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Comparing the toxic potency in vivo of long-chain perfluoroalkyl acids and fluorinated alternatives



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ABSTRACT

Since 2000, long-chain perfluoroalkyl acids (PFAAs) and their respective precursors have been replaced by numerous fluorinated alternatives. The main rationale for this industrial transition was that these alternatives were considered less bioaccumulative and toxic than their predecessors. In this study, we evaluated to what extent differences in toxicological effect thresholds for PFAAs and fluorinated alternatives, expressed as administered dose, were confounded by differences in their distribution and elimination kinetics. A dynamic onecompartment toxicokinetic (TK) model for male rats was constructed and evaluated using test data from toxicity studies for perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), perfluorobutane sulfonic acid (PFBS), perfluorooctanoic acid (PFOA), perfluoroctanesulfonic acid (PFOS) and ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate (GenX). Dose-response curves of liver enlargement from sub-chronic oral toxicity studies in male rats were converted to internal dose in serum and in liver to examine the toxicity ranking of PFAAs and fluorinated alternatives. Converting administered doses into equivalent serum and liver concentrations reduced the variability in the dose-response curves for PFBA, PFHxA, PFOA and GenX. ranking using modeled serum (GenX > PFOA > PFHxA > PFBA) and The toxicity liver (GenX > PFOA ≈ PFHxA ≈ PFBA) concentrations indicated that some fluorinated alternatives have similar or higher toxic potency than their predecessors when correcting for differences in toxicokinetics. For PFOS and perfluorobutane sulfonic acid (PFBS) the conversion from administered dose to serum concentration equivalents did not change the toxicity ranking. In conclusion, hazard assessment based on internal exposure allows evaluation of toxic potency and bioaccumulation potential independent of kinetics and should be considered when comparing fluorinated alternatives with their predecessors.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a family of commercially important chemicals characterized by a fully or partially fluorinated aliphatic chain of different length terminated by a functional group (i.e. sulfonate, carboxylate, alcohol) (Buck et al., 2011). The perfluoroalkyl moiety offers high thermal and chemical stability and unique surface tension lowering properties to the molecule which has made PFASs useful in numerous applications since the 1950s. The same properties that make PFASs economically valuable also result in undesired environmental hazard properties such as persistence, bioaccumulation potential and toxicity, especially for long-chain perfluoroalkyl acids (PFAAs i.e. \geq 7 perfluorinated carbons for perfluoroalkyl carboxylic acids (PFCAs) and \geq 6 perfluorinated carbons for perfluoroalkane sulfonic acids (PFSAs)). Since the early 2000s, a number of regulatory actions (ECHA, 2014; UNEP, 2015; US EPA, 2006) and different substitution strategies by the fluorochemical manufacturers have led to a shift in the production toward shorterchain PFCAs and PFSAs, and the introduction of ether linkages in perfluoroalkyl chains (e.g. in perfluoroether carboxylic and sulfonic acids (PFECAs and PFESAs) (Wang et al., 2013). In this study, we focus on 3 PFCAs, 2 PFSAs and 1 PFECA and we collectively term them all as "PFAAs" for convenience (even if the PFECA is strictly not a PFAA). We refer to all fluorinated alternatives that have been introduced to the market to replace legacy long-chain PFAAs (i.e. "short-chain" PFAAs and those containing ethers linkages) as "alternatives".

The rationale for favoring these fluorinated alternatives is that they are regarded as less toxic and less bioaccumulative compared to legacy long-chain PFAAs (Bowman, 2015). While the relationship between PFAA chain length and bioaccumulation potential is well established,

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the reasons behind the lower toxicity of short-chain PFAAs are still unclear. Some studies suggest a structure-dependent toxicity based on the carbon-chain length (Mertens et al., 2010; Olson and Andersen, 1983), whereas others emphasize differences in elimination half-life as the main contributor for the difference in toxicity among PFAAs homologues (Borg et al., 2013; Iwai and Hoberman, 2014; Kudo et al., 2006). Considering that PFAA alternatives are equally persistent as their predecessors (Gomis et al., 2015; Wang et al., 2015) and will lead to a poorly reversible exposure of humans, it is important to scrutinize the intrinsic toxicity of these chemicals to delineate between their bioaccumulation potential and toxic potency (Cousins et al., 2016; Zhang et al., 2017).

In the context of hazard assessment, the relationship between a specific effect of a chemical and the dose at which it occurs is represented by external dose descriptors, such as the no-observed-(adverse)-effect level (NO(A)EL, i.e. the highest dose that does not differ significantly from the unexposed group) and the lowest-observed-(adverse)-effect level (LO(A)EL, i.e. the lowest dose that does differ significantly from the unexposed group). External dose descriptors are commonly used as a point of departure (PoD) in human risk assessment and as a measure of the potency of a chemical. This permits comparison and ranking of substances for their ability to cause a specific toxic effect and can be used for classification and labelling purposes of substances. As the relationship between administered dose and toxic response is partly defined by the absorption, distribution, metabolism and elimination (ADME) of the substance, this practice can, however, result in misleading conclusions on toxicity, interspecies extrapolations and risk to human health and/or the environment if differences in toxicokinetics is not considered (Arnot and Mackay, 2008; Mackay et al., 2001; Maeder et al., 2004; McCarty and Mackay, 1993). This is especially important when considering substitution strategies for chemicals which can have similar modes of actions but different ADME properties, such as PFASs. Thus, ideally, to further characterize the intrinsic toxicity and facilitate animal-to-human extrapolation in risk assessment of chemical substances such as PFAAs, the dose descriptors would represent the internal dose, i.e. the amount of the chemical in blood or at the target tissue.

In this study, we systemically investigated if alternatives to longchain PFAAs are less potent than their predecessors when considering integrated internal doses. The central hypothesis of this work was that apparent differences in toxicity between legacy long-chain PFAAs and their alternatives, based on administered dose, are minimal if they are compared on an internal dose basis and can be largely accounted for by their differences in the toxicokinetics.

2. Methods

2.1. Overview of the methodology

The first step (Fig. 1, Step 1) consisted of parametrizing the toxicokinetic (TK) model to allow prediction of internal doses of PFAAs from a wide range of administered doses tested. Once the model was shown to be able to reproduce the experimental data from the toxicity studies the TK model was used to convert administered dose into internal dose, by implementing the exposure conditions (i.e. exposure length, repeated/single dose regimen) as defined in the toxicity studies of interest (Fig. 1, Step 2). In a third step (Fig. 1, Step 3), for each PFAA, the toxic response, as measured in the toxicity studies, was plotted against a) the corresponding tested administered dose and b) the corresponding predicted internal dose. The resulting dose-response relationships allowed the LOELs to be expressed as administered and internal dose. The internal dose was investigated in serum and in liver, which is known to be the main target organ for PFAAs (Lau et al., 2007). The LOELs corresponded to the tested dose at which the first significant effect was observed, as indicated in the toxicity studies. For each exposure level, the PFAAs were ranked based on their potencies, with the most potent substance having the lowest LOEL.

2.2. Selection of toxicokinetic and toxicity data

The present study was dependent on pre-existing toxicokinetic and toxicity data available in the scientific literature. The comparability between the collected data was a prerequisite for the validity of the predicted results and of the potency assessment. Two levels of comparability had to be considered. First, to accurately reproduce the internal dose corresponding to the specific administered dose regimen, the parametrization of the TK model was required to integrate the toxicity experimental settings. At that point, the comparability between toxicity and toxicokinetic studies was a prerequisite. Second, comparing different chemicals based on their potencies was only possible if their corresponding dose-response relationships were obtained from similar experimental conditions. As a consequence, the methodology in the toxicity studies had to be similar. The substantial interspecies variability in the elimination of PFAAs prevented the use of studies based on different animal models (Lau et al., 2007). Rats were the most common animal model in both toxicity and toxicokinetic studies and were therefore chosen as the animal reference in this study. Female rats were excluded due to different kinetics in the elimination of PFAAs compared to male rats (Kudo et al., 2001).

Besides the administered dose, external factors influencing the levels of a substance in an organism were the duration of exposure and the exposure route. Therefore, to be able to compare the dose–response curves of different PFAAs, the toxicity studies had to be selected based on similar experimental methodologies. Among the different ways to administer the external doses (i.e. intravenous, intraperitoneal, gavage, dietary, inhalation), toxicity studies with gavage and dietary dosing were selected as they were most common. In addition, sub-chronic experiments (i.e. daily dosing during 90 days) were preferred because the internal levels of PFAAs were assumed to be at steady-state when the toxic effect was evaluated. Finally, for toxicokinetic studies, experiments with oral administration and displaying the serum concentration-time curve were preferred.

As a result of the abovementioned selection criteria, the following three PFCAs and two PFSAs were investigated: perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorobutane sulfonic acid (PFBS) and perfluorooctane sulfonic acid (PFOS). In addition, one PFECA alternative to PFOA, ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoic acid (GenX), was included. Perfluorodecanoic acid (PFDA) could not be included in the analysis because of a lack of experimental data on subchronic toxicity, preventing the comparison with the other homologues. The same problem was encountered with perfluorononanoic acid (PFNA) for which the only toxicological study available on rats was carried out with S-111-S-WB, a mixture of perfluoro fatty acid ammonium salts (C6-C13) with PFNA as the major component (Mertens et al., 2010). Finally, perfluorohexane sulfonic acid (PFHxS) and ammonium 4,8-dioxa-3H-perfluorononanoat (ADONA), another PFECA alternative to PFOA, could not be assessed due to a lack of data on their toxicokinetics in rats. A list of the toxicity studies selected for each of the six PFAAs are presented in Table 1.

2.3. Defining the toxicity endpoints

PFAAs have been linked to numerous toxic effects. Observed effects following PFAA dosing include: decreased body weight, reduced red blood cell count, immunotoxicity, increased hepatic enzyme activity (i.e. alkaline phosphatase, alanine aminotransferase), decreased serum cholesterol and thyroid hormone levels in serum, induction of peroxisomal beta oxidation activity, hepatocellular hypertrophy as well as increases in liver and kidney weight (Chengelis et al., 2009b; Lau et al., 2007). The selection of toxic effect to which the potency of PFAAs were associated was dependent on the availability and comparability of data but also on the target tissues evaluated. Only effects that were specifically known to be downstream consequences or directly linked to



Fig. 1. Conceptual overview of the approach used in this study.

hepatotoxicity were considered. Decreased red blood cell counts can, in addition to hepatotoxicity, be due to other processes such as effects on erythropoiesis in the bone marrow and direct haemolysis by the chemical (Bloom and Brandt, 2001). Since hematopoietic effects are not commonly associated with PFAAs exposure (Lau et al., 2007), we considered effects on red blood cell count to be unrelated to the relatively mild hepatocellular effects discussed in this study. Concerning the decrease in body weight, this effect can be due to loss of appetite following exposure to PFASs (Cui et al., 2010), atrophy of adipose tissue in rodents by PPAR-alpha agonists (Xie et al., 2003) or indirectly linked to effects mediated by liver toxicity. For the effect on thyroid hormone levels in serum, PFAAs are assumed to displace the thyroid hormones from their binding sites in serum transport proteins (Lau et al., 2007). Concerning the immunotoxicity of PFAAs, multiple pathways may be involved (Corsini et al., 2014). Thus, since the etiology of these effects is not necessarily related to hepatotoxicity, immunotoxicity, decreased body weight, decreased thyroid hormone levels and decreased red blood cell count were discarded. Alterations in enzyme activity, serum cholesterol levels as well as induction of peroxisomal beta oxidation activity were also not considered due to the lack of comparable data

among the PFAAs (see Table 1). The only endpoint fulfilling all criteria following exposure to PFAAs was the increase in liver weight. Increased liver weight has been associated with adverse effects at higher doses such as hepatocellular necrosis (Butenhoff et al., 2012b) and, when exceeding 15%, is considered an adverse effect by the World Health Organisation (WHO, 2015). Increased liver weight is a sensitive hallmark response following PFAS exposure in rats and, thus, is a suitable endpoint to compare between different PFASs. The effect at a specific dose was quantitatively expressed as the ratio between the average liver weights in the dosed animals and the control animals. A ratio larger than 1 indicated an increase in the liver weight. In toxicity studies, the liver weights were either communicated in absolute weight or relative to the body weight. In order to address most potential confounding factors (Bailey et al., 2004), the ratio was calculated from the relative liver weights.

2.4. Predicting the internal dose in serum

The conversion of a specific exposure to internal dose is often achieved with TK models. To predict the serum concentrations of

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ist of the selected toxicity studies together with the experimental conditions and evaluated endpoints.

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	Animal	Exposure ^a	Body weight	Liver weight	Cholesterol	Thyroid hormones	Red blood cell count	Enzyme activity (ALT/ALP) ^b	Peroxisome proliferation ^c	Reference		
PFBA	Male rats	G/M/90D	x	x	x	x	x	-/x	-	Butenhoff et al., 2012a		
PFHxA	Male rats	G/M/90D	x	x	x	-	x	x/x	x	Chengelis et al., 2009b		
GenX	Male rats	G/M/90D	x	x	-	-	x	x/x	-	Beekman et al., 2016		
PFOA	Male rats	DD/M/90D	x	x	-	-	х	-/-	х	Perkins et al., 2004		
PFBS	Male rats	G/M/90D	x	x	x	-	х	x/-	-	NICNAS, 2005		
PFOS	Male rats	DD/M/98D	x	x	x	-	х	x/-	x	Seacat et al., 2003		

 a DD = dietary dosing, G = gavage, M = multiple dose with intervals of 24 h, D = days.

^b ALT = alanine aminotransferase, ALP = alkaline phosphatase.

^c Measurements of palmitoyl CoA-oxidase activity.

PFAAs, both one- and multi-compartment TK models have been used in the past (Andersen et al., 2006; Harada et al., 2003; Yoo et al., 2009). Even though a multi-compartment model provides additional information, as long as the multiple input parameters are known and well constrained, a one-compartment model describing first-order kinetics is often sufficient as long as the exposure does not exceed the threshold of saturable processes (Lou et al., 2009). For legacy long-chain PFAAs, and more specifically for their alternatives, the availability of toxicokinetic data on organ distribution, such as exchange rates, was limited. Therefore, to minimize the propagation of error to the predicted results, a simple one-compartment dynamic TK model was used to predict the temporal changes in PFAA serum concentrations following single or multiple dose regimen.

2.5. Model equations

The decrease in serum concentrations over time after a single dose can be described as monophasic or biphasic. In a biphasic curve, a first distribution " α " phase represents the decrease in serum concentration as a result of the migration of the substance to peripheral tissues, and is followed by a terminal " β " phase when a pseudo-equilibrium between the serum and the peripheral tissues has been reached. Each phase is characterized by a disappearance rate, corresponding mainly to the distribution processes in the α phase and to the elimination in the β phase. In a monophasic curve, the α phase is negligible because distribution is instant and, therefore, the β phase is dominant (Toutain and Bousquet-Melou, 2004). With this in mind, a first-order equation defining the mass balance between elimination and administered dose was applied. For PFAAs following monophasic kinetics (i.e. PFBA, PFHxA), the serum concentration time trends were predicted with Eq. (1) (Toutain and Bousquet-Melou, 2004).

$$C_{serum}(t) = \frac{F * \frac{D_{adm}}{U} * k_a}{V d_0 (k_a - k_e)} * (e^{-t * k_e} - e^{-t * k_a})$$
(1)

where C_{serum} (in µg/ml) is the concentration in serum, *F* is the bioavailable fraction (unitless), D_{adm} (in µg/kg-bw/day) is the administered dose, Vd_0 (in ml/kg) is the volume of distribution at time 0, k_a (in 1/h) is the absorption rate, k_e (in 1/h) is the elimination rate and U is a unit conversion factor (24 h/day). Since PFAAs are generally very well absorbed (> 93%), F was assumed to be 1 (Chang et al., 2008; Cui et al., 2010; Gannon et al., 2011, 2016; Olsen et al., 2009). The model was based on a discrete-time approach and the time step was set to 30 min. D_{adm} can represent a single or multiple administration regime. In the latter case, the equation was modified to account for the dosing intervals, which was one dose every 24 h for all toxicity experiments considered in this study.

For PFAAs following biphasic profiles (i.e. GenX, PFOA, PFBS, PFOS), Eq. (1) had to be modified to include both α (Eq. (2)) and β (Eq. (3)) phase. Because the duration of the α phase was not communicated in the toxicokinetic studies, the transition from Eq. (2) to Eq. (3) was fitted to the experimental data.

$$C_{serum_{\alpha}}(t) = \frac{F * \frac{D_{adm}}{U} * k_{a}}{V d_{0}(k_{a} - k_{d})} * (e^{-t * k_{d}} - e^{-t * k_{a}}), t \in [0, t_{\alpha_{end}}]$$
(2)

where $C_{serum\alpha}$ (in µg/ml) is the serum concentration during the α phase, k_d (in 1/h) is the distribution rate into peripherical tissues and $t_{\alpha end}$ is the time corresponding to the end of the α phase.

$$C_{serum_{\beta}}(t) = C_{serum_{\alpha}}(t_{\alpha_{end}}) * e^{(-t*k_e)}, t \in (t_{\alpha_{end}}, t_{end}]$$
(3)

where $C_{serum\beta}$ (in µg/ml) is the serum concentration during the β phase, k_e (in 1/h) is the elimination rate and t_{end} corresponds to the end of the experiment.

The model was parametrized using compound-specific TK data for each of the PFAAs included. The input parameters were collected from single oral dose toxicokinetic studies on male rats, except for PFOA for which the only toxicokinetic experiment presenting serum concentration time trends was based on intravenous administration (Table S1 of the Supplemental Material (SM)). The model was first parameterized and evaluated (step 1 of methodology) for each PFAA by comparing the predicted concentrations in serum with experimental data from single dose toxicokinetic studies. In order to identify potential prediction weaknesses and dose threshold where saturable processes might start, the model estimations for PFAAs with slow elimination rates (i.e. PFOA, PFOS) were compared to the serum concentration obtained from repeated dose subchronic experiments.

2.6. Applying the model to subchronic toxicity experiments

To predict the internal doses corresponding to administered doses (step 2 of methodology), the dose conditions as set in the toxicity experiment were transposed to the model simulations. In this respect, the length of exposure, defined as the length of the experiment, the chemical intake, defined by the administered dose and the dosing intervals (i.e. 24 h), were considered in the model.

The area under the curve (AUC) was considered to be the best measure for the internal dose since this parameter provides an integrated measure of exposure to the chemical over time. The serum AUC (in μ mol * h/ml) at the interval between two doses was calculated from the model predictions, when steady-state was achieved, using the trapezoidal rule. The AUC was expressed in molar concentrations for consistency with Kudo et al. (2006) who suggested the amount of molecule at the target site to determine the toxicity (Kudo et al., 2006).

2.7. Predicting the internal dose in liver

Under steady-state conditions, the changes in serum concentration were assumed to reflect the changes of concentration in the liver. This proportional relationship is supported by the findings of Vanden Heuvel et al. (1991) who measured the concentration of PFOA in plasma and in liver in rats over 24 days after a single intraperitoneal injection (see Section 3 in the SM) (Vanden Heuvel et al., 1991). With this assumption, the equivalent AUC at steady-state (AUC_{ss}) in liver (in μ mol*h/g) was estimated from the predicted serum AUC_{ss} using liver to serum concentration (L:S) ratios obtained from different studies as conversion factor (Beekman et al., 2016; Butenhoff et al., 2012a, 2012b; Gannon et al., 2011; Kudo et al., 2001; Seacat et al., 2003; Tatum-Gibbs et al., 2011; Vanden Heuvel et al., 1991). To be more consistent, the L:S ratio calculated with the closest experimental serum concentration to the predicted serum AUCss were favored. In other cases, the averaged L:S ratio was used. Even though AUCs were more commonly used to reflect the bioavailability of chemicals in serum, several studies have expressed the accumulation over time in liver as equivalent AUC (Kim et al., 2003). The L:S ratios are available in Table S2 of the SM.

2.8. Uncertainty analysis

An uncertainty analysis was carried out on the predicted liver AUC_{ss} based on the Monte Carlo method (Robert and Casella, 1999). L:S ratios were randomly generated according to the range of experimental data collected, assuming a uniform distribution to be conservative. For each compound, the corresponding liver AUC_{ss} were calculated from each generated ratio and the standard deviation was derived. The uncertainty analysis was carried out for PFBA, PFHxA, GenX, PFOA and PFBS. For PFOS, the uncertainty analysis was not necessary since the L:S ratios was provided by the toxicity study under consideration.

3. Results and discussion

3.1. Predicting experimental serum concentrations using the TK model

The model parameterization and evaluation (step 1 of the

methodology) was an important step to ascertain a reasonable predictive power for the range of administered doses tested in the toxicity studies. As shown in Fig. S1 of the SM, the predictions for the six PFAAs were in good agreement with the experimental serum concentrations of the toxicokinetic studies, indicating that the one-compartment TK model was able to reproduce monophasic and biphasic patterns. For PFAAs with relatively short elimination half-lives (i.e. PFBA, PFHxA, GenX and PFBS), accumulation was expected to be minimal following a multi-dose regimen, as demonstrated by Gannon et al. (2016) with GenX. However, PFOA and PFOS, which have long elimination halflives in rats (> 100 h), would gradually accumulate in serum under a multi-dose regimen. As shown in Fig. S3 in the SI, the predicted serum concentration of PFOS at steady-state, after multiple doses, was fairly close to the experimental values, even though the model tend to slightly underestimate the serum concentration of PFOS at lower dose ($< 1 \, \mu g/$ ml difference). For PFOA (Fig. S2), however, the model underestimated the serum concentration by up to a factor of two as the dose decreases. This can be explained by an elimination half-life in serum inversely proportional to the dose, as a result of saturated organic anion transporters (OATs) responsible for the renal reabsorption of PFOA (Weaver et al., 2010). This is illustrated by Vanden Heuvel et al. (1991) and Kudo et al. (2002) who estimated the elimination half-life of PFOA in male rats to be 9 days and 5.6 days from a single intravenous injection of 4 and 20 mg/kg, respectively. Using Kudo et al.'s elimination half-life to parametrize the TK model for PFOA, as it was done in this study, is therefore only valid under saturation conditions, when elimination kinetics are faster. According to Fig. S2, this does not appear to be the case for a multi-dose regimen below 6.5 mg/kg/bw, for which Vanden Heuvel et al.'s estimated elimination half-life would be more suitable.

3.2. Bioaccumulation potential and carbon chain-length

In order to investigate the bioaccumulation potential of the 6 PFAAs, a 10-day oral experiment with a dose of 1 mg/kg/day was simulated using the model. As shown in Fig. 2A), the AUCs in serum increased together with the chain-length among the PFCA and PFSA homologues. PFBA was the only exception due to a four-time longer elimination half-life compared to PFHxA (see Table S1 in the SM). In addition, the model simulation showed that PFBA, PFHxA, GenX and PFBS already reached steady-state conditions at the end of the first 24 h after the first dose. In contrast, PFOA and PFOS were still accumulating at the 10th day of the simulation. As shown in Fig. 2B), the bioaccumulation potential in liver was higher for PFOA and PFOS, compared to serum. PFBA, PFHxA, GenX and PFBS partitioned to liver, but the largest fraction of these substances was in serum.

As opposed to classical lipophilic organic pollutants, such as

dioxins, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) that partition primarily to fatty tissues, PFCAs and PFSAs instead bind strongly to proteins. > 98% of the molecules are bound to serum proteins, principally to albumin, and also interacting with fatty acid-binding proteins in liver (Lau, 2012; Luebker et al., 2002; Ohmori et al., 2003). The binding affinity appears to increase with chain-length and is also dependent on the functional group. For shorter-chain homologues (i.e. PFBS), the binding to the plasma protein fraction decreases as the concentration increases, indicating a potential saturation of the available binding sites (NICNAS, 2005). In addition, for the same chain length, albumin binding sites accommodate more molecules of PFSAs than PFCAs (i.e. 9 for PFOA and 11 for PFOS per protein) (Salvalaglio et al., 2010). Even though interaction with hepatic proteins have been less studied than interaction with albumin, longer chain homologues and sulfonates seem to display a higher affinity (Luebker et al., 2002; Woodcroft et al., 2010). These observations likely explain the preferential accumulation of PFOS and long-chain PFCAs in liver as highlighted in Fig. 2. The primary elimination route of the unbound PFAAs and its efficiency is also dependent on the chemical structure. Compared to the short-chain homologues, which are excreted via urine, the longer-chain PFSAs and PFCAs tend to be eliminated through biliary excretion, as a result of higher accumulation in liver (Ohmori et al., 2003). For both routes, reabsorption processes such as enterohepatic circulation and the binding to OATs can decrease the elimination rate. The affinity to organic apical anion transporters and the ability to enter the enterohepatic circulation increases with chain-length (Goecke-Flora and Reo, 1996; Weaver et al., 2010; Yang et al., 2010). Even though the molecular interactions have not been fully identified and understood yet, the more favorable receptor/binding behavior of longer chain PFAAs seems to contribute to the observed bioaccumulation potential and distribution.

3.3. Comparison of LOELs based on administered dose, predicted levels in serum and in liver

From the toxicity studies reporting increased liver weight, administered doses were converted into equivalent serum AUC_{ss} and liver AUC_{ss} for each of the 6 PFAAs. The corresponding dose–response relationships are presented in Fig. 3 (raw data in Table S3 of the SM).

The observed effect (Y axis) is expressed as the ratio between the relative liver weight in the dosed animal and in the control. An increase in liver weight corresponds to a ratio above 1. For PFBA, PFHxA, GenX and PFOA the lowest observed effect levels (LOELs) based on the administered dose were 30, 200, 10 and 1.94 mg/kg/day, respectively, with similar ratios between 1.2 and 1.3 (Fig. 3, Graph A). Consequently, according to the administered dose, the ranking from the most potent to



Fig. 2. Predicted area under the curves (AUC) of PFBA, PFHxA, GenX, PFOA, PFBS and PFOS in rats following a daily oral dose of 1 mg/kg-bw in A) serum and B) liver. The serum AUCs in µmol * h/ml are reported for each PFASs at 1 (light grey), 5 (dark grey) and 10 (black) days of dosing. The liver AUC in µmol * h/g were calculated from serum AUC at day 1 using the L:S ratios.



Fig. 3. Liver weight ratios – dosed/control – as a function of (A)/(D) administered dose, (B)/(E) predicted serum AUC at steady-state and (C)/(F) predicted liver AUC at steady-state. Dose response relationships for PFCAs and alternatives are presented in A,B,C, and dose–response relationships of PFSAs are presented in D, E, F. The standard deviations are indicated for each predicted AUC in liver. The studies which results are reported in graph A, D and G are indicated in the legend. The black symbols represent the doses where a significant effect was reported by the studies. In order to plot both curves together, the administered dose in graph D) is expressed in log scale.

the least potent substance was PFOA > GenX > PFBA > PFHxA. Converting these LOELs into equivalent serum levels (Fig. 3, Graph B), the rank order changed to GenX > PFOA > PFHxA > PFBA. At the same time, the difference of LOELs between PFAAs in serum (Fig. 3, Graph B) was reduced compared to the range of LOELs expressed as administered dose (Fig. 3, Graph A), which varied over 3 orders of magnitude. When looking at the target tissue (Fig. 3, Graph C), the LOELs expressed as AUCss in liver indicated that, for PFBA, PFHxA and PFOA, the same internal dose (AUCss range: 1.9-2.8) led to the same increase in liver weight (ratio range: 1.2-1.25). In other words, according to the LOELs in liver and within the uncertainty range, the same amount of substance triggered the same effect, indicating that PFBA, PFHxA and PFOA had the same potency to induce increased liver weight. GenX was the most potent substance since its LOEL in liver (Fig. 3, Graph C) was the lowest of the examined PFAAs and was associated to a higher effect compared to the three other substances. However, the rank order of substances should be treated with caution as LOEL values in liver displayed small differences between the different

substances. Furthermore, it should be noted that PFOA, for which the serum AUC_{ss} predictions were underestimated at dose below 6.5 mg/ kg/day, consequently shifting the dose–response curve toward falsely more potent characteristics. Correcting the predicted serum AUC_{ss} of PFOA according to the experimental serum concentration at the LOEL (Perkins et al., 2004) resulted in an increase in serum AUC_{ss} of 1.4 µmol*h/ml compared to the serum AUC_{ss} presented in Fig. 3B. The standard deviations of all AUC_{ss} in liver at LOEL obtained from the Monte Carlo simulations varied between 0.002 and 0.9. Considering this relatively small uncertainty range, the abovementioned observations for LOELs are still valid.

On the basis of administered dose, the LOEL of PFOS corresponded to 1.34 mg/kg/day, whereas no significant effect was reported up to 600 mg/kg/day for PFBS (Fig. 3, Graph D) (NICNAS, 2005; Seacat et al., 2003). Looking at the corresponding AUC_{ss} in serum, PFOS was estimated to be more potent than PFBS (Fig. 3, Graph E). However, the predicted AUC_{ss} in liver indicates that PFBS has been tested at concentrations that resulted in lower levels in the liver than those that

triggered an effect for PFOS (Fig. 3, Graph F). Therefore, no robust conclusions could be drawn on the relative potencies of the two compounds. In addition, due to the scarcity of information on L:S ratio, the predicted liver AUC_{ss} for PFBS was associated with high uncertainty (SD $\pm~20\,\mu\text{mol}*h/\text{g}$). It should be noted, however, that for PFBS, the administered dose should be up to 2000 times higher to achieve the same magnitude in serum and liver concentrations as PFOS, which is due to the higher bioaccumulation potential of PFOS in serum and in liver, as demonstrated in Fig. 2.

In conclusion, the results showed that the difference in LOELs decreased from the administered dose to the levels in liver, indicating that the difference in potency decreased when approaching the target tissue. These results confirmed the hypothesis that, for this specific endpoint, the amount of molecules at the target site, regardless of their structure, determined the effect. This hypothesis was also supported previously by Kudo et al. (2000).

3.4. Methodological limitations

Despite the benefit of being less complex, a one-compartment TK model describes first-order kinetics and does not consider saturation of protein binding sites. The model was suitable for short-chain PFASs for which linear kinetics apply, even at a very high dose (Chang et al., 2008; Chengelis et al., 2009a; Gannon et al., 2011). However, for GenX, it was not known if enhanced elimination due to saturation processes occurred beyond a dose of 10 mg/kg (Gannon et al., 2016). Additionally, in the case of long-chain PFASs, that tend to accumulate in the organism, internal concentrations could reach saturation thresholds in repeated dose studies. It was therefore necessary to identify the dose range where the one-box model was applicable in order to avoid underor overestimation of the internal doses. Another aspect that could lead to erroneous estimations came from the type of dosing regimen applied in the toxicity study. To be consistent with the experimental conditions, the model needed to consider the daily dose, the dosing intervals as well as the length of the experiment. Any changes in these parameters would modify the internal concentrations and should therefore be implemented in the model accordingly for higher accuracy of the predictions. In the oral dose studies, the daily intake was better controlled through gavage compared to diet since, in the latter, a daily variability in food consumption can occur through loss of appetite resulting in a decreased intake (Perkins et al., 2004; Seacat et al., 2003). The decrease in serum concentration over time resulting from a reduced dose could not be estimated by the model, which assumed the same dosing over time. Finally, the criteria required to retrieve comparable toxicokinetic and toxicity data hampered the inclusion of additional compounds and toxic endpoints. Due to the shortage of comparable studies, the relationship between internal dose and effect could only be investigated for one toxic endpoint and a limited number of PFAAs.

There is an inherent uncertainty in the predictions of serum AUC_{ss} and liver AUC_{ss} from the modelling method used in this study. While the uncertainty for liver AUC_{ss} could be calculated for the six PFAAs based on the range of their respective L:S ratios, the uncertainty of the predicted serum AUC_{ss} could not be quantified. The quantification was not possible because the toxicokinetic studies used to parametrize the model provided only averaged values for the volume of distribution, the distribution and elimination half-lives and the absorption rate. Furthermore, information on the toxicokinetic parameters for individual rats was lacking which prevented from estimating the interindividual variability in ADME. Nevertheless, since the model predictions were thoroughly validated by independent data sets, we believe that the uncertainty in predicted internal doses is reasonably low. In addition, the model limitations have been identified and the results have been discussed accordingly.

3.5. Implication for human health risk assessment

The results of this study demonstrate that the apparent lower toxicity (toxicity assessment based on administered dose) of fluorinated alternatives in rats compared to legacy PFAAs was primarily caused by their faster elimination and lower distribution to the liver. As a result, the assessment and comparison of toxicity of these PFAAs according to REACH regulation standards (EC 1907/2006) will be heavily influenced by their kinetics. Methodologies of varying complexity to correct for differences in toxicokinetics have been applied to assess intrinsic toxicity, in the field of aquatic ecotoxicology (Landrum et al., 2013; McCarty and Mackay, 1993; Meador et al., 2011) and also for mammalian toxicity studies (Arnot and Mackay, 2008; Mayer, 1995; Ploemen et al., 2007). Nevertheless, even though the implementation of these methods in the risk assessment of bioaccumulative chemicals has been requested by US EPA for aquatic ecosystems (US EPA, 2005), the standard guidance of toxicological studies under REACH (or OECD) do not require internal dose-response data (ECHA, 2012; OECD, 2008). Since the manifestation of a toxic effect depends on the dose of the toxicant at the target site, PFASs that have a fast clearance and thus a low bioaccumulation potential can still be intrinsically toxic as demonstrated in this study. Whether toxic effects are triggered would depend on the level of exposure. Toxicity risks linked to persistent short-chain PFAAs is therefore not to be excluded since these chemical are expected to accumulate in the environment with low reversibility (Cousins et al., 2016) and lead to highly elevated exposures (Zhang et al., 2017).

The benefit of using internal dose measurements in the hazard assessment of PFAAs is that it allows to focus on the intrinsic toxic potency of the substance and less on toxicokinetics and facilitates animalto-human extrapolations (Borg et al., 2013; Butenhoff and Rodricks, 2015). In terms of human health risk assessment, the guidance values for human exposure are derived from oDs such as LOELs obtained from in-vivo experiments. However, due to large interspecies variability in toxicokinetics, the concentration of PFAAs in serum and target tissues will likely differ between human and rats for the same exposure conditions. For example, the L:S ratios of PFAAs tend to be smaller in humans whereas the elimination half-lives increase from hours in rats to years in humans (Olsen et al., 2003; Olsen and Burris, 2007). As a consequence, short-chain PFASs that are very rapidly excreted in a species such as the rat may not reach internal concentrations sufficient to result in toxic effects that it could in other species with a longer halflife, such as humans. By considering the internal dose during human health risk assessment, the inter-PFASs, species and sex variability is reduced allowing a more accurate extrapolation from animal data to humans (Borg et al., 2013). Furthermore, despite some limitations, internal dose descriptors, such as serum concentrations, are a better approximation of the concentration of the chemical at the target site as compared to the administered dose. Nevertheless, it should be noted that human exposure to PFAAs, which can be characterized as low-level chronic exposure, differs largely from the exposure settings in in vivo sub-chronic studies, where dosing occurs one time daily at levels up to 5 orders of magnitude higher than the estimated human daily intake.

Finally, using the internal dose approach facilitates cumulative risk assessments, which address simultaneous exposure to multiple chemicals. Since biomonitoring studies indicate that human serum contains a mixture of PFAAs, mainly PFOA, PFDA, PFOS and PFHxS (CDC; Toms et al., 2009), it is necessary to consider the potential cumulative effects of these chemicals. Provided the model applicability to the dose range investigated, the use of a TK model such as the one presented in this study for the conversion of the tested administered dose into a corresponding internal dose can overcome the lack of systematic internal dose measurements in the toxicity literature. Even though additional endpoints should be investigated, the present study and Kudo et al. (2006)'s, where the amount of molecules at the target site rather than the chemical structure for determining the toxic response was demonstrated, suggests that the different PFAAs do not differ much in potency when toxicokinetics are taken into account. Our results support, therefore, the use of cumulative assessment and limit values in the regulation of PFAAs, as developed by the Swedish National Food Agency and the US Environmental Protection Agency, although further evaluation is needed for additional endpoints (National Food Agency, 2016; U.S. EPA., 2016).

4. Conclusions

The potency ranking among the PFAAs and their fluorinated alternatives gradually disappeared when internal doses, closer to the target tissues, were used for the assessment. These results indicate that toxicokinetics is an important factor in the toxicity of PFAAs and that alternatives to legacy PFAAs could likely be intrinsically as potent as their predecessors. Despite some limitation, using a one-compartment toxicokinetic model to convert administered dose into internal dose in rats lead to reasonable results. This methodology could be an efficient alternative for toxicity studies and risk assessments to obtain internal doses when systematic measurements are not possible. Finally, attention should be drawn to the lack of toxicokinetic data for PFHxS and ADONA which made it currently impossible to assess their toxic potency. Since PFHxS was recently proposed for listing under the Stockholm Convention (Stockholm Convention, 2017) and ADONA is being used as a replacement of PFOA (Fromme et al., 2017), we strongly encourage further research to establish the toxicokinetic parameters of both compounds following oral dosing of male rats.

Conflict of interest

The authors declare they have no actual or potential competing financial interests.

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Appendix A. Supplemental Material

The parametrization and evaluation of the one-compartment PK model for each PFAA, the relationship between serum and liver concentrations, and the predicted serum and liver concentrations are available in the supplemental material. Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.envint.2018.01.011.

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