin and PFAA contents are available, the correlations are slightly better than with phospholipids for PFOA in the rat and harbor seal, worse for PFDoA and PFOS in the harbor seal, and considerably better for all PFAAs in the rainbow trout. Clearly the strength of the albumin-PFAA correlations is driven by the good agreement between high albumin content in the blood and high PFAA accumulation in blood. We can also identify two tissues for which the model shows poor performance but for which other mechanisms may be at work: liver and kidney.

The albumin content alone cannot explain the high PFAA concentrations that are observed in the liver, since the tissue-to-plasma albumin ratio in the liver is not much higher than in other tissues, including those that have low PFAA concentrations. However, in the previously published trout bioconcentration model, the very good agreement that was obtained with empirical data for the liver compartment was not due solely to the influence of albumin binding, but also to binding to liver fatty acid binding protein (L-FABP). High affinities (comparable to albumin binding affinities) have been observed for PFCAs of different chain lengths to L-FABP in vitro.[27,29] Since L-FABP is highly expressed in the liver, it could provide a possible explanation for the accumulation of PFAAs in liver tissues.

Relatively high PFAA concentrations have also been reported in kidneys for a number of species, including the harbor seals and rainbow trout included here. Although named for the organ in which it was first discovered, L-FABP is also expressed in the kidney and in the intestine.[72] Thus, binding of PFAA to this protein may also occur in these tissues. Indeed, Bogdanska et al. 2014[57] report high relative concentrations of PFBS in the intestines of mice. High PFAA concentrations in the kidneys of some species may also be related to interactions with renal transporter proteins that have been shown to mediate the elimination and reabsorption of anions in the kidneys. These proteins and their potential influence on observed variability in PFAA half-lives across different species and genders are discussed in more detail in the next section.

When liver and kidneys are excluded from the correlations of PFAA concentration with tissue-to-plasma albumin ratios, the correlation coefficients (r^2*) are substantially higher—between 0.52 and 0.96 for harbor seal and rat, and 0.99 for all PFAAs for rainbow trout. However, it should be noted that for the rainbow trout, because not many tissues were available for the comparison, removing liver and kidneys from the data set results in the correlation being made on the basis of only three data points. Therefore, the very high r^2* values that result are not a good measure of model performance. The overall correlations are best for trout and worst for harbor seals. The correlation with PFDoA in the harbor seal is particularly poor due to its relatively low concentration in the blood compartment.

When comparing the "central hypotheses" of the PL and PB models in this way, neither model emerges as clearly superior. The PL model performs best for the liver compartment, while the PB model (when considering only albumin) performs best for blood. The most problematic tissues for the PL model are muscle, because the PL content of muscle is 10 times higher than in adipose (see SI Table S2) but muscle and adipose typically have similar very low PFAA concentrations; spleen, which has a relatively high PL content (similar to that in kidneys) but typically some of the lowest PFAA concentrations; blood, as was discussed above. For the PB model, accumulation via protein binding only works if we assume that L-FABP binding can explain PFAA accumulation in liver and in kidneys. Further assessment based on other tissues is difficult because so few data exist on extravascular albumin contents. Ideally, a single data set using the same method that included other important compartments such as lungs (or gill), spleen, and gonads would be particularly useful.

It is certainly conceivable that a combination of the two models might provide a good structure for predicting PFAA tissue distribution, that is, one which includes albumin binding as an important mechanism for PFAA distribution and recognizes the blood compartment as an important sink for PFAAs, but which also uses the phospholipid content to predict the subsequent tissue distribution of free PFAAs. Given the inconsistencies in both approaches, however, more research is needed. In particular, there is need for more and more consistent physiological data for multiple species, which would allow for more systematic comparison to PFAA tissue distributions.

2.3. Influence of Chain Length on Bioaccumulation and Protein Interactions. Only a limited number of studies currently exists that allow for the calculation of a bioconcentration factor (BCF) or bioaccumulation factor (BAF) from concentration data. For field studies in particular it is rare to find both concentrations in organisms and in surrounding media reported. Here, we have extracted data from four laboratory studies: two for rainbow trout,[52,73] one for black

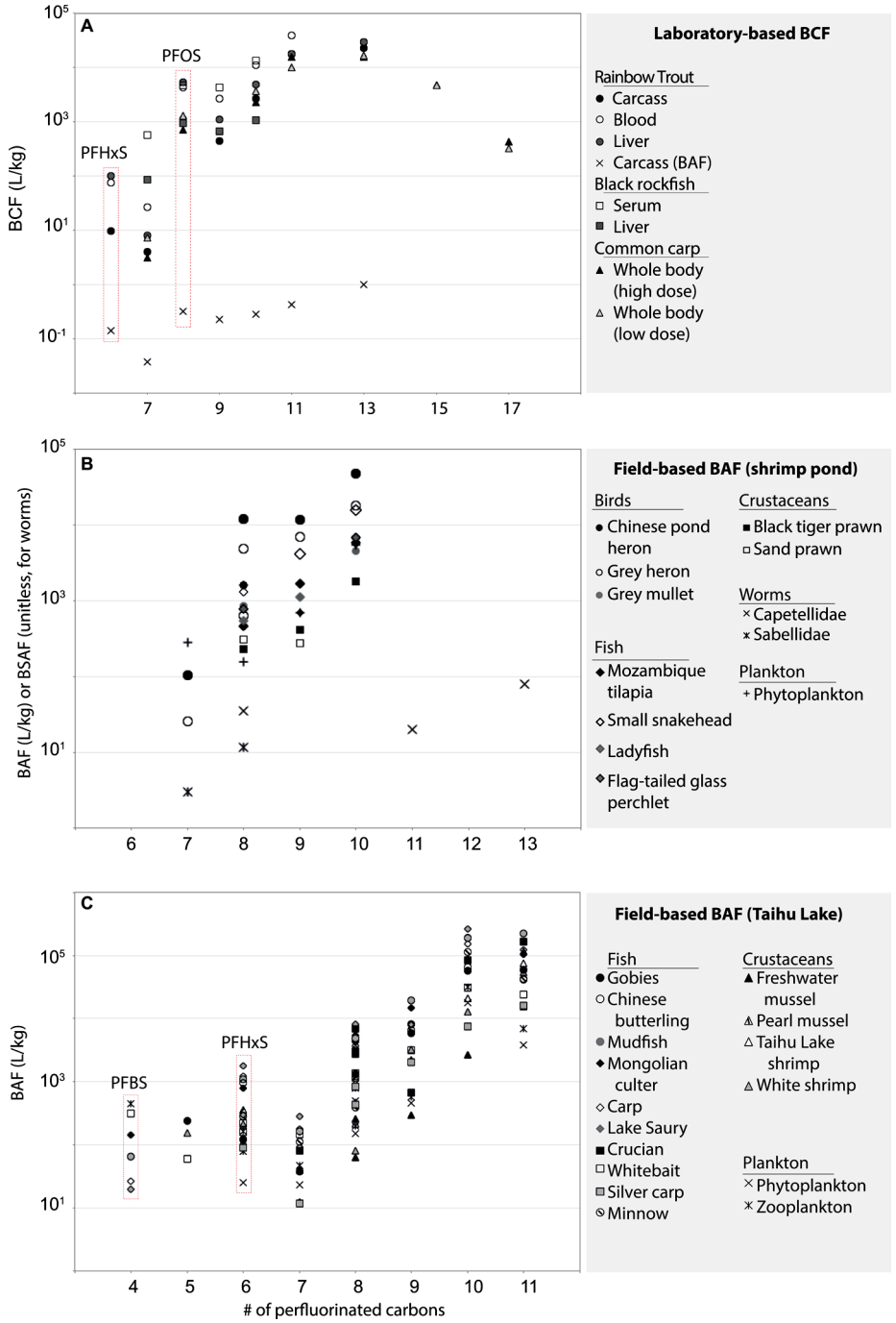

Figure 3. Observed relationship between perfluorinated chain length and bioconcentration factors (BCF) and bioaccumulation factors (BAF) extracted from (**A**) laboratory[52,53,74] and (**B**, **C**) field[75,76] studies.

rockfish,[74] and one for common carp[53]; and from two field studies that also reported concentrations in water and in a number of aquatic species.[75,76] In spite of the small number of studies, it is clear from the data, which cover at least 29 different species, that a relatively consistent picture of PFAA bioaccumulation has emerged. When considering bioaccumulation as a function of perfluorinated chain length (Figure 3), the BCF and BAF peak at a value between 10^4 and 10^6 at around a chain length of 10−13 (that is, between PFUnA and PFTeA). In addition, the bioaccumulation potential of PFHxS and PFOS are higher than what would be expected on the basis of perfluorinated chain length alone, suggesting an influence of the acidic headgroup.

For the PL model, the chain length dependence of bioaccumulation is based on the chain-length dependence of the membrane−water and octanol−water partition coefficients. Thus, the distinction, for example, between PFOS and PFNA will depend on the method used to estimate the K_{OW} (from which the D_{OW} and D_{MW} are derived). According to the Supporting Information provided by Armitage et al.,[25] the COSMOTherm software assigns a higher log K_{OW} to PFOS, whereas EPISuite's KOWWIN versions 1.67 and 1.68 predict a considerably higher log K_{OW} for PFNA than PFOS.

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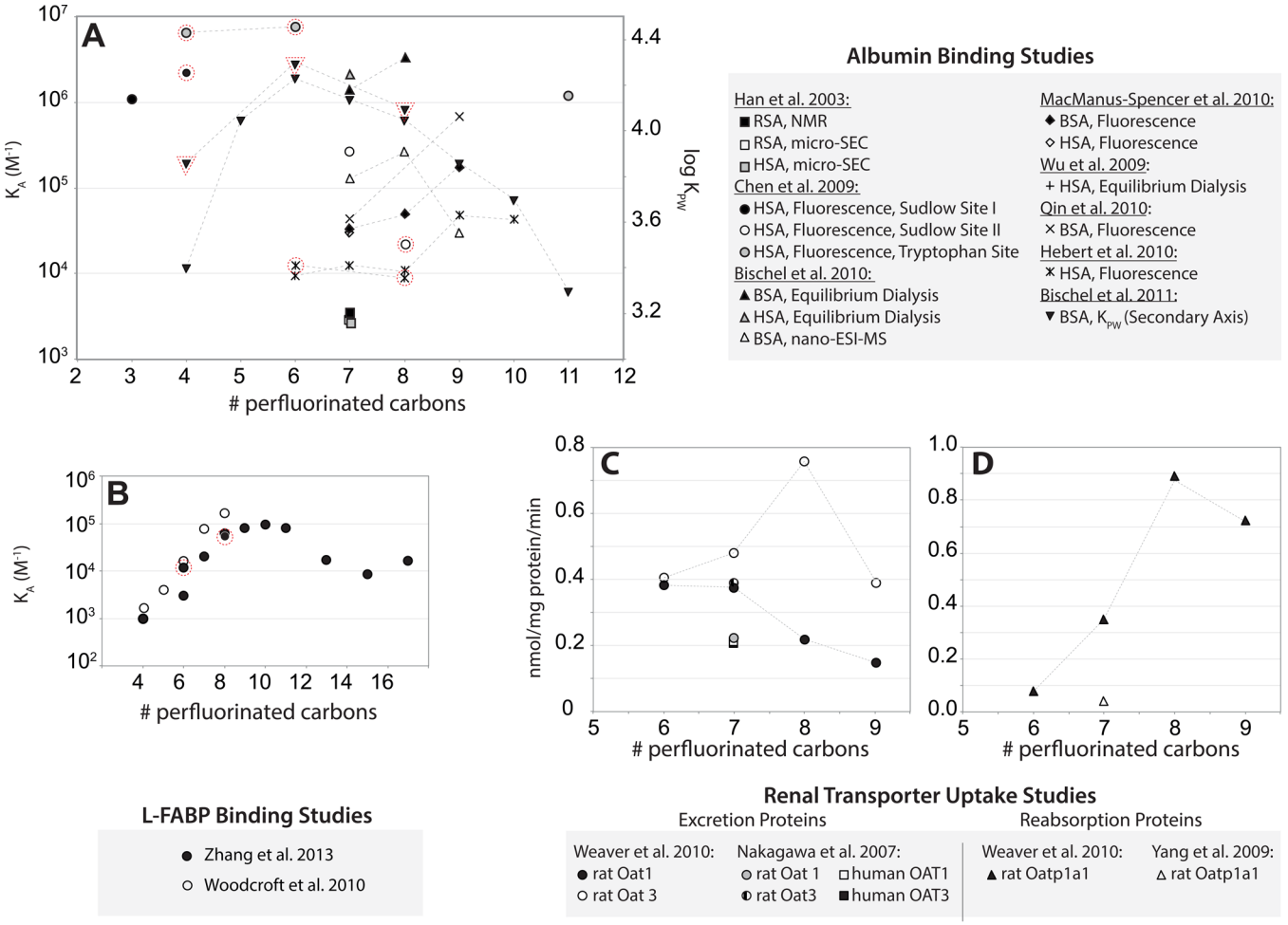

Figure 4. Influence of perfluorinated chain length on (**A**) PFAA-albumin association constants; (**B**) PFAA-LFABP association constants; cellular uptake of PFCAs mediated by renal transporters (**C**) responsible for excretion to urine and (**D**) responsible for reabsorption from urine back to blood.

For the PB model, the chain length dependence of bioaccumulation is somewhat more complicated. It depends not only on the relationship between perfluorinated chain length and the affinity for albumin, but also on the relationship between chain length and affinity for liver fatty acid binding protein (L-FABP) and for the interaction with renal transporter proteins that control the rate of PFAA excretion to and reabsorption from urine.

For the binding affinity of PFAAs with serum albumin, a relatively large number of studies exist, but their results are far from consistent.[10−12,38,40,42−44] Depending on the method and the species to which the serum albumin belongs the PFAA-albumin association constants (K_A) can vary over many orders of magnitude (Figure 4A). This variation stems in part from the presence of a large number of possible binding sites with different binding strengths.

Serum albumin controls the circulation of numerous endogenous and exogenous substances in the body, including fatty acids, hormones, bile acids, metals and a wide variety of drugs. The molecule has a heart-shaped conformation with three distinct domains. To date, 10 important binding sites have been identified in serum albumin: seven fatty acid binding sites (FA sites 1−7), the tryptophan (Trp) site, and Sudlow's drug binding sites I and II.[77−80]

Early studies investigating PFAA binding to albumin found relatively large numbers of binding sites (on the order of 10) for PFOA and PFOS, with binding strengths varying from 10^2 to 10^3 M^{-1}.[38] As techniques developed, studies reported both fewer binding sites (on the order of one to four) and higher binding affinities, but these affinities still span a range of more than 2 orders of magnitude (typically 10^4−10^6 M^{-1}).[10−12,40] One of the only site-specific studies available has shown binding affinities on the order of 10^6 M^{-1} for Sudlow sites I and II, while the Trp site shows lower affinities (10^4 to 10^5).[10] One of the most important factors behind this variability appears to be the PFAA-to-albumin ratio. At low concentrations of PFAA, binding occurs to a smaller number of primary binding sites, with high affinity. At higher PFAA concentrations, as primary sites become saturated, PFAAs bind to a larger number of low-affinity sites.[40]

For the few studies for which a series of PFAAs were tested using the same type of albumin and method (data points connected by dotted lines in Figure 4A), the binding affinity, K_A, appears to level off or decrease between 6 and 9 perfluorinated carbons. This is also consistent with what was

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observed by Bischel and co-workers[44] for the protein−water distribution coefficient, K_{PW}. It is not yet clear whether this indicates a shift from higher-affinity to lower-affinity binding sites or simply a reduction in the affinity at the same site. Binding affinities for PFAA to albumin binding sites are likely constrained by their ability to fit into hydrophobic binding pockets,[13] leading to lower binding affinities than for fatty acids which have highly flexible hydrocarbon tails. PFAAs with fewer than eight fluorinated carbons can have similar "zigzag" conformations to fatty acids,[28] whereas eight-carbon and longer PFAAs become increasingly rigid and begin to adopt helical conformations.[81] This may explain the reported peak in affinity between 6 and 9 perfluorinated carbons, though uncertainties remain about this peak given the high variability in the association strength data.

Three studies have reported the interaction of PFAAs with liver fatty acid binding protein (L-FABP).[13,27,76] Fatty acid binding proteins are a class of small (14−15 kDa) proteins that are exclusively expressed within cells, and constitute between 4 and 18% of soluble cytosolic protein concentration.[72,82,83] They bind primarily to long-chain fatty acids, eicosanoids, and other lipids,[84] and have highly tissue-specific expression. Their expression shows lower variation from species to species in the same tissue than they do within a single species across different tissues.[72,82,83] Not all FABPs may be able to bind PFAAs, due to constraints in their binding site conformations and the stiffness of the fluorocarbon tail. For example, endogenous fatty acid substrates bind to intestinal FABP (I-FABP) in a bent conformation, and heart and brain FABPs require a U-shaped conformation. Given the rigid nature especially of longer-chain PFAAs, they may not have high affinity for these FABPs. However, it has been noted that in mammals (though not in fish) L-FABP is distinct from other FABPs in that it can bind two fatty acid molecules, as well as other bulky ligands such as bile acids.[72,79,85] Thus, L-FABP may be an ideal candidate for PFAA binding.

A 2002 study by Luebker et al. showed that PFOS and PFOA both bound to the secondary binding site (a nonpolar site) on rat L-FABP. They noted that binding to the primary binding site might not be possible due to the U-shaped conformation needed. However, they also did not rule out the interaction of the highly polar sulfonate headgroup with the polar residues of the primary binding site, which might help explain the higher affinity of L-FABP for PFOS than for PFOA.[13] More recently, Zhang et al. (2013)[29] used molecular docking analysis to show that short-chain PFAAs *could* bind to L-FABP at the primary binding site, with their perfluorocarbon tails fully extended in the binding cavity. For PFAAs with more than 11 carbons the fluorocarbon tails would need to bend to fully contact the hydrophobic binding pocket. Given the rigidity of the fluorocarbon tail, this may further support the idea that not all FABPs would be able to accommodate PFAA binding, as discussed above. In addition, they found that while the carboxylate headgroup of PFCAs formed two hydrogen bonds with amino acid residues at the L-FABP binding site, sulfonates formed three. This supports observations that PFSAs have stronger protein interactions than PFCAs with the same perfluorinated chain length.

Only Woodcroft et al. (2010) and Zhang et al. (2014) report quantitative association constants between PFAAs and L-FABP for a series of PFAAs.[27,29] However, their results are quite consistent for perfluorinated chain lengths between 4 and 8 (Figure 4C). For the longer-chain PFAAs, which are only included in the study by Zhang et al.,[29] the K_A increases up to a perfluorinated chain length of about 11 (PFDoA) and decreases thereafter. Based on this study, L-FABP appears to have a higher affinity for long-chain PFAAs than does albumin. Given that the available data for albumin are so variable, however, it is difficult to say this with any certainty.

Finally, a number of studies have focused on the interaction of PFAAs with organic anion transporters. The family of organic anion transporters (OATs in humans, otherwise Oats) is a class of transporters with very broad substrate specificity that favors smaller molecules (about 100−600 g/mol).[86−88] They have been intensively studied because of their importance in the excretion of drugs and their metabolites via hepatic and renal elimination.[86,87] All OATS are expressed in the kidneys and/or liver, and some members are expressed in other tissues as well. The organic anion transporting polypeptides (OATPs in humans, otherwise Oatps) are a similar family of transporters that are ubiquitously expressed, and generally mediate the uptake of bulkier (>350 g/mol) amphipathic substrates, including bile acids, steroid conjugates, hormones, and a number of drugs.[88,89]

As reviewed by Han et al. 2012, uptake proteins expressed in kidney cells can be expressed either at the basolateral membrane or at the apical (brush border) membrane.[9] The basolateral proteins aid elimination by transporting organic anions from the blood side to the urine side of kidney cells. The apical membrane proteins are responsible for the reabsorption of anions from the urine back to the blood. They have been well studied in the human, mouse, and rat, and include: OAT1 (Oat1), OAT3 (Oat3), and OATP4C1 (Oatp4c1) in the human, rat and mouse, and OAT2 in the human, all in the basolateral membrane; OAT4, URAT1, and OATP1A2 in the human, Oat2, Oat5, Urat1, and Oatp1a1 in the rat and mouse, all on the apical membrane. Interestingly OAT2 is a basolateral transporter in the human but an apical one in the rat and mouse.

The first study showing in vitro PFOA transport by organic anion transporters was published in 2007 by Katakura et al., with Oat3 and Oatp1a1 in the rat.[45] Nakagawa et al. (2008) subsequently used in vitro studies with human embryonic kidney cells to show that OAT1 and OAT3, but not OAT2, were capable of transporting PFOA.[46] In 2008, Han et al. used freshly isolated rat hepatocytes to show that the uptake of PFOA in the liver was at least partially mediated by active protein transport.[47] In a follow-up, Yang and Han (2009) showed that Oatp1a1, a rat renal transporter responsible for reabsorption of anions from kidney filtrate back to the blood, was capable of transporting PFO. That same year Weaver et al. (2010) published a study of PFCA transport by rat renal Oats that directly measured uptake rates for the C7, C8, C9, and C10 PFCAs, this time with rat transporters Oat1, Oat3, Urat1, and Oatp1a1.[50]

One of the more interesting features of both OATs and OATPs are gender-specific differences in expression that can lead to markedly different pharmacokinetics, and can thus help to explain observed differences in PFAA excretion in some species.[9] In Figure 4C and D, we summarize the results from three studies that quantified transporter-mediated PFCA uptake in cells (no similar studies exist for the PFSAs.).[46,48,50] For the proteins responsible for transfer to urine for excretion (Figure 4C), it is evident that different proteins for different species have varying levels of affinity for different PFAAs. In general the rat proteins (Oat1 and Oat3) have higher activity than their

human counterparts (OAT1 and OAT3), and there is a substantial difference in the activity of Oat1 and Oat3 for chain lengths between 7 and 9. For transporters responsible for reabsorption from urine back to blood (Figure 4D), uptake rates were only available for Oatp1a1.[50] It showed a steep increase of activity from 6−8 perfluorinated carbons followed by a decreased but still high activity for PFDA. In other words, the rate of reabsorption is substantially higher for longer chain length PFCAs. This is particularly interesting because Oatp1a1 shows male-dominant gender expression in rats, and could help explain why renal excretion of PFAAs in female rats is substantially higher, leading to much lower elimination half-lives (for example, approximately 50 h vs approximately 1000 h for PFNA in female and male rats, respectively).[9]

Inhibition studies have been performed for a wider range of perfluorinated chain lengths with renal excretion and reabsorption proteins,[49,50] and are summarized in the SI (Figures S11 and S12). Rather than measure the cellular uptake of PFAAs directly, these studies probed the ability of PFAAs to suppress the transport of native substrates of the transporter proteins. Inhibition studies with estrone-3-sulfate (E3S) showed that interaction with PFCAs was chain-length-dependent, with essentially no inhibition occurring for 3−6 perfluorinated carbons (at low concentrations). Activity of the excretion proteins peaked at seven to eight perfluorinated carbons, while those for reabsorption proteins peaked at 9. Moreover, the activity of the human reabsorption transporters, OAT4 and URAT1, was substantially greater than that of Oatp1a1. Again this points to a possible explanation for differences in observed elimination half-lives, this time between rats and humans.

Taken together, the observed patterns of PFAA interactions with albumin, L-FABP and renal transporters show highest albumin binding for PFAAs of medium chain length (6−9 perfluorinated carbons), an increasing affinity for L-FABP up to a perfluorinated chain length of about 11, and an increasing rate of reabsorption from urine back to blood which peaks at about nine perfluorinated carbons. These interactions all lead to increasing bioaccumulation (by increasing uptake or storage), and are balanced by the activity of renal excretion proteins, which peaks at a chain length of between 7 and 8 and then decreases substantially. Thus, it is evident that for the PB model, which seeks to take all of these processes into account, the curvilinear relationship between bioaccumulation and chain length observed in the laboratory and field (Figure 3) results from the combination of different curvilinear relationships between affinity strengths of PFAAs with albumin, L-FABP, and renal transporters.

By incorporating species- and gender- specific expression of renal transporters, the PB model may be able to reproduce the species- and gender-specific variability that has been reported for PFAAs. The PL model, on the other hand, has no inherent mechanism with which to explain these variable half-lives. Elimination mechanisms considered in the PL model include respiratory exchange back to the water, which was defined as the ratio of the uptake rate constant to the sorption capacity, a fecal elimination rate based on relative sorption capacities for the organism and feces, and biotransformation, which is expected to be negligible for PFAAs.[25] Thus, elimination would generally decrease as a function of chain length (or K_{OW}). However, the small variations in sorption capacity that would be expected among species (see for instance the phospholipid distributions we present for rats, rabbits and humans in SI Figure S9) and certainly between genders could not explain the very large differences observed in half-lives between rats, rabbits, and humans.

3. DISCUSSION

Perfluorinated alkyl substances in general and PFAAs in particular have been the subject of intense research for more than a dozen years. Much of this research attention has focused on their unique bioaccumulation behavior. In this review, we have looked at three key aspects of this behavior: (1) the tissue distribution of different PFAAs in different species, (2) the relationship between bioaccumulation potential and perfluorinated chain length, and (3) the high gender- and species-specific variability in elimination half-lives. We have examined these characteristics in the context of the two mechanistic models that currently exist for PFAA bioaccumulation: the phospholipid-based (PL) model of Armitage et al. (2013) and the protein-binding (PB) model of Ng and Hungerbühler (2013).

Our analysis has shown that the two models have a similar ability to predict tissue distribution, with each showing strengths for particular tissues (e.g., the liver compartment for the PL model and the blood compartment for the PB model), and poor performance for others (e.g., blood for the PL model and liver for the PB model when L-FABP is excluded). However, for each model plausible explanations could be found for the observed weaknesses. Overall, the analysis of tissue distribution alone does not lead to one model clearly outperforming the other.

For the curvilinear relationship that has been observed between bioaccumulation factors (BAF) or bioconcentration factors (BCF) and perfluorinated chain length in both the laboratory and in field studies, the mechanisms included in the two models are very different. For the PL model the increase in bioaccumulation results from increasing hydrophobicity which leads also to decreasing elimination rates (the BCF in this model is determined by the sorption capacity of the organism, D_{BW}, which is in turn determined by the fractions of water, storage lipids, nonlipid organic matter and membrane lipids, as well as the octanol−water and membrane−water distribution coefficients). Thus, for this model there is no clear mechanism to explain the decrease observed in BCF and BAF for perfluorinated chain lengths greater than 11. For the PB model, bioaccumulation is determined based on the balance of affinities for albumin, L-FABP and renal transporter proteins, all of which have their own curvilinear relationship with perfluorinated chain length. Although more complicated than the PL approach, the PB model incorporating these three protein interactions showed good agreement with tissue-specific bioconcentration factors observed in rainbow trout and common carp.[51−53] In a more general sense, the PB model seems to show greater potential to explain the relationship between bioaccumulation and PFAA structure, which could also be extended to other features possibly affected by protein binding affinities, such as the variability in bioaccumulation observed for branched versus linear versions of the same PFAA.

Finally, in considering observed differences in elimination half-lives, only the PB model can propose a mechanistic explanation. Interaction with fatty acid transport proteins, which is included in the PB model, has been hypothesized as a driver of differences in half-lives. A recent review of PFCA interactions with OAT proteins by Han et al. (2012) nicely illustrates how such gender differences might emerge.[9]

However, many open questions still remain. Both the PL and PB models were developed based on the bioaccumulation of PFAAs in fish. As our analysis of tissue distributions showed, there can be considerable differences between tissue distributions of some PFAAs in mammals and nonmammals. However, mammals are much better studied in terms of both physiological parameters (including phospholipid distribution) and protein expression and binding affinities. The PB model would benefit greatly from more data for fish and birds. Given the high variability in reported values for PFAA-albumin association constants, studies that are specific to certain types of albumin would also be useful. In addition, the ability of PFAAs to bind to other types of fatty acid binding protein should be explored, as this could have a profound influence on tissue distribution. Finally, membrane transporters in other key tissues, including the gut, gills, blood-brain-barrier, and placenta, warrant further investigation.

Finally, it should be emphasized that the PL and PB models are by no means mutually exclusive. It may well be that the best model to mechanistically describe the bioaccumulation of PFAAs should include both a protein component, particularly in order to describe the accumulation in the blood compartment and elimination and reabsorption as mediated by transporter proteins, as well as a phospholipid component to describe the distribution into tissues where little or no specific binding occurs. However, we would underscore here that without including any protein interactions at all, a model would have little chance of capturing all three key features of PFAA bioaccumulation.

Although there is still much that remains unknown or poorly described about PFAAs, they represent only a single class of perfluorinated alkyl substances. Moreover, long-chain PFAAs and their precursors are being largely phased out. There is therefore a need to shift our focus to a much broader range of structures, and, ideally, move toward more predictive models. Although we are hampered by a general lack of data regarding exactly which perfluorinated structures are being used and released into the environment,[90] modeling studies must begin to incorporate research that looks into these alternatives to the long-chain PFAAs, both old and new.[64,90,91] This will require a concerted effort to produce new data regarding their properties, tissue distributions, and elimination half-lives as well as their distribution coefficients in membrane lipids and affinities for different proteins in different tissues.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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