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Abstract

Prenatal exposures to perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been associated with child health outcomes, but many of these associations remain poorly characterized. The aim of this work was to provide new indicators of foetal exposure for the Spanish INMA birth cohort. First, a pregnancy and lactation physiologically based pharmacokinetic (PBPK) model was calibrated in a population framework to provide quantitative estimates for the PFOA and PFOS placental transfers in humans. The estimated distributions indicated that PFOA crosses the placental barrier at a rate three times higher than PFOS and shows a higher variability between mothers. The PBPK model was then used to back-calculate the time-varying daily intakes of the INMA mothers corrected for their individual history from a spot maternal concentration. We showed the importance of accounting for the mothers' history as different dietary intakes can result in similar measured concentrations at one time point. Finally, the foetal exposure was simulated in target organs over pregnancy using the PBPK model and the estimated maternal intakes. We showed that the pattern of PFOA and PFOS exposures varies greatly among the foetuses. About a third has levels of either one compound always higher than the levels of the other compound. The other two thirds showed different ranking of PFOA and PFOS in terms of concentrations in the target organs. Our simulated foetal exposures bring additional information to the measured maternal spot concentrations and can help to better characterize the prenatal exposure in target organs during windows of susceptibility.

Keywords: PBPK model; PFAS; reverse dosimetry; in utero; exposure assessment; pregnancy.

1 Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds extensively used in industrial and commercial products because of their specific chemical properties, including chemical stability, oil and water repellence, and fire resistance (Lindstrom *et al.*, 2011; Kotthoff *et al.*, 2015). Numerous biomonitoring studies have shown worldwide exposure of humans to PFAS, including in the general population and sensitive groups (e.g., Calafat *et al.*, 2007; Brantsaeter *et al.*, 2013; Manzano-Salgado *et al.*, 2016). Early life is of high interest when it comes to PFAS. A large number of epidemiological studies have investigated associations between prenatal and early childhood exposure to PFAS and adverse health outcomes. While there is stronger evidence for potential effects of PFAS on vaccine efficacy and dyslipidemia, findings are less conclusive for other health outcomes such as asthma, allergies, metabolic function, and cognitive and behavioural deficits (Rappazzo *et al.*, 2017; Agency for Toxic Substances and Disease Registry (ATSDR), 2018; Liew *et al.*, 2018; Meng *et al.*, 2018). Prenatal exposures to PFAS can be directly assessed by their presence in umbilical cord at delivery (Monroy *et al.*, 2008; Arbuckle *et al.*, 2013; Manzano-Salgado *et al.*, 2015), but maternal spot plasma or serum concentrations during pregnancy are most commonly used in large populations as surrogate for PFAS prenatal exposures (e.g. Granum *et al.*, 2013; Mora *et al.*, 2017; Timmermann *et al.*, 2017). Several studies showed that maternal plasma levels of some PFAS evolve over the course of pregnancy (Kato *et al.*, 2014; Pan *et al.*, 2017) indicating that the timing of the sampling can impact the estimation of foetal exposure. A complementary approach for foetal exposure assessment is to interpret the direct spot maternal measurements by accounting for the factors that cause variations in maternal plasma concentration during pregnancy. For instance, one can cite: the modifications of the mother's physiology due to pregnancy, the transfers of chemicals to the foetus, and the variations in PFAS intake by the mother.

The impact of pregnancy on the maternal plasma levels can be handled by physiologically based pharmacokinetic (PBPK) models that simulate the fate of compounds in the body by accounting for their absorption, distribution, metabolism and excretion, and for the anatomy and physiology of the individual (Reddy *et al.*, 2005; Bois and Brochot, 2016). Several PBPK models have been developed successfully to predict the internal dosimetry of PFAS in rodents (Loccisano *et al.*, 2012a; Loccisano *et al.*, 2012b; Worley and Fisher, 2015; Cheng and Ng, 2017), monkeys (Loccisano *et al.*, 2011), and humans (Loccisano *et al.*, 2011; Fabrega *et al.*, 2015; Worley *et al.*, 2017). Loccisano *et al.* (2013) also developed the first PBPK model for PFOA and PFOS in pregnant and breastfeeding women and observed that the placental transfer rate of the compound is a key parameter for foetal exposure in PBPK modelling. In their model, Loccisano *et al.* (2013) estimated empirically the human placental transfers based on animal data and did not account for inter-individual variability. Recently, several biomonitoring studies reported human paired maternal and foetal concentrations that could be used to better characterize the human transfers to PFAS and highlighted the high variability of placental transfers (computed as the ratio of the foetal over maternal concentrations) between women (Zhang *et al.*, 2013; Cariou *et al.*, 2015; Chen *et al.*, 2017). Population PBPK modelling offers an adequate framework to statistically calibrate transfer rates from these data (Gelman *et al.*, 1996; Bois *et al.*, 2010), by accounting for uncertainties and variability between women and life histories. The resulting calibrated PBPK model will offer predictions of foetal exposure during the whole pregnancy that will be more representative of the population variability.

In the context of exposure assessment, PBPK models associated with a realistic exposure scenario can be used to back-calculate from an internal concentration (here, in plasma) an external exposure of an individual, corrected for his/her personal characteristics (Clewell *et al.*, 2008; Ulaszewska *et al.*, 2012). The reconstructed maternal exposure can be compared to

reference values (as tolerable daily intakes) or used as input of a PBPK model to simulate indicators of internal dose at target tissues (where the toxic effect occurs) during critical windows of exposure (Clewell *et al.*, 2008; Ulaszewska *et al.*, 2012). Those internal indicators are rarely accessible to measurement in humans even though they may better predict health issues (Verner *et al.*, 2010; Sasso *et al.*, 2013). As PFAS half-lives in plasma are of the order of years (Olsen *et al.*, 2007), the maternal plasma concentration reflects the current and past exposure of the woman. A realistic scenario for pregnant women should then encompass the variations in intake and the individual factors that impacted the internal exposure over few years before the concentration's measurement. Indeed, several studies showed that PFAS maternal concentrations is associated with many traits of the mother such as parity (Brantsaeter *et al.*, 2013; Manzano-Salgado *et al.*, 2016), breastfeeding (Fei *et al.*, 2010; Sagiv *et al.*, 2015), maternal age (Sagiv *et al.*, 2015; Lauritzen *et al.*, 2016; Manzano-Salgado *et al.*, 2016), and maternal bodymass index (Lauritzen *et al.*, 2016). In case of multiparous mothers, interpreting their concentration with a PBPK model then requires accounting for mother's history in terms of previous pregnancies and breastfeeding, and therefore having a PBPK adequate for both pregnancy and breastfeeding.

In this work, we aimed at estimating the non-occupational exposure of pregnant women and their foetuses of the Spanish INMA birth cohort to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) using spot maternal concentrations collected during pregnancy and at providing new calculated indicators of foetal exposure in target organs. First, we improved the estimation of the human placental transfers for PBPK models using a subset of INMA mother-child pairs for which concentrations were measured in mothers during pregnancy and in cord blood at delivery and evaluated the model predictions using biomonitoring data. Then, we defined for each INMA mother an individualized exposure scenario based on her personal history and used it together with the PBPK model to back-

calculate her daily intakes. These estimates were then used as an input to the PBPK model to simulate the prenatal exposure of children in several target organs over the whole pregnancy. Figure 1 presents the workflow with the different steps.

2 Materials and methods

2.1 Study population

The Environment and Childhood Project (INMA, www.proyectoinma.org) is a network of prospective population-based birth cohorts in Spain aiming to understand the associations of pre- and postnatal environmental exposures and child health (Guxens *et al.*, 2012). From 2003 to 2006, women from the Spanish Sabadell region were recruited in their first trimester of pregnancy and were followed-up until delivery. A written informed consent was obtained from the participants prior to inclusion. Details on the recruitment and follow-up have been described elsewhere (Guxens *et al.*, 2012). All participants were healthy at enrolment. The regional hospital ethics committees approved this study.

Among the cohort, we selected a first subsample of 52 mother–child pairs that had available samples of maternal blood and cord serum for estimating the placental transfers of PFOS and PFOA during pregnancy (Group 1). A second subsample of 355 mother–child pairs with available samples of maternal blood during pregnancy was selected to characterize the maternal and prenatal exposure (Group 2). For both groups, maternal blood samples were drawn during the first or the second trimester of pregnancy. Cord blood was drawn at delivery. Detailed information on mothers and newborns was obtained from questionnaires and medical records. Table 1 presents a summary of those data for the two groups.

2.2 Analytics

The PFOA and PFOS plasma concentrations were determined as previously described (Manzano-Salgado *et al.*, 2015). Briefly, plasma concentrations of PFOA and PFOS were determined by column-switching liquid chromatography (Agilent 1100 Series HPLC apparatus) coupled with tandem mass spectrometry (Sciex API 3000 LC/MS/MS system in ESI negative mode) according to a modified protocol described by Kato et al. (2011). After addition of labelled internal standards (13C8-PFOA and 13C4-PFOS) to 250 μL of plasma,

the proteins are precipitated using 500 µL of 2 mM ammonium acetate (pH 4) in acetonitrile. After centrifugation of the sample, 300 µL of the supernatant are mixed with 700 µL of 2 mM ammonium acetate (pH 4) in water. 100 µL of this solution are injected into the column-switching LC/MS/MS-system. First, the analytes are enriched on a Lichrospher® RP-8 ADS-phase (25 µM, 24 mm x 4mm) and transferred after 2 min to the analytical phase (Phenomenex Luna C 8(2), 150 mm x 4.6 mm, 3 µM particle size) at a flow rate of 0.3 ml/min. The limit of quantification (LOQ) was 0.20 ng/mL for both PFOA and PFOS. Calibration was carried out using matrix-matched calibration standards in bovine serum. The between day imprecision during the study ranged from 5.9 % - 11.1 % for PFOA and 7.1 % - 9.9 % for PFOS using spiked quality control samples as well as a native human sample, respectively. The laboratory regularly participates successfully in round robins for the determination of PFOA and PFOS in plasma in the environmental concentration range (www.g-equas.de).

PFOA and PFOS concentrations measured in maternal plasma and cord serum for the two groups are reported in Table 1 and in Table S1 and S2 (Supplementary Data) according to maternal characteristics. In Group 1, both compounds were detected and quantified in the 52 mother-child pairs. We calculated the individual plasma:serum ratios that were on average close to 1 with a high variability as a 10-factor was observed between the minimum and maximum values (Supplementary Data, Table S1). No correlations were found between the maternal concentrations and the plasma:serum ratios for PFOA and PFOS. The concentrations in serum cord were converted to plasma cord concentrations using the individual plasma:serum ratio of each woman. For the 355 mothers of Group 2, all the samples were above the LOQ for PFOS, and nine PFOA samples were below (Table 1 and Table S2 in Supplementary Data).

2.3 The PBPK Model for PFOA and PFOS

A generic PBPK model (Supplementary data, Figure S1) was used to describe the toxicokinetics of PFOA and PFOS during the lifetime of the women, including pregnancy and breastfeeding (Beaudouin *et al.*, 2010). This model has already been applied to persistent compounds such as polychlorinated biphenyls, to estimate the human exposure over long time periods (Ulaszewska *et al.*, 2012; Radomyski *et al.*, 2016). The foetus model of Beaudouin *et al.* (2010) was modified to make the compartmentalization of the foetal PBPK model similar to the maternal PBPK model. New equations were derived for the relative organ weights using data reported by the International Commission on Radiological Protection (2002) (Supplementary Data). The chemical transfers between the mother and the foetus (represented by the parameter $K_{Uter2Pla}$) take place between the sexual organs (uterus) and the placenta, are scaled to the placental volume (V_{Pla}), and reach a maximum rate at the end of placenta growth:

$$K_{Uter2Pla} = K_{Uter2Pla\_max} \times \frac{V_{Pla}(t)}{V_{Pla\_max}} \quad (1)$$

with $K_{Uter2Pla\_max}$ the transfer rate and V_{Pla_max} the placenta volume at the end of the placenta growth.

The physiological parameters of the PBPK model, *e.g.* organ volumes and perfusion, were implemented as proposed by Beaudouin *et al.* (2010). The PBPK model was parameterized for PFOA and PFOS based on the study performed by Loccisano *et al.* (2013). The absorption was assumed to occur via ingestion of contaminated food or drinking water. A direct input in the liver was modelled with a complete absorption of both PFOA and PFOS.

PFOA and PFOS are distributed in the compartments by plasma flow. Cardiac output was then corrected with the haematocrit. Since the haematocrit was not integrated in the initial model, a relationship was derived to model its time evolution (Supplementary Data). Because these compounds are highly bound to plasma proteins, only the free fraction in plasma can

distribute in the compartments. The values of the free fraction in plasma were obtained from Loccisano *et al.* (2013) that used values fitted to monkey data. The extent of distribution in a compartment is driven by the tissue:plasma partition coefficients that were calculated using measured concentrations of PFOA and PFOS in human tissues and blood (Maestri *et al.*, 2006; Zhang *et al.*, 2013). Tissue:plasma partition coefficients were obtained by scaling the tissue:blood partition coefficients with a plasma to blood ratio equal to 2.0 for PFOA and 2.3 for PFOS (Ehresman *et al.*, 2007). Partition coefficients were missing for some tissues. In that case, kidney and muscle were used as surrogate for the rapidly and slowly perfused tissues, respectively. Partition coefficients in the foetus were assumed to be equal to maternal ones. The milk:plasma partition coefficients were determined with the study by Liu *et al.* (2011) that measured simultaneously the concentrations in both media. They were set to 0.11 for PFOA and 0.02 for PFOS. Other studies observed similar ratios between maternal plasma (or serum) and breast milk concentrations (Karrman *et al.*, 2007; Kim *et al.*, 2011).

PFOA and PFOS are eliminated by urinary excretion and breastfeeding. The urinary elimination was modelled in plasma and parameterized with half-lives derived using serum data in workers. The studies of Olsen *et al.* (2007) and the re-analyses of these data by Russell *et al.* (2015) were used. Excretion through breast milk was modelled as the milk production (function of the postpartum age) times the concentration in breastmilk (Beaudouin *et al.*, 2010). An additional route of excretion was integrated to account for blood loss during delivery. Stafford *et al.* (2008) calculated that loss using the haematocrit values measured pre- and post-delivery and obtained a median estimate of 632 mL for 677 women. The extent of the loss differed between the types of delivery and can reach about 1 L for caesarean delivery. In our model, a blood loss of 500 mL was assumed, that corresponds to about 10% of the maternal blood volume at the end of pregnancy.

All values of the PFOA- and PFOS -specific PBPK model parameters are reported in Table 2 and the PBPK model code is given in Supplementary Data.

2.4 A dynamic exposure model for PFOA and PFOS daily intakes

Emission and production data show that human exposure to PFOA and PFOS has changed since about the year 2000, notably due to their phase out by their major producer (Prevedouros *et al.*, 2006; Paul *et al.*, 2009). The studies of Paul *et al.* (2009) and Wang *et al.* (2014) showed a fast decrease in PFOS and PFOA production around the year 2000 followed by a slower decrease. This is in concordance with the temporal variations of the PFOA and PFOS levels in humans measured in several European studies (Haug *et al.*, 2009; Sundstrom *et al.*, 2011; Glynn *et al.*, 2012; Gebbink *et al.*, 2015). A dynamic exposure model for the maternal daily intakes of both compounds was built to reproduce these variations: a constant exposure (per kg of bodyweight) from birth until the year 2000, then a 20% decrease for PFOA (Wang *et al.*, 2014) and 66% decrease for PFOS (Paul *et al.*, 2009) in the year 2000, and finally a further 4% decrease for PFOA and 7% decrease for PFOS in each year after 2000 (Glynn *et al.*, 2012).

2.5 Women's personal history

Several individual determinants of PFOA and PFOS exposure were taken into account to build the women's history and to individualize the PBPK model. Those determinants include the birth year, the age of the mother, the bodyweight before pregnancy, the weight gain during pregnancy, and the bodyweight and birth date of the newborn. For multiparous women, the parity and breastfeeding history was built using the following information: the number of children, the sibling position of the newborn, the dates of the previous pregnancies, the duration of the pregnancies, and the duration of all the exclusive and mixed breastfeeding periods.

2.6 Estimation of the placental transfer rates of the PBPK model

The dataset of the mother-child pairs (Group 1) was analysed prior to the mothers' dataset (Group 2) to provide estimates for the placental transfer rates of PFOA and PFOS. Two model parameters were unknown: the maximum placental transfer rate ($K_{Uter2Pla\_max}$) of the PBPK model and the constant daily intakes (before year 2000) of the exposure model. These parameters were estimated for each mother-newborn pair. The model calibration was performed in a Bayesian population framework. For the placental transfer rates, a log-normal distribution was used to model inter-individual variability. The population mean and individual $K_{Uter2Pla\_max}$, and the population standard deviation were estimated. Non-informative uniform prior distributions were assigned to the population parameters of $K_{Uter2Pla\_max}$ (between 1×10^{-8} and 0.1 L/min for the mean, and 0 and 500% for the coefficient of variation) and to the individual daily intakes (between 0.001 and 15 ng/kg/day). Markov Chain Monte Carlo (MCMC) sampling was used to numerically obtain a sample of parameter values from their joint posterior distribution. Three independent MCMC chains were run for 5,000 iterations. The first 3,000 iterations were not considered as convergence was not reached, and then one in two of the last 2,000 iterations was recorded and used to produce the results (yielding to 1,000 parameter vectors per MCMC chain). The $\hat{R}$ criterion was used to check convergence to a stable posterior distribution (Gelman *et al.*, 1995). At perfect convergence, all the $\hat{R}$ should be equal to 1. Convergence was very likely and was assumed to be achieved when they were all below 1.2.

Correlations of the estimated placental transfer rates with individual characteristics (bodyweight of the newborn, and weight, height, age, and weight gain during pregnancy of the mother) were computed. Moreover, one-way ANOVA was used to test the independence of estimated placental transfer rates to the parity of women.

2.7 Model evaluation using biomonitoring data

The dynamic exposure model for daily intakes and the PBPK model were evaluated using biomonitoring data. First, we tested if the models were able to reproduce the temporal trends of PFOA and PFOS plasma concentrations observed between 1995 and 2015 in the general population of European countries, Sweden and Norway (Haug *et al.*, 2009; Gebbink *et al.*, 2015). For each year between 1995 and 2015, we predicted the PFOA and PFOS concentrations of a non-pregnant 35-year-old woman. A deterministic simulation was performed with the PBPK model parameters set to their average values (Table 2). To harmonize the different datasets, we considered the evolution of the relative compounds levels, *i.e.* the concentrations were normalized by the year 2000 concentrations.

The models were also evaluated for predicting the evolution of the maternal concentrations over the course of a pregnancy, between two pregnancies and during breastfeeding. In all simulations described below, the maternal exposure started several years before the pregnancy in order to reach a pseudo steady-state concentration at the start of pregnancy. Several studies that measured the maternal concentrations of PFOA and PFOS in the same mothers at different times of their pregnancy were used for the evaluation during a pregnancy (Monroy *et al.*, 2008; Fromme *et al.*, 2010; Glynn *et al.*, 2012; Kato *et al.*, 2014; Fisher *et al.*, 2016; Pan *et al.*, 2017). A deterministic simulation of the PBPK model combined with the model for daily intakes was run. As the plasma concentrations differ between the studies, the comparison was based on the evolution of the concentration normalized by the concentration at the start of pregnancy.

The study of Papadopoulou *et al.* (2015) was used to evaluate our model predictions of the PFOA and PFOS plasma concentrations for women that had two consecutive pregnancies. In their study, one blood sample was taken during the second trimester (weeks 17-18) of each pregnancy. About half of the women (49%) did not breastfeed the newborn, whereas the other half (51%) did for at least six months. The characteristics of this population were used

as input of our PBPK model to simulate the plasma concentration of PFOS and PFOA. Three types of simulation were performed: no breastfeeding between the two pregnancies, 6 months and 10 months of breastfeeding. The exposure dose was estimated in order to fit the first measured concentration.

Finally, we evaluated our model for its capabilities to describe the toxicokinetics in breast milk. Several studies reported that the duration of breastfeeding lowers the PFOA and PFOS concentrations in maternal plasma (Brantsaeter *et al.*, 2013; Mondal *et al.*, 2014) and in breast milk (Thomsen *et al.*, 2010; Kang *et al.*, 2016). The study of Thomsen *et al.* (2010) was selected for a comparison with our model predictions. The population characteristics (*i.e.,* 2006 as the starting date of pregnancy, and 29 years old as the age of the mothers at the beginning of pregnancy) and the dynamic exposure model as defined above were used to run the PBPK model. The initial dose of the exposure model was set to correspond to realistic serum levels observed in European populations. Monte Carlo simulations were performed to model inter-individual variability of the PFOA and PFOS transfer to milk, *i.e.* the milk:plasma partition coefficient was assumed to follow a normal distribution with the mean value reported in Table 2 (PFOA: 0.11, PFOS: 0.02) and a coefficient of variation of 50%. The normal distribution was truncated with the lower bound to 0.0001 and the upper bound to 10.

2.8 Estimation of the daily intakes and the internal exposure of the INMA cohort

Once the models were calibrated and evaluated, they were used to estimate the external and internal exposure of the mother-foetus pairs of the INMA cohort. First, the daily intakes were estimated individually for each of the 355 mothers of Group 2. Reverse dosimetry analyses were performed in a Bayesian framework and a uniform prior distribution was used for the daily intakes. MCMC sampling was used to obtain a sample of parameter values for each woman. Three independent MCMC chains were run for 3,000 iterations. One in two of the

last 2,000 iterations was recorded and used to produce the results (yielding to 1,000 parameter vectors per MCMC chain). The $\hat{R}$ criterion was used to check convergence (Gelman *et al.*, 1995). One-way ANOVAs were performed to assess the independence of the estimated daily intakes with respect to individual characteristics of the mother, *i.e.* parity, age and pre-pregnancy body mass index (BMI). Three classes were defined for parity (1, 2 and 3 children), three for the age of the mother (≤30, 31-35, >35), and two for the pre-pregnancy BMI (≤18.5 and >18.5). For the multiparous women, additional tests were run to test the influence of the time since last pregnancy (less or more than 4 years), and the time since last breastfeeding (less or more than 3.5 years). The bounds of the groups were chosen to have a similar number of women in each group.

The daily intake estimates were then used as input of the PBPK model to simulate the foetal exposure in several target organs over the whole pregnancy. Monte Carlo simulations were run to simulate inter-individual variability of the placental transfer rates. The maximal transfer rates were taken from the posterior distribution. Several variables were predicted: the foetal concentration in plasma at different times of the pregnancy, the area under the curve in several organs and the total body burden at birth.

2.9 Software

The GNU MCSim software (www.gnu.org/software/mcsim/) was used for all simulations with the PBPK model, *i.e.* MCMC simulations to calibrate the model in the Bayesian framework and to estimate the women's exposure, and Monte Carlo simulations to predict the internal exposure of the foetus. R CRAN (https://cran.r-project.org/) was used to perform statistical analyses.

3 Results

3.1 Evaluation of the dynamic exposure model for daily intakes

The adequateness of the dynamic exposure model together with the PBPK model to reproduce the temporal trends of PFOA and PFOS exposure was evaluated against serum levels measured in the general population (Haug *et al.*, 2009; Gebbink *et al.*, 2015). Figure 2 shows the observed and simulated temporal trends for PFOA and PFOS. To facilitate the comparison between the model predictions and the biomonitoring measurements, the ratio between serum levels at a given year and those observed at year 2000 was calculated. Our exposure model captures well the decrease in serum levels reported in European biomonitoring studies between 1995 and 2010.

3.2 Estimation of placental transfer rates of the PBPK model

The maximum transfer rate (*i.e.* at the end of placenta's growth, $K_{Uter2Pla\_max}$ in Equation 1) was estimated using the dataset of maternal and cord plasma sampling (Group 1). The measured concentrations of both compounds were well predicted (results presented in Supplementary data, Figure S2). The median ratio between the predicted and observed concentrations and the coefficient of determination, R^2, were close to 1 for maternal and cord plasma. Figure 3 presents the histograms of the estimated maximum transfer rates for PFOA and PFOS. The average population rate was estimated at $1.9\times10^{-5}\pm0.2\times10^{-5}$ L/min for PFOA and at $6.2\times10^{-6}\pm0.4\times10^{-6}$ L/min for PFOS, indicating that PFOA crosses the placental barrier at a rate three times higher than PFOS. A high inter-variability was observed between individual transfer rate estimates. The coefficients of variation of the maximal transfer rate across subjects were estimated at 75% for PFOA and 55% for PFOS. Factors of 8 and 5 were obtained between the lowest and highest values for PFOA and PFOS respectively. Figure 3 presents also the log-normal population distribution defined with the estimated population mean and standard deviation. The adequateness of this distribution to describe the inter-

individual variability of the placental transfers was assessed by testing the distribution of the estimated individual rates with the *fitdistrplus* package in *R*. The best adjustment was obtained for a log-normal distribution whose parameters were close to the estimated values (mean = 2.2×10^{-5} and CV=54% for PFOA and mean=6.8×10^{-6} and CV=41% for PFOS).

The individual placental transfer rates were highly correlated with the cord serum/maternal serum (CS/MS) ratio ($R^2 > 0.97$ for both compounds), but the correlations were weak with the measured concentrations in mothers (R^2=0.04 for PFOA, 0.10 for PFOS) and in umbilical cord (R^2=0.15 for PFOA, 0.17 for PFOS). Correlations of the placental transfer rates with recorded individual characteristics (bodyweight of the newborn, and weight, height, age, and weight gain during pregnancy of the mother) were null or weak ($R^2<0.1$). A one-way ANOVA showed that the placental transfer rates were not significantly different between primipara and non-primipara women (F(1,50)=0.18 and p-value=0.68 for PFOA, F(1,50)= 0.25 and p-value=0.62 for PFOS).

3.3 Evaluation of the models during pregnancy and breastfeeding

Ratio of cord serum over maternal serum

The measured mean CS/MS ratio was 0.79±0.29 for PFOA and 0.33±0.13 for PFOS. They were model-estimated to be 0.76±0.23 and 0.32±0.11, respectively. Figure 4 presents CS/MS ratios that were observed in several studies with the maternal samples collected at different times of the pregnancy (Inoue *et al.*, 2004; Fei *et al.*, 2007; Midasch *et al.*, 2007; Monroy *et al.*, 2008; Fromme *et al.*, 2010; Beesoon *et al.*, 2011; Gutzkow *et al.*, 2012; Lee *et al.*, 2013; Porpora *et al.*, 2013; Zhang *et al.*, 2013; Kato *et al.*, 2014; Cariou *et al.*, 2015; Yang *et al.*, 2016; Chen *et al.*, 2017; Pan *et al.*, 2017; Zhao *et al.*, 2017). The average INMA cohort PFOA ratio is a bit high compared to ratios reported at a similar period of the pregnancy but is similar to ratios observed later in pregnancy. For PFOS, the INMA cohort ratios fall in the range of the other studies. The evolution of the CS/MS is also represented on Figure 4. Monte

Carlo simulations were run with the estimated population distribution for the placental transfer rates. For both PFAS, the ratio increases with the age of pregnancy and a 30% increase is observed between the lowest and highest ratios. The confidence intervals of the predictions encompass the observed ratios, and the variability observed in our study was similar to the ones reported in most of the studies.

Maternal PFOA and PFOS *toxicokinetics in plasma during pregnancy*

The impact of pregnancy on PFOA and PFOS toxicokinetics was assessed by running simulations with the mean values for each parameter (Figure 5). Three phases are observed on the plasma concentration curve during a pregnancy: first a decrease that accelerates with the age of pregnancy, then a rapid increase after delivery due to the decrease of the volumes of some organs and fluids (plasma, uterus and mammary tissues), and finally a slow increase that reflects the diminution of the adipose tissues volume over 6 months after delivery. At the end of pregnancy, the plasma concentration is reduced by 28% for PFOA and 25% for PFOS, compared to the concentration at the start of the pregnancy. This decrease mainly reflects the dilution of the body burden due to the increase of the bodyweight and therefore of the volume of distribution. After the period of the physiological changes due to pregnancy (*i.e.*, about 15 months after the beginning of the pregnancy), the plasma concentration is reduced by 11% and 8% for PFOA and PFOS, respectively, compared to the plasma concentration of a non-pregnant woman at the same period. This decrease encompasses the blood loss at delivery (2% for both compounds) and the transfer to the foetus. We also compared our model predictions to the data on the evolution of maternal plasma concentration over the course of a pregnancy (Table 3). Overall, our predictions are in good agreement with the experimental studies for the different periods of pregnancy. The decline is more pronounced during the first two trimesters than later in the pregnancy.

The study of Papadopoulou *et al.* (2015) was used to evaluate our model predictions of the PFOA and PFOS plasma concentrations for women that had two consecutive pregnancies (Figure 5). Our predictions encompassed the data for PFOA, but the decrease for PFOS is a bit under-predicted compared to the data. Nevertheless, the predictions represent well the general tendency regarding the uncertainties due to the use of aggregated population and exposure data.

Maternal toxicokinetics in plasma during breastfeeding

Figure 6 shows our model's predictions for the concentration in plasma and breast milk (as the percentage of the milk concentration at the start of breastfeeding) during breastfeeding together with the data of Thomsen *et al.* (2010). In our model, the concentration in blood and in milk are linearly correlated, and the milk:plasma partition coefficient is their proportional factor. On average, the monthly decrease is about 8% and 10% for PFOA, and 3% and 4% for PFOS in blood and milk respectively. This yields to a decrease over a year of 65% and 28% in blood, and of 73% and 33% in milk for PFOA and PFOS respectively. Regarding the data collected in other studies (Brantsaeter *et al.*, 2013; Mondal *et al.*, 2014), the decrease in blood predicted by our model seems to be a bit high for PFOA and of similar ranges for PFOS. For instance, Mondal *et al.* (2014) reported that the maternal serum concentrations of PFOA and PFOS were decreased by 3% each month of breastfeeding. Brantsaeter *et al.* (2013) observed similar trends for PFOA and about 1% of decrease for PFOS. In milk, the predicted decrease over a year is similar to the one reported by Thomsen *et al.* (2010) for PFOS (33% vs. 37%), but a bit lower for PFOA (73% vs. 94%).

3.4 Prediction of the daily intakes of the INMA women

The PFOA and PFOS daily intakes of the INMA mothers before year 2000 were estimated to be 0.79±0.76 ng/kg/day (IC95% [0.07; 1.81]) for PFOA and 1.16±0.70 (IC95% [0.10; 2.77]) for PFOS. In 2004, year of the birth of the first children of the cohort, the daily intakes

decreased down to 0.54±0.52 ng/kg/day (IC95% [0.05; 1.23]) for PFOA and 0.29±0.18 (IC95% [0.03; 0.71]) for PFOS. A high inter-individual variability was observed with a 200-fold factor between the lowest and the highest estimated daily intakes for PFOA, and a 80-fold factor for PFOS. For comparison, the factors between the lowest and highest measured maternal concentrations were 158 for PFOA and 100 for PFOS (Table 1). We assessed the importance of accounting for breastfeeding for multiparous women in reconstructing their exposure by comparing the estimated plasma PFOS concentrations for two women: each gave birth to two children, their second child is part of the INMA cohort, the women were approximately of the same age at the time of sampling, and their measured PFOS concentrations were similar (year 2006). The only difference between these two women is that one of them breastfed her first child for 8 months and the other did not. The impact of the pregnancies and breastfeeding is visible on the plasma concentrations (Supplementary Data, Figure S3). We observed that after the first pregnancy and breastfeeding, both women exhibit similar estimated plasma levels. Using our approach, the daily intake of the breastfeeding woman was estimated to be 17% higher than the daily intake of the non-breastfeeding woman. If breastfeeding after the first pregnancy was not considered in the individualized scenario, the daily intakes of these two women would have been estimated at similar levels and that would lead to underestimate the exposure of the woman that breastfed for her previous child.

Statistical tests were performed to assess the independence of the estimated daily intakes with respect to some individual characteristics (detailed results in Supplementary data, Table S3). For the maternal PFOA concentrations, a statistically significant difference between the women according to their parity was found ($F(2,343)=8.9$, $p = 1.7E\text{-}4$). A Tukey post-hoc test revealed that parity was associated with a low maternal concentration, and that there were significant differences between the primipara and non-primipara women ($p<0.05$).

The age of the mother was also found to be a factor influencing PFOA maternal concentration. A Tukey post-hoc test revealed that the groups of youngest (< 30) and oldest (>35) women were statistically different (p=0.03). For PFOS, the maternal concentrations were independent of all the tested individual characteristics. This difference between both PFAS could be explained by a longer residence of PFOS in the body, as its serum half-life is higher, and the transfers to the foetus or during breastfeeding are lower than for PFOA. Dissimilarities in their exposure patterns could also explained these results. For both PFAS, the daily intake estimates were independent of any of the individual characteristics.

For the multiparous women, additional tests were run to test the influence of the time since last pregnancy and last breastfeeding. The detailed results are reported in Supplementary data (Table S4). One-way ANOVA revealed statistical differences between the groups for both variables on the maternal concentrations, but not on the daily intake estimates. For PFOS, it should be noted that the p-values for this latter were just above the 5% threshold.

3.5 Prediction of prenatal exposures of the INMA cohort

The PBPK model was then used to predict the internal exposure of the foetus given the estimated daily intakes of his/her mother. Figure 7 presents the predictions of several indicators of foetal exposure: the foetal concentration in plasma at different times of the pregnancy, the area under the curve (AUC) over the pregnancy in several organs, and the total body burden at birth. The AUC represents the cumulative exposure in an organ. The predictions are represented for the INMA mother-foetus pairs (Group 2, 355 for PFOS and 346 for PFOA) and for one mother-foetus pair selected randomly. The population indicators were highly inter-correlated (R^2> 0.9) and were also individually correlated with the maternal concentrations measured during the first trimester of pregnancy (R^2> 0.9). At the population level, the foetal plasma concentrations at the end of pregnancy were rather similar for both PFAS despite the 2-factor between the measured maternal concentrations (Table 1). This

similarity is also observed for the AUC in brain and the total body burden at birth. However, the foetal exposure in liver is higher for PFOS than for PFOA (the liver:plasma partition coefficient is twice higher for PFOS than for PFOA), and the situation is inversed in kidneys.

At the individual level, diverse situations are found. For example, the AUC in the three organs is represented on Figure 7 for one foetus, for which all the PFOA indicators were above the PFOS ones. We then compared the ranking of PFOA and PFOS in terms of exposure levels among the foetal indicators of exposure (mean values) for all the foetuses for which their mothers had plasma concentrations above the LOQ for both compounds (n=346). Table 4 presents the results of the comparison for whole group. For all indicators except AUC in brain and liver, the PFOA levels are superior of the PFOS ones in most of the individuals. We then compared this ranking for each individual by computing the number of individuals that have similar patterns (Figure 8). We observed that about 10% of the population have all their PFOA indicators superior to PFOS ones, and that 20.2% have the PFOS indicators always superior. This means that about 70% of the individuals showed different PFOA and PFOS ranking among the different indicators.

4 Discussion

The consequences of PFAS exposure on child health are still unclear despite a relatively high amount of research published for the last two decades. Monitoring programs have focused on the assessment of the PFAS exposure by the means of blood sampling in populations and associations between exposures and child health outcomes have been reported (Braun *et al.*, 2016; Manzano-Salgado *et al.*, 2017; Rappazzo *et al.*, 2017; Lauritzen *et al.*, 2018). Yet many of these associations remain inconsistent between studies or are poorly characterized (Bach *et al.*, 2015). Here we present a mechanistic modelling approach (exposure model, PBPK model, and construction of individual scenarios) to reconstruct the PFOA and PFOS exposure of pregnant women and foetuses from maternal data usually collected in biomonitoring studies (spot measures of biomarkers of exposure and questionnaires). A first step was to characterize placental transfers of the PBPK model at the individual and population levels, and to evaluate the model predictions during pregnancy and breastfeeding. Then a reverse dosimetry approach was used to estimate the daily intakes of the INMA mothers, corrected for their individual history. Quantitative estimates of foetal exposures were computed in several organs at different time periods.

4.1 PFOA and PFOS placental transfers of the PBPK model

The placental transfer rates are a key determinant of PBPK models to predict foetal exposure. In this work, we adapted an existing lifetime PBPK model (Beaudouin *et al.*, 2010) to PFOA and PFOS. This previous model already includes the physiological changes in women that are related to pregnancy and breastfeeding as well as the development of the foetus, and it has been evaluated using independent datasets for several chemicals and using sensitivity analyses. The model is based on precise anatomical and physiological data that has made possible a detailed description of the body. Regarding the limited data in some organs for PFOA and PFOS (e.g., tissue partitioning), the model structure could have been

simplified and restricted to a tenth of compartments as done in other published models (Loccisano *et al.*, 2012b; Fabrega *et al.*, 2015). It should be underlined that model predictions in organs for which data are missing are associated with a relatively high uncertainty. Because we assumed similar partitioning according to the organ perfusion as also done in the other models, such a detailed body compartmentalization should not impact the model performance. However, if new data become available, they could be included easily in our model to improve predictability in these organs.

In this PBPK model, the rates were estimated quantitatively at the individual and population levels for 52 mother-child pairs of the INMA cohort, for which both maternal and cord plasma concentrations were available. The estimated population probability distributions for the placental transfer rates for both PFAS captured well the observed inter-individual variability with coefficients of variation of the maximal transfer rate across subjects estimated at 75% for PFOA and 55% for PFOS. Such an inter-individual variability is commonly observed in a number of physiological or ADME processes. The studied population was quite diverse regarding the parity and age of the women. Nevertheless, we showed that this diversity had little impact on the transfer rates' estimates (unlike the measured maternal plasma concentration). The estimation of the placental transfer rates was driven mainly by the measured CS/MS ratios that exhibited a reduced variability (less than 40% for the two compounds) compared to the estimated transfers (75% for PFOA and 55% for PFOS). We also showed that these ratios predicted by our PBPK model increase slightly over the course of pregnancy, as observed by Pan *et al.* (2017) in 100 women followed at different times of their pregnancy. Even if the variation of the CS/MS ratio is less marked in case of semi- or persistent compounds such as PFAS (as the maternal concentration is stable over a longer period of time) than for non-persistent and rapidly eliminated compounds (Zhang *et al.*, 2017), the sampling time still has an impact on the estimation of the placental transfer rates.

Interpreting the CS/MS ratios without accounting for the toxicokinetics in mother and foetus could then lead to erroneous estimates of the placental transfer rates.

The modelling of placental transfers among PBPK models can be quite heterogeneous. In our PBPK model, the placental transfers were assumed to increase with the placenta's growth whereas others assume a dependence to the foetus bodyweight (Loccisano *et al.*, 2013) or a constant rate through the whole pregnancy (Verner *et al.*, 2016). These modelling assumptions have a direct impact on the resulting estimates of the transfer rates. The location means of the distribution provided here therefore depends on our model and cannot be directly used in other toxicokinetic models built under different assumptions. Nevertheless, the form of the distribution (log-normal) and its variance could be kept if it needs to be used in other models. The population distribution of these rates estimated with our approach could be ultimately used for risk assessment, and conservative or even worst-case scenarios could be run with high percentiles (e.g., 95^{th}) of the distribution to assess the foetal exposure.

The model of placental transfer strongly conditions the predicted exposure of the foetus. Because our placental transfer rate is equivalent to a partition coefficient between the placenta and the foetus blood at any time of the pregnancy, the exposure in the foetus correlates well with the measured concentrations in the mother. Recently, new models have been proposed for placental transfers of compounds, especially in the field of drug development (Dallmann *et al.*, 2017b; Zhang *et al.*, 2017). These models make use of a growing literature on the physiology of human pregnancy (Dallmann *et al.*, 2017a). They account for a detailed description of the placenta (*e.g.* eight compartments are described in (Dallmann *et al.*, 2017b)), the evolution of the placenta physiology and functionality that may impact compound transfer, and they provide *in silico* tools for parameterizing the placental transfer rates based on physicochemical properties of the compounds. However, uncertainties still remain on the processes that drive the placental transfers especially in the first months of

pregnancy when the uteroplacental circulation is not fully established and the keratinization of the foetal skin is not complete. Dedicated data on the mother-foetus exchanges during this period of rapid changes would help to improve the quality of pregnancy PBPK model predictions of the foetal exposure.

4.2 Model evaluation with monitoring data

Our modelling approach combines two types of models (PBPK, daily intakes) and was evaluated using monitoring data in pregnant or breastfeeding women non-occupationally exposed to PFOA and PFOS. Over the course of a pregnancy or a breastfeeding period of several months, the plasma and breastmilk concentrations are sensitive to changes in the physiology of the women and in external exposure (*i.e*, daily intake). It was not possible to dissociate the impact of these two phenomena on the biomonitoring data. The use of such data, for which exposure is not controlled, makes the model evaluation difficult as it encompasses the contributions of all models and prevent a direct evaluation of each model separately. The PBPK and exposure models were developed using specific data that were not all derived from monitoring data (*e.g.*, production and emission data for the exposure model, and extrapolated animal to human data for the PBPK model), giving some confidence in their predictability capabilities. The dynamic exposure model was defined to comply with the decline observed in PFOA and PFOS exposure in several European countries (Glynn *et al.*, 2012), and therefore should be adequate for the INMA cohort. This decline may not be relevant for other parts of the world and might explain the discrepancies between several biomonitoring studies. For example, Thomsen *et al.* (2010) estimated monthly reductions of breastmilk by 7.8% for PFOA and 3.1% for PFOS in Norwegian women. In South Korea, Kang *et al.* (2016) observed decreased levels during lactation for PFOA but not for PFOS, and Lee *et al.* (2018) observed constant levels during breastfeeding except during the first 7 days for which the concentrations were lower than during the rest of breastfeeding. As the

PFOA and PFOS exposure patterns may differ between these countries, the comparison of their results is not straightforward. As showed by our analysis of the INMA cohort, the internal maternal PFOA and PFOS levels (e.g., in plasma or milk) depends on the mother's intake, the toxicokinetics of the compounds, and the individual traits and the life history of the women. Another source of uncertainty in the evaluation process was the use of population aggregated data for which detailed information on the mothers was not available. Despite all the uncertainties that could affect the quality of predictions, the models capture well the PFOA and PFOS toxicokinetics in pregnant and breastfeeding women. The declines in plasma due to the pregnancy and breastfeeding observed in European populations were well reproduced in terms of timing and magnitude.

4.3 Daily intakes for the IMNA mothers

Dietary intakes evolving with time were estimated for each INMA woman. PFOS exposure was estimated to be higher than PFOA one's before the year 2000 decline, after which the trend reversed with the decline being more pronounced for PFOS. Our exposure model was defined to reproduce specifically the PFOA and PFOS exposure between 1995 and 2006 (year of birth of the last INMA child). The temporal trends before and after this period were not considered, and that should prevent the use of our exposure model for analysing data collected outside the studied period. However, the exposure model could easily be updated to describe exposures before 1995, period for which PFAS production volumes have been documented (Paul *et al.*, 2009). Regarding recent exposure, the continuous decline over the years after 2001 should be verified, but seems to be adequate for the USA according to the study of Olsen *et al.* (2017) on the trends of PFAS blood levels in US donors until 2015.

As an example, the daily intakes of the INMA mothers were estimated at 0.49 ng/day/kg (min: 0.03; max: 6.04) for PFOA and 0.25 ng/day/kg (min: 0.01; max: 0.93) for PFOS in 2006. All the estimates were far below the tolerable daily intakes established by the European

Food Safety Authority, *i.e.* 1,500 ng/day/kg for PFOA and 150 ng/day/kg for PFOS (European Food Safety Authority, 2008). Our estimates are in the same range of dietary intakes based on concentrations in foodstuffs and beverages associated with their consumptions in Spain and other European countries (Fromme *et al.*, 2007; Ericson *et al.*, 2008; Haug *et al.*, 2010; Noorlander *et al.*, 2011; Domingo *et al.*, 2012). Ericson *et al.* (2008) estimated the PFOS diet intake to be about 1 ng/day/kg with food samples collected on the Spanish market in 2006, whereas the PFOA intake was not estimated in that study due to a high number of samples below the limit of quantification. These are very close to the diet intakes of the Norwegian population estimated by Haug *et al.* (2010) for year 2008 (0.41 ng/day/kg for PFOA and 0.21 ng/day/kg for PFOS for females between 16 and 29 years old), a study in which most of the food samples were above the limits of quantification. This last point must be considered when comparing diet intakes obtained with different methods, as the common imputation of non-detected samples to the limit of detection divided by 2 or to 0 can over- or under-estimate the intake estimates. For example, Fromme *et al.* (2007) compared diet intakes computed using duplicate diet samples or using TK modelling of plasma concentrations. In that study, less than 50% of the diet samples were above the limit of detection and their concentration was assigned to half of the limit of detection. They observed that the diet intakes estimated with TK modelling were lower than the ones derived with the diet samples, especially for PFOA.

Among the INMA pregnant women, we showed that similar measured concentrations at one time point can correspond to differences in external (diet intakes) and internal exposure over a long period. This effect is more prevalent among multipara women as the women's history, like their age or previous pregnancies and breastfeeding, influences the maternal plasma concentrations of PFOA and PFOS. The estimated intakes were independent of the

women's traits, supporting the use of our diet intake estimates in predictive exposure scenarios for a wider population.

4.4 New indicators of foetal exposure

The final aim of our work was to compute new indicators of foetal exposure using the PBPK model for the woman-foetus with the estimated dietary intakes of the woman. In the context of biomonitoring and epidemiological studies, such indicators predicted by PBPK modelling can help to estimate the internal dosimetry in target tissues during windows of susceptibility in order to better understand the onset of adverse effects through the mechanism of action of chemical compounds (El-Masri *et al.*, 2016). Here, we showed that the ranking of PFOA and PFOS in terms of levels of exposure differs between the foetal indicators. Due to the maternal exposure and the mother-to-foetus transfers, the PFOA and PFOS foetal plasma concentrations are quite similar at the end of pregnancy for the whole cohort (355 foetuses/newborns). This similarity is predicted in brain, but not in kidneys and liver. At the individual level, we also showed that the ranking of PFOA and PFOS exposure varies greatly among the foetuses/newborns. About a third of the population has levels of either one compound always higher than the levels of the other compound. The other two thirds can exhibit different patterns, but the majority has PFOA levels superior to PFOS levels for most of the indicators of foetal exposure. These differences of tissue exposure in foetus cannot be suspected only with the measurement of the maternal concentration during pregnancy. These simulated foetal exposures then bring additional information to the measured maternal spot concentrations that could help to better characterize the prenatal exposure in target organs of the compounds.

4.5 Conclusions

Our study shows the contribution of PBPK modelling to chemical exposure assessment in large cohorts. From a maternal spot concentrations and women personal history, the PBPK

model simulates the internal exposure in mothers throughout pregnancy and breastfeeding, as well as the internal exposure of the foetus in several target organs. While our study focuses on the prenatal life, our PBPK model would also be a valuable tool to estimate plausible PFOA and PFOS plasma levels in infants given the body burden at birth and subsequent exposures, including breastmilk ingestion. This application of the model would appear to have the ability to account for the continuum of exposures of the same individual. Our work comes in support to the current emphasis on human biomonitoring studies in Europe. Several initiatives have been carried out at national and European levels to encourage the development of new technologies, tools and models to reduce the uncertainties in exposure assessment. Even though the integration of internal dosimetry simulated with a PBPK model in the exposure-response relationship is a recognized promising methodology to reduce uncertainty in risk assessment (Gibb *et al.*, 2002; Smith, 2002), there are still very attempts to integrate this in population (epidemiological) studies (Verner *et al.*, 2010). The next step will be to apply such an approach on large cohorts to test the simulated indicators of internal exposure as predictors of child health effects.

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Conflict of Interest Statement

The authors have nothing to disclose.

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Figure 1: Workflow presenting the three steps of our study: 1) the calibration and evaluation of the PBPK model with the estimation of the PFOA and PFOS placental transfers, 2) the estimation of the maternal exposure (as daily intakes) for INMA mothers; 3) the simulation of the foetal internal exposure.

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Figure 3: Histograms of the individual maximal placental transfer rates for PFOA (left) and PFOS (right) together with the estimated population distribution (- -) and the distribution that best fits the individual rates (–).

Figure 4: Ratios of cord over maternal serum concentration (CS/MS), from maternal concentrations measured at different times during pregnancy. Our study is represented by an open circle, and plain circles represent ratios reported in the literature (Inoue *et al.*, 2004; Fei *et al.*, 2007; Midasch *et al.*, 2007; Monroy *et al.*, 2008; Fromme *et al.*, 2010; Beesoon *et al.*, 2011; Gutzkow *et al.*, 2012; Lee *et al.*, 2013; Porpora *et al.*, 2013; Zhang *et al.*, 2013; Kato *et*

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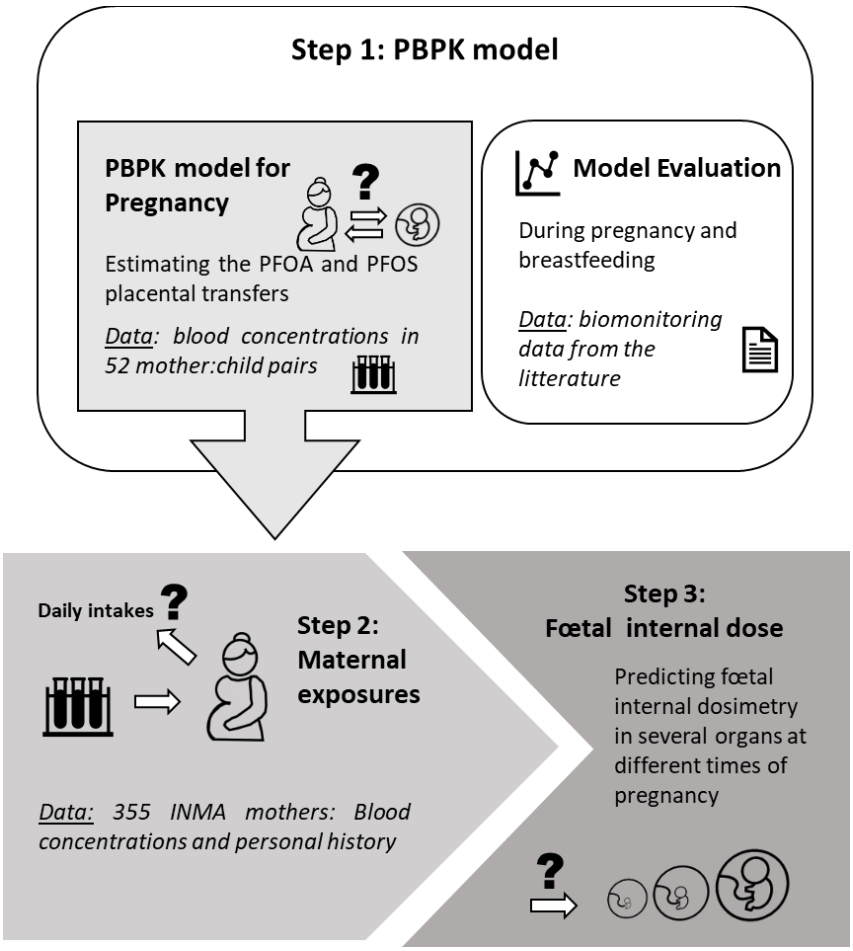

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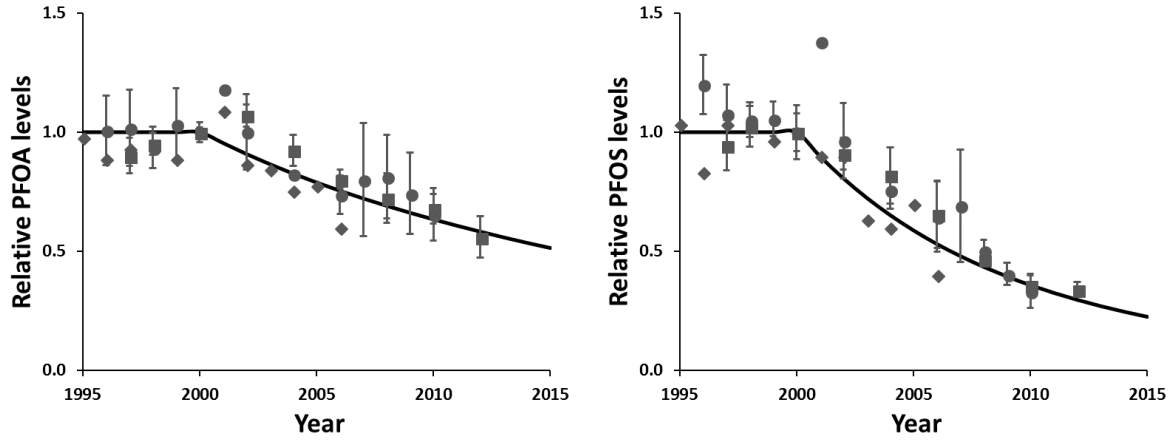

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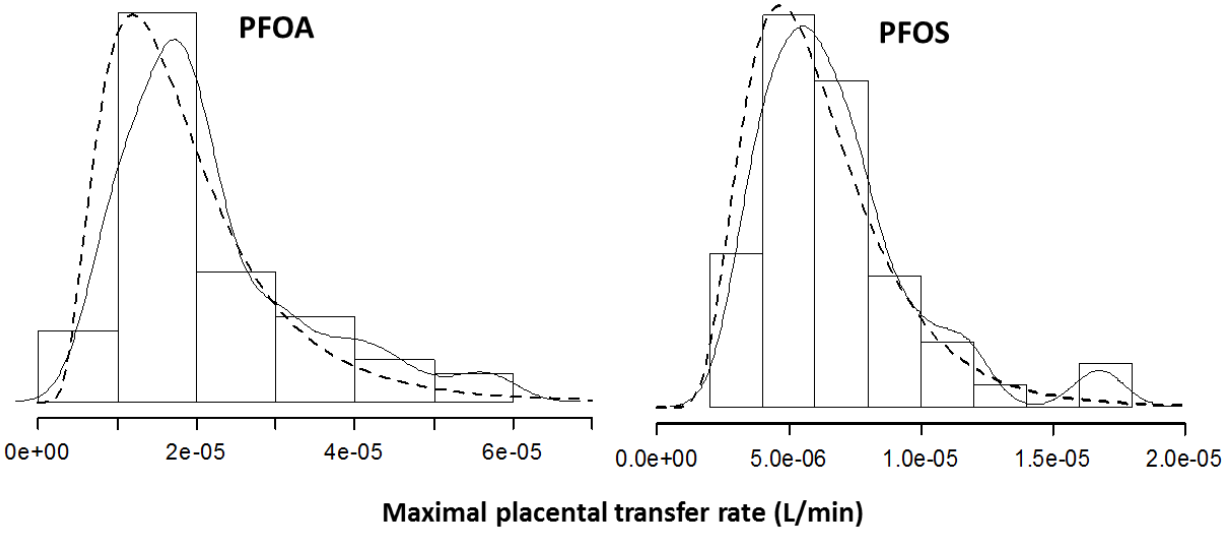

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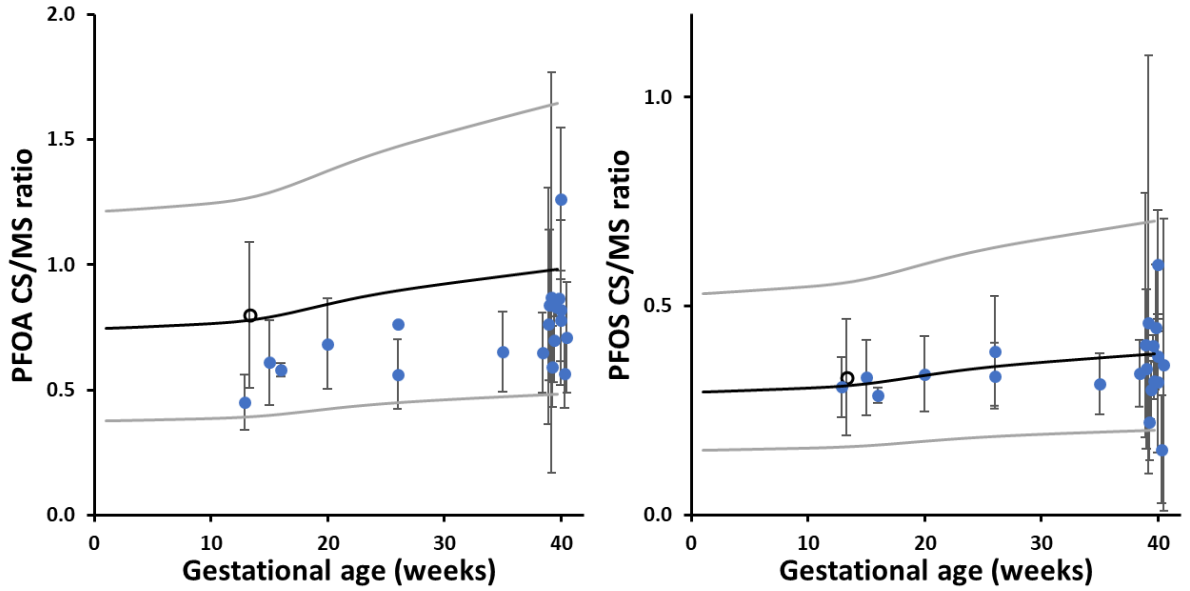

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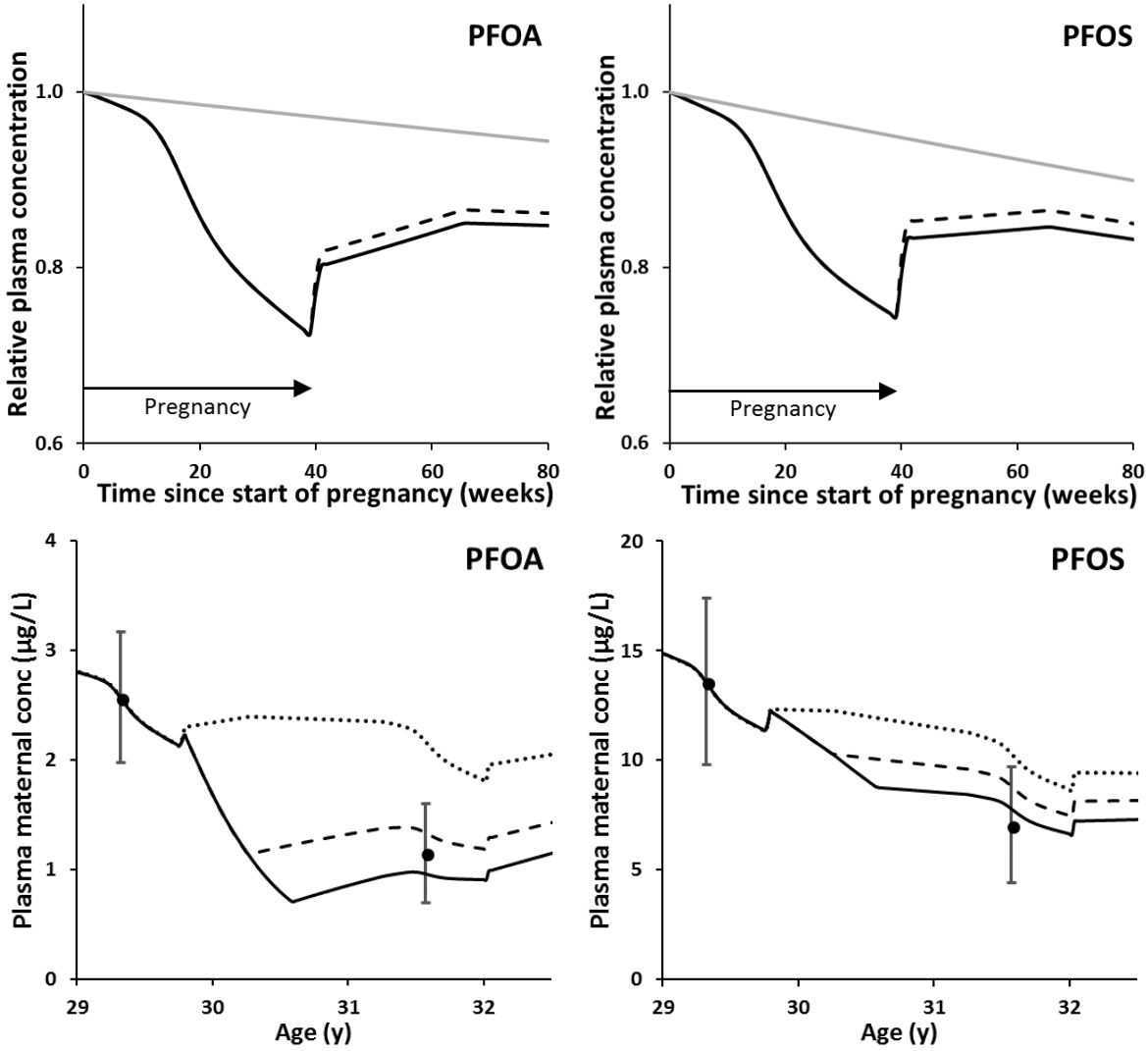

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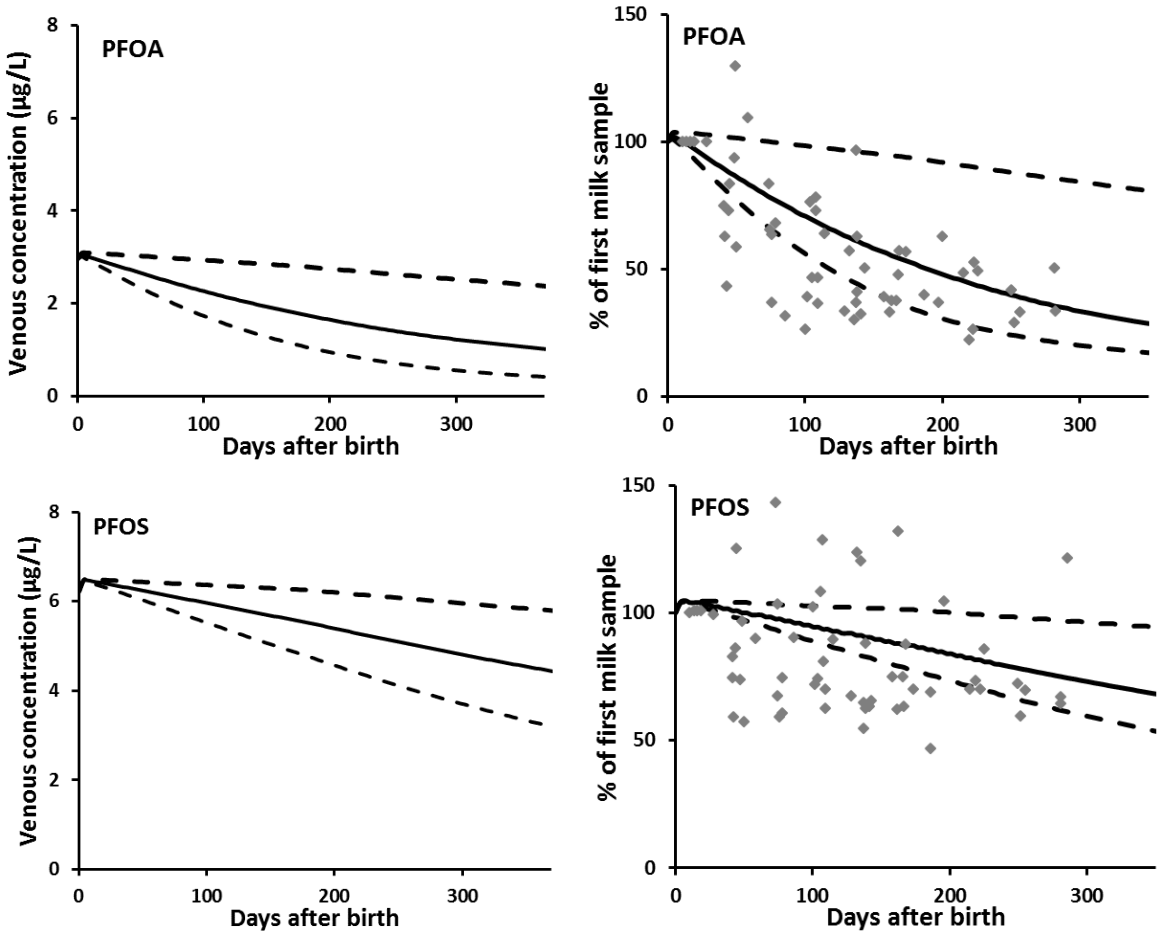

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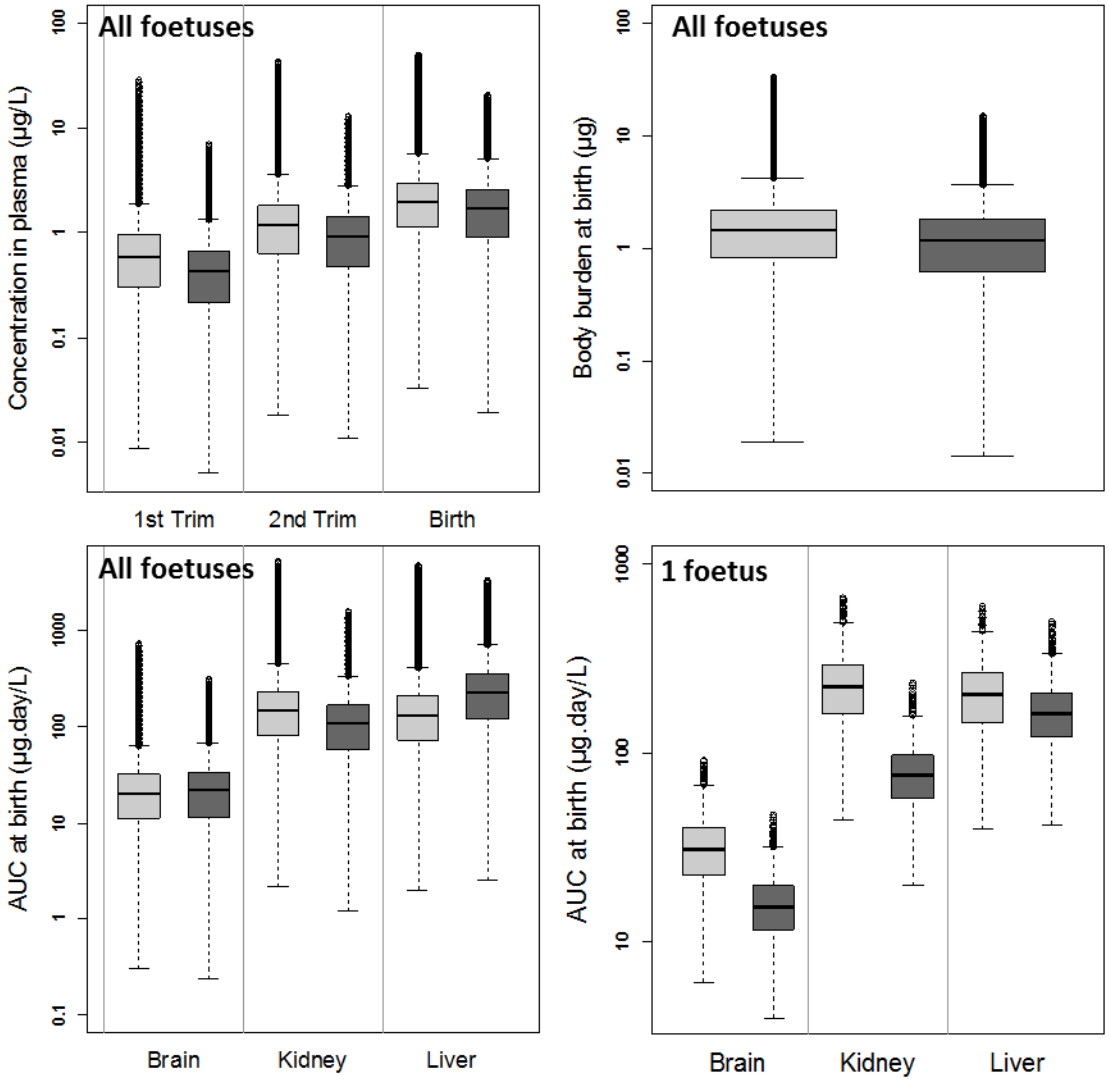

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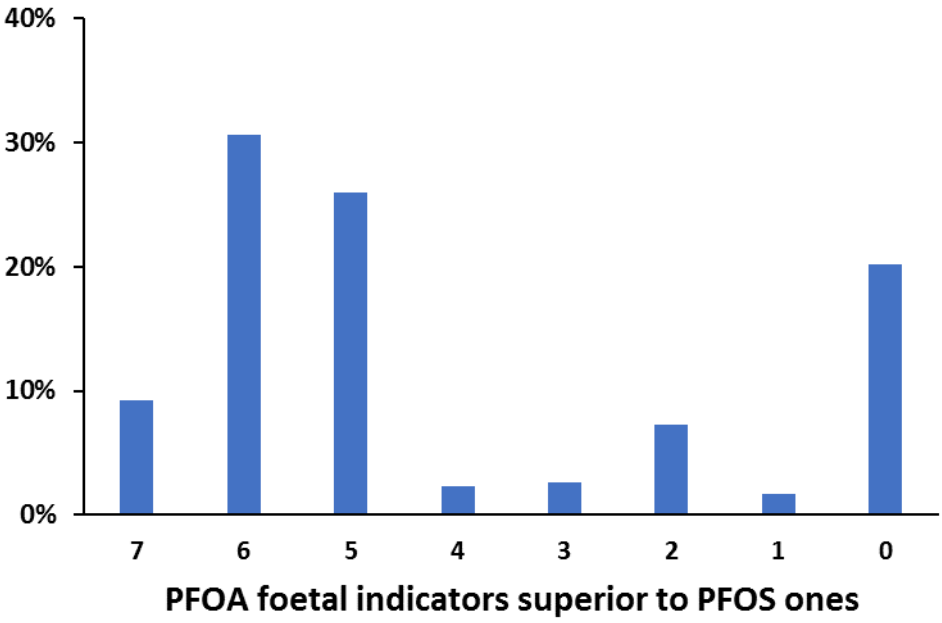

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List of tables

Table 1: Summary characteristics of IMNA mothers and newborns. The mean, standard deviation, minimum and maximum are reported.

Variables	Group 1	Group 2
Number of participants	52	355
Age at the start of pregnancy (year)	31.3±4.9 [18.3-40.9]	31.0±4.0 [17.4-42.2]
Pre-pregnancy bodyweight (kg)	61.6±10.8 [43.0-94.0]	62.9±13.2 [39.0-143.0]
Weight gain at the end of pregnancy (kg)	14.1±5.4 [0.0-26.5]	14.1±4.8 [2.1-30.4]
Age of pregnancy at maternal sampling (week)	13.3±1.5 [11.1-17.7]	13.5±1.8 [8.6-25.7]
Duration of pregnancy (week)	39.8±1.4 [35.3-42.1]	39.7±1.3 [34.3-42.3]
Bodyweight of the newborn (g)	3314±470 [2300-4480]	3258±401 [1920-4400]
Year of the newborn's birth	2005 [2004-2006]	2005 [2004-2006]
PFOA maternal plasma concentrations (µg/L)	3.26±1.87 [0.39 -11.93]	2.78±2.18 [0.20-31.64]
PFOS maternal plasma concentrations (µg/L)	7.14±5.35 [0.69-38.58]	5.70±3.45 [0.26-25.98]
PFOA cord serum concentrations (µg/L)	2.54±1.64 [0.86-10.56]	–
PFOS cord serum concentrations (µg/L)	2.08±1.00 [0.53-4.71]	–

Group 1 was used to estimate the placental transfer rates; Group 2 was used to estimate the maternal and foetal exposure.

Table 2. Values of the compound-specific parameters of the human PBPK model for PFOA and PFOS.

Parameters	PFOA	PFOS
Intestinal absorption (%)[a]	1	1
Free fraction in plasma[a]	0.02	0.025
Half-life in plasma (years)[b,c]	3.0	4.8
Tissue:plasma partition coefficient[d-g]		
Adipose	0.23	0.14
Adrenal glands	0.58	0.55
Bone	0.58	0.55

Brain	0.08	0.11
Breast	0.10	0.09
Gut	0.58	0.55
Heart	0.58	0.55
Kidney	0.58	0.55
Liver	0.52	1.16
Lung	0.63	0.67
Marrow	0.58	0.55
Muscle	0.10	0.09
Pancreas	0.22	0.30
Sexual organs	0.58	0.55
Skin	0.10	0.09
Spleen	0.58	0.55
Stomach	0.58	0.55
Thyroid	0.38	0.26
Thymus	0.58	0.55
Urinary tract	0.58	0.55
Placenta	0.24	0.26
Milk	0.11	0.02

[a]Loccisano *et al.* (2013); [b]Olsen *et al.* (2007); [c]Russell *et al.* (2015); [d]Maestri *et al.* (2006); [e]Zhang *et al.* (2013); [f]Ehresman *et al.* (2007); [g]Liu *et al.* (2011)

Table 3: Percentage of decline in PFOA and PFOS maternal plasma concentration over a pregnancy simulated by our model and measured in several studies.

	PFOA		PFOS	
	Predictions	**Observations**	**Predictions**	**Observations**
Trimester 1/ Trimester 2	-16%	-20%[a]	-15%	-8% [a]
Trimester 1/ Trimester 3	-23%	-29% [*,a,b,c]	-21%	-18% [*,a,b,c]
Trimester 2/ Trimester 3	-9%	-13% [a]	-7%	-2% [a]
Trimester 2/Delivery	-15%	-21% [*,d,e]	-13%	-12% [*,d,e]
Trimester 3/Delivery	-3%	-12% [f]	-2%	0% [f]

*: average of several observations; [a](Pan *et al.*, 2017); [b](Fisher *et al.*, 2016); [c](Glynn *et al.*, 2012); [d](Kato *et al.*, 2014); [e](Monroy *et al.*, 2008); [f](Fromme *et al.*, 2010).

Table 4: PFOA and PFOS ranking of the seven foetal indicators of exposure based on their mean values for the foetuses of Group 2 (n=346). The results are presented in percentage of the individuals.

	PFOA > PFOS	**PFOA ≤ PFOS**
Body burden at birth (µg)	68.2%	31.8%
Concentration in plasma (µg/L)		
End of trimester 1	79.8%	20.2%
End of trimester 2	70.8%	29.2%
Birth	65.9%	34.1%
Area under the curve (µg.day/L)		

Brain	39.9%	60.1%
Kidney	78.0%	22.0%
Liver	9.2%	90.8%

Highlights

- We estimated the PFOS and PFOA placental transfers in a PBPK model using human data
- Our PBPK model reproduced blood and milk data measured in biomonitoring studies
- Mothers' history impacts the estimation of their dietary intakes
- The new indicators of foetal exposure differed from the maternal concentrations
- Foetal exposure exhibits different patterns in target organs among the IMNA cohort