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PBPK modeling for PFOS and PFOA: Validation with human experimental data

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HIGHLIGHTS

- A preexistent PBPK model was here validated to estimate PFOA and PFOS human levels.
- Coefficient partition (*Pk*) values from human experimentation studies should prevail.
- Uncertainty and variability associated to PFAS body burdens is too high.
- Further efforts should be made to reduce parametric uncertainty of PBPK models.

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ABSTRACT

In recent years, because of the potential human toxicity, concern on perfluoroalkyl substances (PFASs) has increased notably with special attention to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Unfortunately, there is currently an important knowledge gap on the burdens of these chemicals in most human tissues, as the reported studies have been mainly focused on plasma. In order to overcome these limitations, the use of physiologically-based pharmacokinetic (PBPK) models has been extended. The present study was aimed at testing an existing PBPK model for their predictability of PFOS and PFOA in a new case-study, and also to adapt it to estimate the PFAS content in human tissue compartments. Model validation was conducted by means of PFOA and PFOS concentrations in food and human drinking water from Tarragona County (Catalonia, Spain), and being the predicted results compared with those experimentally found in human tissues (blood, liver, kidney, liver and brain) of subjects from the same area of study. The use of human-derived partition coefficient (*Pk*) data was proven as more suitable for application to this PBPK model than rat-based Pk values. However, the uncertainty and variability of the data are still too high to get conclusive results. Consequently, further efforts should be carried out to reduce parametric uncertainty of PBPK models. More specifically, a deeper knowledge on the distribution of PFOA and PFOS within the human body should be obtained by enlarging the number of biological monitoring studies on PFASs.

1. Introduction

Perfluoroalkyl substances (PFASs) are a group of man-made substances, whose chemical structure is a carbon backbone, where the hydrogen has been substituted by fluorine. The carbon fluorine bound is among the strongest covalent bounds, conferring a high molecular stability. PFASs have widely used in consumer and industrial applications, including protective coatings for fabrics and carpets, paper coatings, insecticides, paints, cosmetics, and fire-fighting foams (Domingo, 2012). However, the properties making these chemicals useful, turns into environmental problems. Thus, PFASs show a high persistence and spreading capacity in the environment (Fujii et al., 2007). Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are among the most widely spread PFASs, having been detected in a number of environmental matrices, including water, sediments, soils and biota (Post et al., 2009; Rumsby et al., 2009; Shi et al., 2012; Zareitalabad et al., 2013). Detectable concentrations of PFOA and PFOS have been found in food (Domingo et al., 2012a), while measurements in whole blood, plasma or serum samples from humans are also available from the scientific literature (Ehresman et al., 2007). Because of their low degradation, high bioaccumulation, potential toxicity and long-range transport capacity, both PFOS and PFOA are considered as persistent organic pollutants (POPs) (Chaemfa et al., 2010). In fact,

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PFOS was included as a POP under the Stockholm Convention in 2009, while PFOA remains a serious candidate to enter that list shortly. Very recently, PFOS was also identified as a priority hazardous substance according to the European Directive 2013/39/EU, in the field of water policy (European Commission, 2013). Consequently, regulatory agencies are paying considerable attention to the presence of PFOA and PFOS in the environment, as well as to the human health risks associated to their exposure.

Pharmacokinetic and pharmacodynamic characteristics of PFOA and PFOS have been studied in animals (Lau et al., 2007; Vanden Heuvel et al., 1991). These compounds are highly absorbed, not metabolized and poorly eliminated, being plasma, liver, kidney and lungs the main distribution tissues (Cui et al., 2009; Hundley et al., 2006). Both compounds have a high affinity to albumin, being therefore accumulated in plasma. Specific PFOA half-lives in a number of animal species are known. Elimination half-lives of PFOA, ranging 2–6 h, 17–19 days, and 30–21 days, have been estimated for rats, mice, and monkeys, respectively (Lau et al., 2007; Lau, 2012). Contrastingly, PFOA half-life in the human body is markedly higher, with an estimated value of 3.8 years. Regarding PFOS, half-lives have been established in 1-2 months, 4 months, and 4.8 years in rodents, monkeys, and humans, respectively (Chang et al., 2012). Although the reasons of these differences are not conclusive, it has been hypothesized that it can be due to a saturable process of resorption of PFASs from urine to plasma (Andersen et al., 2006). In addition to bioaccumulation, toxicity of PFOS and PFOA has been also characterized in animals (Lau et al., 2004; Stahl et al., 2011). A number of developmental effects, such as reduction of fetal weight, edema, cardiac abnormalities, and delayed ossification, as well as behavioral effects, have been reported following acute exposure to PFOS (Fuentes et al., 2006, 2007a,b). Subchronic and subacute toxicities induce hepatotoxicity, reduction of body weight, reduction of the levels of triglycerides and cholesterol in serum, liver hypertrophy and thyroid hormones reduction, while the neuroendocrine system seems also to be affected in rats exposed to PFOS (Austin et al., 2003). Recently, PFOA exposure has been suggested to be associated with kidney and testicular cancer in human populations (Barry et al., 2013).

The time dependent concentration of a certain pollutant in human tissues can be predicted using physiologically-based pharmacokinetic (PBPK) models. These models consider the human body as a set of well stirred compartments linked by the blood flow. Physiological processes are represented by a set of ordinary differential equations describing the processes of administration, distribution, metabolism and elimination of a specific chemical (Loizou et al., 2008; Péry et al., 2013). The final result is a model that simulates the time course distribution of a substance in the human body (Nadal et al., 2013), which helps to quantify the relationship between measures of external exposure and internal dose. Detailed species-specific physiological, chemical, and biochemical parameters have been obtained. It allows not only extrapolating data to humans, but also assessing the possible sources of variability and uncertainty in model parameterization (Huizer et al., 2012). Due to their potential power, PBPK models have been largely used in pharmacological development and health risk assessment (Chiu et al., 2007; Clewell and Clewell III, 2008).

The objectives of the present study were the following: (1) to test an existing PBPK model for their predictability of PFOS and PFOA in a new case-study, and (2) to adapt the PBPK model to estimate the burdens in various human tissue compartments. The performance of this PBPK model, when using different partition coefficients (*Pk*), either from rats or humans, was studied in detail. In PBPK models, *Pk* is the main regulator parameter of the distribution of chemicals in the human body. Model validation was conducted using data on PFOS and PFOA levels in food and drinking water from Tarragona County (Tarragona, Spain). The model was calibrated and validated by using experimental data from autopsy tissues of subjects residing in the Tarragona County area.

2. Materials and methods

A new PBPK model for PFOA and PFOS was developed based on a previously reported model (Loccisano et al., 2011, 2013). The key process adopted in the model is the kinetics of resorption by renal transporters in the filtrate compartment, where chemicals are reabsorbed back to plasma through a saturable process (Andersen et al., 2006; Tan et al., 2008). This resorption mechanism could be responsible for the high persistence of PFOA and PFOS in human blood, compared to the low persistence found in other animal species (e.g., rat, monkey). In addition to plasma, gut, liver, fat, kidney, filtrate, and the remaining body compartments, the adapted PBPK model included lungs and brain. However, since it is not a potential site of absorption/accumulation for PFASs, skin was removed. The final compartmental structure of the adapted PBPK model is depicted in Fig. 1. The human tissues were selected due to their toxicokinetic relevance: plasma as a carrier tissue of PFASs in the human body, gut as an absorption site, kidney for its role in elimination (Barry et al., 2013), brain as target organ of PFASs neurotoxic effects (Mariussen, 2012), liver as an accumulative tissue for organic chemicals, lungs because they may exhibit immaturity after PFOS exposure (Grasty et al., 2005), and fat as a main site of accumulation in lipophilic tissues. The rest of the body was considered as an independent compartment to include PFASs remaining in other tissues of the human body. In plasma, more than 90% of PFOA and PFOS is bound to albumin, while <10% is free to move to other tissues (Han et al., 2003).

The PBPK model was based on a series of differential equations. The expression to estimate the levels of PFOA and PFOS in non-elimination tissues (fat, brain, lungs, and rest of the body) was the following:

$$\frac{dCi}{dt} = \frac{Qi \times \text{free} \times \left(Ca - Ci/Ki : p\right)}{Vi} \quad (1)$$

where *Ci* is the cellular concentration in each tissue (pg/mL), *Qi* is the blood flow (mL/h), free means the free amount of PFASs in plasma (unitless), *Ca* is the arterial concentration (pg/mL), *Ki*:*p* is the partition coefficient (unitless), and *Vi* is the tissue volume (mL).

The cellular concentrations of PFOA and PFOS in gut were obtained by applying this equation:

$$\frac{dCg}{dt} = \frac{\left(Qg \times \text{free} \times \left(Ca - Cg/Kg : p\right) + \text{intake}\right)}{Vg} \quad (2)$$

where *Cg* is the cellular concentration in gut (pg/mL), *Qg* is the blood flow to gut (mL/h), free means the free amount of PFASs in plasma (unitless), *Ca* is the arterial concentration (pg/mL), *Kg*:*p* is the gut partition coefficient (unitless), Intake is the oral daily intake (pg/h), and *Vg* is the gut volume (mL).

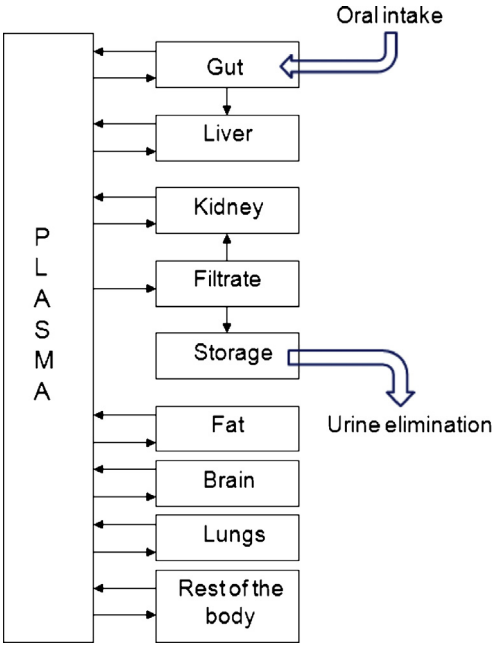

Fig. 1. Structure of the PBPK model for PFOA and PFOS.

With respect to the liver compartment, estimated values of PFASs were estimated by means of the following expression:

$$\frac{dCl}{dt} = \frac{\text{free} \times \left(Ql \times Ca + Qg \times Cg/Kg : p - (Ql + Qg) \times \left(Cl/Kl : p\right)\right)}{Vl} \quad (3)$$

where *Cl* is the cellular concentration in liver (pg/mL), free means the free amount of PFASs in plasma (unitless), *Ql* is the blood flow to liver (mL/h), *Ca* is the arterial concentration (pg/mL), *Qg* is the blood flow to gut (mL/h), *Cg* is the cellular concentration in gut (pg/mL), Kg:p is the gut partition coefficient (unitless), *Kl*:*p* is the liver partition coefficient (unitless), and *Vl* is the liver volume (mL).

For the kidney compartment the following equation was used:

$$\frac{dck}{dt} = \frac{\left(Qk \times \text{free} \times \left(Ca - Ck/Kk : p\right) + Tm \times Cfil/(Kt + Cfil)\right)}{Vk} \quad (4)$$

where *Ck* is the cellular concentration in kidney (pg/mL), *Qk* is the blood flow to kidney (mL/h), free is the free amount of PFASs in plasma (unitless), *Ca* is the arterial concentration (pg/mL), *Kk*:*p* is the gut partition coefficient (unitless), *Tm* is the resorption maximum (pg/h), *Cfil* is the cellular concentration in filtrate (pg/mL), *Kt* is the affinity constant (ng/mL), and *Vk* is the kidney volume (mL).

Finally, PFAS concentrations in the filtrate compartment were simulated by applying this equation:

$$\frac{dCfil}{dt} = \frac{Qfil \times (\text{free} \times Ca - Cfil) - Tm \times Cfil/(Kt + Cfil)}{Vfil} \quad (5)$$

where *Cfil* is the cellular concentration in filtrate (pg/mL), *Qfil* is the blood flow to filtrate (mL/h), free is the free amount of PFASs in plasma (unitless), *Ca* is the arterial concentration (pg/mL), Tm is the resorption maximum (pg/h), *Kt* is the affinity constant (ng/mL), and *Vfil* is the filtrate volume (mL).

Flow limited PBPK equations were used in all tissues (Thompson et al., 2012). Physiological data of volumes and cardiac output, which were time-constant, were obtained from Brown et al. (1997) (Table 1). Plasma concentrations of PFOA and PFOS were adjusted by calibrating the elimination values to fit experimental values from a case-study (Ericson et al., 2007). Thus, our PBPK model was applied to estimate the burdens of PFOS and PFOA for a population living in Tarragona County (Catalonia, NE of Spain), from which data on human intake and body burdens were available (Domingo et al., 2012a,b; Ericson et al., 2007; Pérez et al., 2013). Human exposure to PFOA and PFOS was evaluated through two different pathways: water consumption and food intake. Water ingestion was calculated as the product of the concentration in human drinking water (2.40 and 1.81 ng/L for PFOA and PFOS, respectively) in Catalonia (Domingo et al., 2012b) and the most typical value of water daily intake (1.23 L/day), according to the US EPA (2011). Similarly, dietary exposure was estimated based on the mean PFAS concentration in 40 food items, which are representative of the Catalan diet, and the respective daily consumption by the general population (Domingo et al., 2012a). In agreement with previous findings (Ericson Jogsten et al., 2012; Fromme et al., 2009; Kim et al., 2013), food intake was found to be the most important contributive route to the exposure of PFOS and PFOA, with percentages of 97% and 98% of the total intake, respectively. Dietary exposure of PFOA and PFOS was estimated in 1.55 and 1.80 ng/kg body weight/day, respectively. Overall, the total daily intake of PFOA through water and food consumption was estimated to be 0.11 μg/day for PFOA, while that of PFOS was found to be 0.13 μg/day.

For validation purposes, data on PFOA and PFOS in human tissues from people living in the area of study (Tarragona County) were used. Ericson et al. (2007) reported the levels of 13 PFASs, including PFOA and PFOS, in blood samples of 48 residents in that same area. The mean PFOS concentration in blood was 7.64 ng/mL, while PFOA mean level was 1.80 ng/mL. Recently, Pérez et al. (2013) analyzed the concentrations of 21 PFASs in 99 samples of autopsy tissues (brain, liver, lung, bone, and kidney) from subjects who had been living in Tarragona County (Catalonia, Spain). At the time of death, the mean age of subjects was 57 years, with minimum and maximum values of 28 and 86 years, respectively. A summary of the levels of PFOA and PFOS is shown in Fig. 2. Although PFASs have been largely monitored in human blood/plasma (Ehresman et al., 2007; Stahl et al., 2011), studies in other human tissues are certainly very limited, except some data on breast milk and liver (Kärrman et al., 2010; Lau et al., 2007; Yeung et al., 2013). To the best of our knowledge, to date only two studies have reported burdens of PFOS and PFOA in other human tissues. In Italy, Maestri et al. (2006) analyzed the concentrations of these compounds in samples of human liver, kidney, adipose tissue, brain, basal ganglia, hypophysis, thyroid, gonads, pancreas, lung, skeletal muscle, and blood.

In addition to the model validation, a particular study on the best *Pk* was conducted. Hence, the model was tested by using, as input data, *Pk* from studies conducted with either rats (Loccisano et al., 2011) or humans (Maestri et al., 2006). Data sets were compared to detect any improvement in the performance of both original and adapted PBPK models. In a flow limited equation, *Pk* is the main parameter governing the distribution of a chemical in the human body. However, this variable is usually obtained in animal experimentation studies without taking into account allometric scaling factors (Knaak et al., 1995), and assuming a steady state condition between tissue concentration and blood level. The final results by applying both data sets were compared with those previously observed in human tissues from people living in Tarragona County (Ericson et al., 2007; Pérez et al., 2013). The PBPK model was coded and simulated by using Berkeley Madona™ v8.3.18 (University of California at Berkeley, USA).

Table 1
Physiological parameters used in the PBPK model for PFOS and PFOA, and pharmacokinetic data.

	Volume (L)	Cardiac output (L/h)
Plasma	2.78	–
Fat	1.43	19.03
Brain	1.50	36.50
Lungs	1.00	10.61
Rest of the body	61.59	64.27
Gut	1.14	56.47
Liver	1.64	58.97
Kidney	0.29	55.22
Filtrate	0.03	10.92
Total	71.4	312
	PFOA	PFOS
Tmc	147.4	86.0
Kt	0.116	0.0176
Free	0.03	0.03

Data taken from Brown et al. (1997) and Loccisano et al. (2011).
Tmc: resorption maximum (μg/h).
Kt: affinity constant (μg/L).
Free: free fraction in plasma (unitless).
Conversion from weight to volume was assumed to be 1.

3. Results and discussion

3.1. Original PBPK model

In the first stage, the PBPK model developed by Loccisano et al. (2011) was used to predict PFOA and PFOS concentrations in human tissues for the current case-study. Parameterization data were taken from Loccisano et al. (2011), excepting those values regarding oral intake of food and drinking water, which were obtained from Domingo et al. (2012a). Simulation results were compared with experimental values regarding PFOA and PFOS in autopsy tissues from residents in the area of Tarragona (Table 2). Based on the model, the presence of PFOA and PFOS in plasma was mostly overestimated. The steady-state concentration of PFOA was estimated in 28.7 ng/g, while that of PFOS was 21.3 ng/g. Experimental values of PFOS and PFOA in plasma were reported to be 3.2 ± 1.2 and 13.6 ± 6.3 ng/g, respectively. Similarly, some disagreements were found for other human tissues when comparing modeled and monitored levels of both chemicals. In liver, the simulated PFOA concentration was 4.5-fold higher than the monitored mean value (13.6 ± 35.2 ng/g), while the predicted steady-state level of PFOS in liver was lower than empirical concentrations (79.6 vs. 102 ± 123 ng/g). Similar relationships were also detected in kidney, where simulated concentrations of PFOA and PFOS were 36.1 and 20.5 ng/g, respectively. By contrast, experimental mean levels of PFOA and PFOS in kid-

Table 2
PFOS and PFOA concentration (in ng/g) found in human tissues. Experimental vs. simulated data after applying the original PBPK model.

	Tissue	Experimental			Simulated
		Mean ± SD	Min–max	LOD	
PFOA	Plasma	3.2 ± 1.2	1.4–5.6	0.66	28.7
	Liver	13.6 ± 35.2	<3–98.9	3.0	63.1
	Fat	n.a	n.a	n.a.	1.15
	Kidney	2.0 ± 2.7	<3–11.9	3.0	36.1
PFOS	Plasma	13.6 ± 6.3	1.4–28.9	0.09	21.3
	Liver	102 ± 123	<3–405	3.0	79.6
	Fat	n.a	n.a	n.a.	3.01
	Kidney	75.6 ± 61.2	<6–269	6.0	20.5

LOD: limit of detection; SD: standard deviation; n.a.: not available.

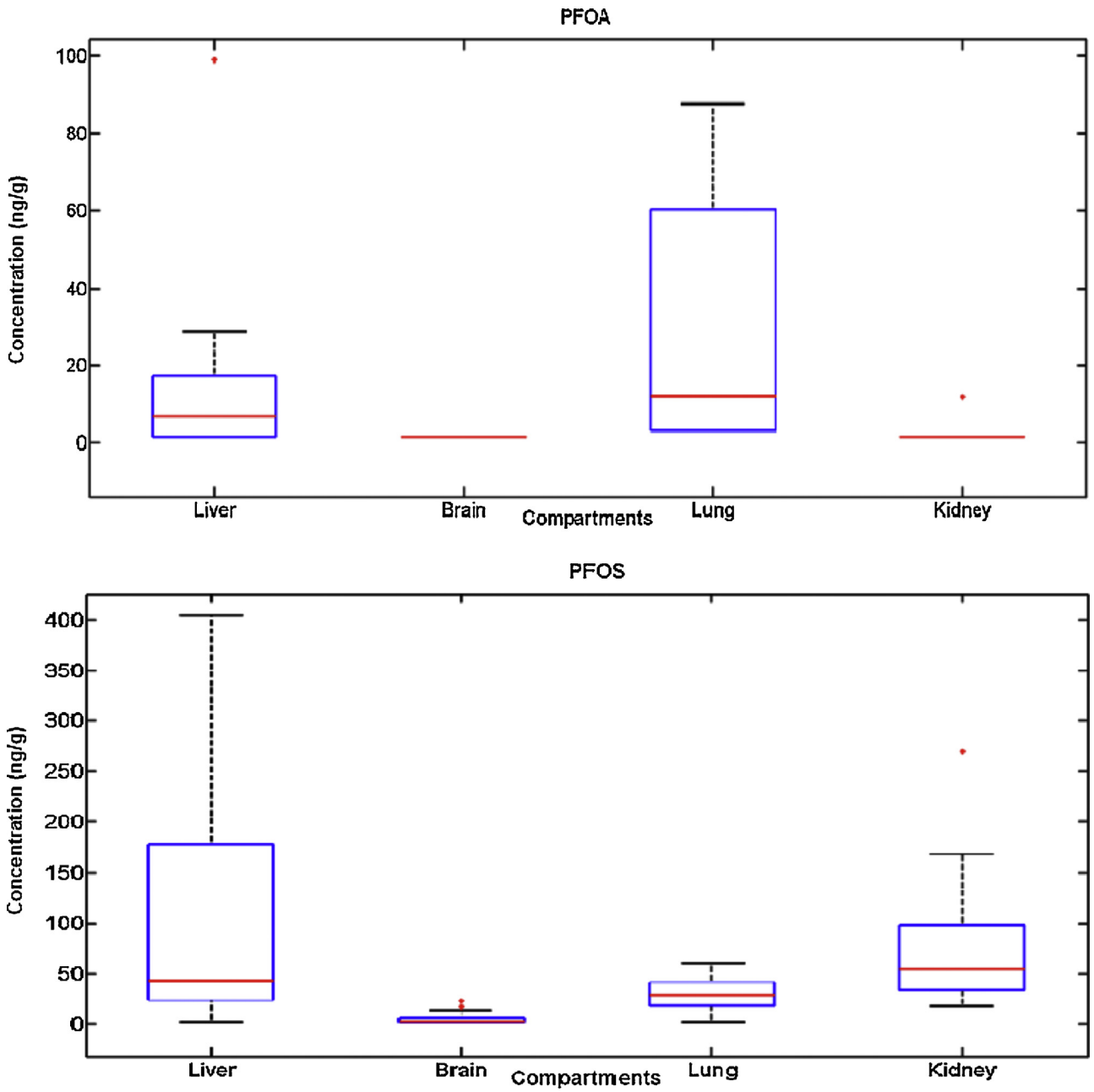

Fig. 2. Concentrations of PFOA and PFOS in autopsy tissues of 20 residents from Tarragona County (Catalonia, Spain). Data from Pérez et al. (2013) Simulated vs. measured concentrations of PFOA.

ney were 2.0 ± 2.7 and 75.6 ± 61.2 pg/g. Consequently, the ratio of estimated:observed kidney PFOA concentration was 18, while that of PFOS was 0.27. According to the model outcomes, PFOA and PFOS concentrations in adipose tissue were 1.2 and 3.0 ng/g, respectively. Unfortunately, since no experimental values are available for this specific compartment, a comparison could not be carried out in adipose tissue. In general terms, the model simulation somehow overestimated the concentration for PFOA and PFOS, with the exception of PFOS in liver and kidney. Simulations results of PFOA in plasma, liver and kidney were >4-fold higher than the mean experimental values. However, predicted and empirical concentrations were of the same order of magnitude. Considering that analytical results were highly uncertain and variable, the results of the PBPK model should be considered as reasonably good.

3.2. Adapted PBPK model

Because of the toxicological significance of PFOS and PFOA (Hu et al., 2012; Sato et al., 2009; Yahia et al., 2010), brain and lung were also included in the current model (Fig. 1). New *Pk* values

Table 3
Partition coefficients (*Pk*) used in the PBPK model.

	PFOA		PFOS	
	Human-based	Rat-based	Human-based	Rat-based
Liver	1.03	2.20	2.67	3.72
Fat	0.47	0.04	0.33	0.14
Brain	0.17	0.01	0.26	0.01
Lung	1.27	0.15	0.15	0.15
Kidney	1.17	1.05	1.26	0.80

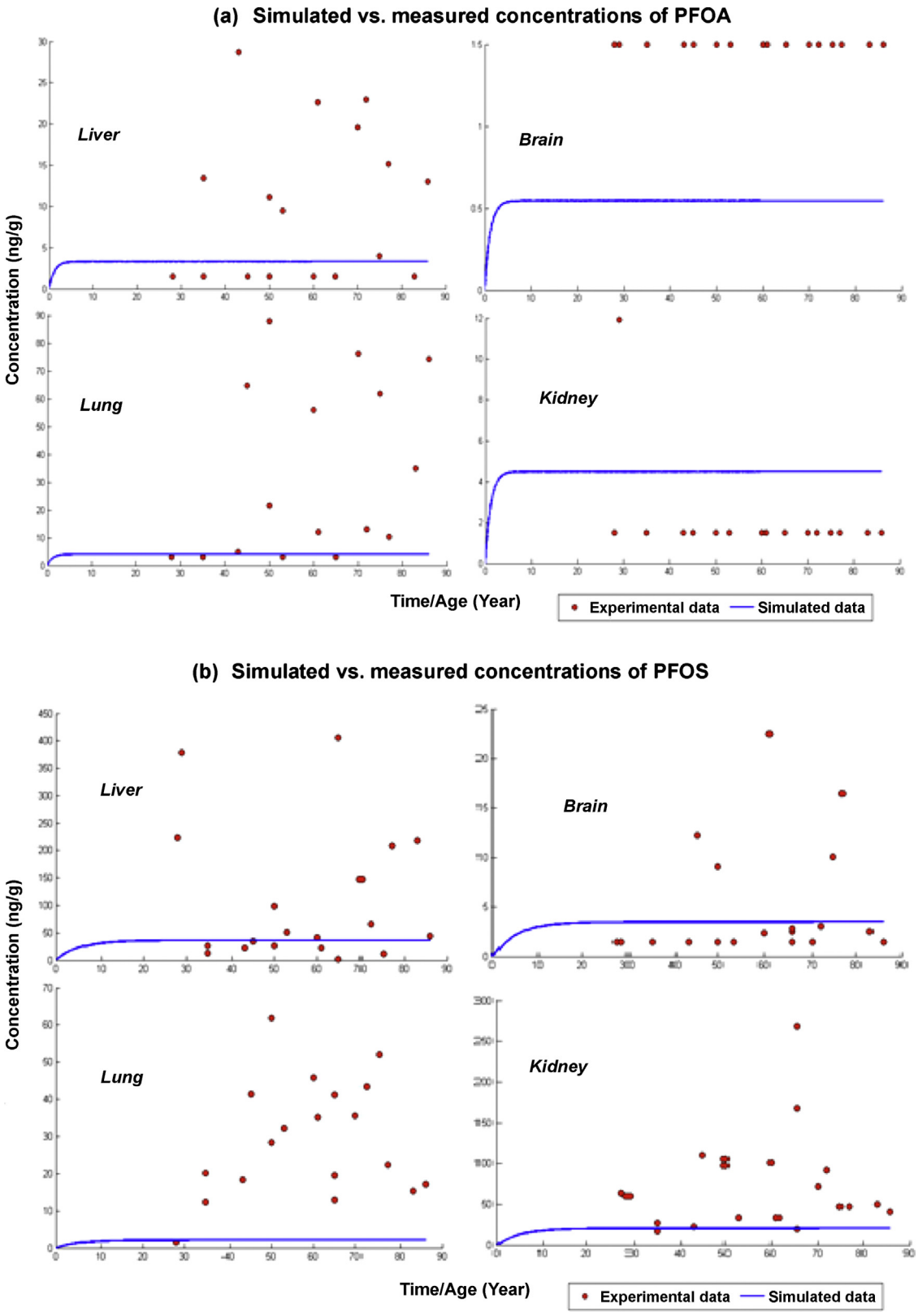

Fig. 3. Time course concentration of (a) PFOA and (b) PFOS in four human tissues from subjects who had been living in Tarragona County (Catalonia, Spain).

coming from human autopsy tissues (Maestri et al., 2006) were also applied and compared with *Pk* data from rats (Loccisano et al., 2011) (Table 3). As abovementioned, a constant oral intake of both PFOA and PFOS was considered. Descriptor parameters of the urinary elimination were calibrated by means of plasma concentrations (Ericson et al., 2007). Again, simulation results including the steady-state curve were compared with those recently reported in the same population group living in Tarragona County (Pérez et al., 2013). To understand the quality of experimental data, a statistical analysis was performed. Outliers (one for PFOA in liver and

Table 4
Steady-state concentration (in ng/g) of PFOA and PFOS. Experimental vs. simulated data after applying the adapted PBPK model, considering rat- and human-derived *Pk* values.

	Tissue	Experimental		Simulated	
		Mean ± SD	Min–max	Rat-based *Pk*	Human-based *Pk*
PFOA	Liver	13.6 ± 35.2	<3–98.9	7.03	3.33
	Brain	>1.5	<1.5	0.04	0.54
	Lung	29.2 ± 32.2	<6–87.9	0.47	4.06
	Kidney	2.0 ± 2.7	<3–11.9	4.02	4.50
PFOS	Liver	102.3 ± 122.9	<3–405	50.7	36.4
	Brain	4.9 ± 6.6	<1.5–22.5	0.16	3.48
	Lung	29.1 ± 16.8	<3–61.8	2.04	2.11
	Kidney	75.6 ± 61.2	<6–269	13.1	20.5

SD: standard deviation.

kidney, two for PFOS in brain, and one for PFOS in kidney) were eliminated from data treatment to avoid any potential distortion of the results. Predicted vs. observed concentrations of PFOA and PFOS, calculated by considering *Pk* values from human tissues are depicted in Fig. 3a and b, respectively. In the cases in which PFOA or PFOS levels were not detected in a sample, the concentration was assumed to be equal to the respective limit of detection (LOD). PFOA was not detected in brain, while it was only quantified in a single sample of kidney. Simulation results were clearly closer to empirical values when using *Pk* values from human autopsy tissues, rather than when applying *Pk* data coming from experimental animal studies (Table 4). In general terms, simulated results were found inside the uncertainty range of experimental values, with the exception of PFOS in lung. This fact support the hypothesis that PFOS and PFOA are resorbed from urine back to plasma in a saturable process, as it was parameterized in the model. Although *Pk* is a key parameter in PBPK modelling, *Pk* values are usually obtained from studies in rodents. Our results highlight the importance to obtain *Pk* data from humans in order to estimate more accurately the body burdens of PFASs in particular, and chemical contaminants in general. For example, simulated liver concentrations for PFOA in steady-state were 7.03 and 3.33 ng/g for rat- and human-derived *Pk* values, respectively, while the measured PFOA concentration was 13.6 ± 35.2 ng/g. Liver PFOS levels were estimated to be 50.7 and 36.4 ng/g, taking into account the rat- or human-derived Pk datum, respectively (observed value: 102 ± 122.9 ng/g). In brain, mean PFOS content ranged between 0.16 and 3.48 ng/g, depending on the use of *Pk* values from animal and human studies, respectively. According to the experimental investigation, brain contained PFOS in a concentration of 4.9 ± 6.6 ng/g. Simulation concentrations in lungs, using both *Pk* values from rats and humans, were notably lower than those experimentally obtained for PFOA (0.47 and 4.06 ng/g vs. 29.2 ± 32.2 ng/g) and PFOS (2.04 and 2.11 ng/g vs. 29.1 ± 16.8 ng/g). Important disagreements were also observed when comparing PFAS concentrations in kidney from the modeling and monitoring studies. Overall, the results from our adjusted model seemed to underestimate the real concentrations of PFOA and PFOS in the steady-state, contrasting with the original PBPK model (Loccisano et al., 2011). Notwithstanding, the good performance of the latter model was validated by comparing only measurements of PFOA and PFOS in human serum. In the current study, validation was conducted by considering other tissue compartments, which increases the complexity in the model calibration. Although the use of data coming from studies with rodents or other animals (i.e., monkeys), is a common practice in PBPK modeling, the improvement in our model predictions confirms the World Health Organization (WHO) general guideline stating that the application of human data is always more desirable (WHO/IPCS, 2010).

The results obtained in the present work are in agreement with previous results where a resorption mechanism also presented a reasonably good matching in the plasma concentrations of PFOS and PFOA (Loccisano et al., 2011). Nonetheless, the high half-lives of both compounds cannot lay only on this fact, as protein binding may be also a key process. In the present model, a high binding between PFAS and plasma proteins was taken into account, as suggested by Loccisano et al. (2011). However, the variation of the protein binding percentage can cause a variation in the plasma concentration. Therefore, further studies should demonstrate the weight of the protein binding and renal resorption in the long half-lives of some PFASs.

The significance of the model prediction when using two different partition coefficients was tested by performing a two-sample *t*-test. In both cases (PFOA and PFOS), the tests failed in rejecting the null hypothesis at the alpha significance level. It means that both data sets came from independent random samples of normal distributions, with equal means and equal, although unknown, variances. Further high *P*-values (0.92 and 0.95 for PFOA and PFOS, respectively) strongly validate the null hypothesis. To test the significance of the results, the correlation coefficient (*R*), which measures the linearity of relationship between observed and predicted values, was calculated. Regarding PFOA, values of $R = -0.12$ and $R = 0.44$ were found for rat- and human-derived *Kp* data, respectively. On the other hand, regarding PFOS, R values were 0.88 and 0.95 considering rat- and human-*Kp* data, respectively. No significant difference was observed in the distribution mean of predicted values, clearly indicating that a better prediction may be achieved by using human partition coefficients.

4. Conclusions

In the current study, a previously developed PBPK model was modified by considering the main target tissues of toxicological relevance for PFOA and PFOS. In agreement with the original model (Loccisano et al., 2011), trends in the simulation results indicate that the urinary PFAS resorption-based PBPK model seems to be a reliable approach to explain the relatively longer half-life of PFOA and PFOS in human plasma.

Although the model had been successfully validated by using experimental data in human blood, good validation results were not achieved for other human tissues. Anyhow, current knowledge on the levels of PFASs in human tissues, other than blood/plasma, is very limited. Uncertainty and variability of experimental data, together with that limited knowledge, means an additional difficulty to analyze those data. Despite only blood is commonly used for PBPK modeling validation, the comparability with other human compartments would ensure the reliability of the model with respect to target tissues (WHO/IPCS, 2010). The present results clearly indicate the need to acquire more information concerning body burdens of PFASs in general and those of PFOA and PFOS in particular. Biological monitoring of these POPs is necessary, as they provide fundamental support for the development of PBPK models

as well as other *in-silico* tools. The parameterization of the partition coefficient was also validated by comparing values derived from animal and human experimental studies. The use of human-derived *Pk* data was more suitable for application to this PBPK model than rat-based values. The model simulation assessed in the present study showed a huge uncertainty. Although results from PBPK modeling are usually uncertain (Barton et al., 2007), the characterization of variability and uncertainty in PBPK models is not a common practice. The uncertainty in PBPK models can be predicted by using Monte Carlo simulations, but also applying Bayesian inferences, or even fuzzy simulations (Gueorguieva et al., 2004). In order to assess the impact of the parameterization uncertainty in the model, a sensitivity analysis was not performed. The reduction of uncertainty can be done by using more precise measurements of the parameterization data (Huizer et al., 2012), while the variability cannot be reduced, but only described. Therefore, the separate characterization of variability and uncertainty should be considered to address more appropriately this issue (Kumar et al., 2009). Finally, further research must be carried out to improve the performance of the PBPK model by introducing temporal dynamics of exposure concentration and physiological parameters for the long-term exposure to PFASs. Being the most sensitive known target organ for PFOA toxicity in animals (Post et al., 2012), the inclusion of the mammary gland within the PBPK model should be also considered in future studies. On the other hand, as humans are really exposed to multiple chemicals, future studies should be focused on assessing the suitability of PBPK modelling for the evaluation of mixtures of PFASs, instead of individual compounds, assuming that different PFASs show a relatively comparable toxicological profile (Borg et al., 2013).

Transparency document

The authors declare that there are no conflicts of interest.

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