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► To cite this version:

Aude Ratier, Maribel Casas, Regina Grazuleviciene, Remy Slama, Line Småstuen Haug, et al.. Estimating the dynamic early life exposure to PFOA and PFOS of the HELIX children: Emerging profiles via prenatal exposure, breastfeeding, and diet. *Environment International*, 2024, pp.108621. 10.1016/j.envint.2024.108621 . hal-04532832

HAL Id: hal-04532832

<https://u-picardie.hal.science/hal-04532832>

Submitted on 6 May 2024

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Full length article



Estimating the dynamic early life exposure to PFOA and PFOS of the HELIX children: Emerging profiles via prenatal exposure, breastfeeding, and diet

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ARTICLE INFO

Handling Editor: Shoji Nakayama

Keywords:

PBPK model

Reverse dosimetry

In utero

Longitudinal cohorts

Children

Human biomonitoring

ABSTRACT

In utero and children's exposure to per- and polyfluoroalkyl substances (PFAS) is a major concern in health risk assessment as early life exposures are suspected to induce adverse health effects. Our work aims to estimate children's exposure (from birth to 12 years old) to PFOA and PFOS, using a Physiologically-Based Pharmacokinetic (PBPK) modelling approach.

A model for PFAS was updated to simulate the internal PFAS exposures during the *in utero* life and childhood, and including individual characteristics and exposure scenarios (e.g., duration of breastfeeding, weight at birth, etc.). Our approach was applied to the HELIX cohort, involving 1,239 mother-child pairs with measured PFOA and PFOS plasma concentrations at two sampling times: maternal and child plasma concentrations (6 to 12 y.o).

Our model predicted an increase in plasma concentrations during fetal development and childhood until 2 y.o when the maximum concentrations were reached. Higher plasma concentrations of PFOA than PFOS were predicted until 2 y.o, and then PFOS concentrations gradually became higher than PFOA concentrations. From 2 to 8 y.o, mean concentrations decreased from 3.1 to 1.88 µg/L or ng/mL (PFOA) and from 4.77 to 3.56 µg/L (PFOS). The concentration-time profiles vary with the age and were mostly influenced by *in utero* exposure (on the first 4 months after birth), breastfeeding (from 5 months to 2 (PFOA) or 5 (PFOS) y.o of the children), and food intake (after 3 (PFOA) or 6 (PFOS) y.o of the children). Similar measured biomarker levels can correspond to large differences in the simulated internal exposures, highlighting the importance to investigate the children's exposure over the early life to improve exposure classification.

Our approach demonstrates the possibility to simulate individual internal exposures using PBPK models when measured biomarkers are scarce, helping risk assessors in gaining insight into internal exposure during critical windows, such as early life.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) have been widely used

since 1950 in large industrial and commercial applications, leading to their detection in the environment, wildlife and humans (Buck et al., 2011; OECD, 2015; Panieri et al., 2022). Many PFAS have been

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<https://doi.org/10.1016/j.envint.2024.108621>

Received 26 October 2023; Received in revised form 29 March 2024; Accepted 31 March 2024

Available online 2 April 2024

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recognized as extremely persistent, bioaccumulative and toxic (Cousins et al., 2020; OECD, 2015). *In utero* and children's exposure to PFAS is a major concern in health risk assessment (EFSA, 2018, 2020), as early life exposure is suspected to induce diverse effects (Rappazzo et al., 2017), such as cardiometabolic (Halldorsson et al., 2012; Timmermann et al., 2014), neurological, neurodevelopmental and attention effects (Rappazzo et al., 2017), as well as asthma and impact on immune response (Grandjean and Budtz-Jørgensen, 2013, e.g., vaccine response), thyroid changes (Rappazzo et al., 2017) and altered timing of puberty onset (Barry et al., 2013; Jensen et al., 2015; Joensen et al., 2012).

Early life exposure to these compounds results from several sources (i.e., maternal transfer via placenta and breastfeeding, diet, drinking water) and the associated internal exposure likely changes as the child grows. Several studies have shown that PFAS are able to cross the placental barrier as they have been detected in cord blood samples at birth (Cariou et al., 2015; Fisher et al., 2016; Horikoshi et al., 2021; Inoue et al., 2004; Kang et al., 2021; Monroy et al., 2008; Rovira et al., 2019). Infants are thus exposed through the mother during prenatal life and also during breastfeeding (Cariou et al., 2015; Fromme et al., 2010; Haug et al., 2011; Kim et al., 2011; Liu et al., 2011; Mosch et al., 2010; Roosens et al., 2010). During childhood, diet and drinking water are the major sources of PFAS exposure, as for the general population (EFSA, 2020). To assess the children's exposure to PFAS, these compounds are usually measured in blood. It has been shown that the internal exposure of children can greatly differ from other population sub-groups, especially those under two years old (Gao et al., 2022; Winkens et al., 2017). However, there are knowledge gaps during this period and a mismatch in current HBM data collections (Allegaert et al., 2022; Batchelor and Marriott, 2015; Lee et al., 2021). As invasive blood sampling is challenging in children, it is beneficial to interpolate the exposure and its potential variations between measurements. Toxicokinetic (TK) modelling helps in linking external exposure to chemicals to internal concentrations that condition health effects. Several approaches are available to model and predict the PFAS TK, such as physiologically based pharmacokinetic (PBPK) models (also called physiologically based toxicokinetic (PBTk) or physiologically based kinetic (PBK)).

PBPK models associated with an exposure scenario enable the reconstruction of the internal exposure of individuals to chemicals over a time period. PBPK models rely on a physiological description of the human body to simulate the fate of compounds by describing their absorption, distribution, metabolism, and excretion (ADME) (Bois and Brochot, 2016; Reddy et al., 2005). They also allow the incorporation of biochemical and physiological changes occurring during pregnancy or childhood. PBPK models link an external exposure to the internal dosimetry (e.g., concentration of the parent compound or its metabolite in blood, urine or target organs) and vice-versa. Individualized PBPK model predictions can be obtained when physiological and exposure data on individuals are available, allowing to explain a part of the apparent inter-individual variability in the biomarker's measurements and paving the way for more individualized risk assessment (Verscheijden et al., 2020). PBPK modelling has already been used for adults or pregnant women exposed to PFOA and/or PFOS (e.g., Brochot et al., 2019; Chou and Lin, 2020; Fàbrega et al., 2014; Fàbrega et al., 2016; Loccisano et al., 2011; Loccisano et al., 2013; Rovira et al., 2019; Worley et al., 2017). To our knowledge, only a few papers (Deepika et al., 2021; Verner et al., 2015) have reported an age-specific PBPK model and provided PFAS kinetic profiles for infants or children. Despite the growing number of studies on PFAS and their fate in the body, to our knowledge, only a few of them (Brochot et al., 2019; Chou and Lin, 2020; Deepika et al., 2021; Rovira et al., 2019) focused on the entire human lifetime and have developed PBPK models from mother to foetus or child. A generic PBPK model was previously developed by our team for men and women (Beaudouin et al., 2010). This PBPK model was updated to include differences in physiology for women from birth to adulthood, accounting for uncertainties and variability between women and their individual history (e.g., in terms of previous pregnancies and

breastfeeding) when exposed to PFOA and PFOS (Brochot et al., 2019). The resulting PBPK model accounting for maternal individual exposure scenarios (i.e., accounting for their individual history, as explained above) is able to predict prenatal exposure to PFAS during the whole pregnancy.

In this study, we aimed to extend this previous work (Brochot et al., 2019) by accounting for the early years of the children. Our approach was applied to the HELIX subcohort of 1,239 mother-child pairs from six European countries. In HELIX, the child's exposure to PFOA and PFOS was measured during the prenatal life (maternal concentration in plasma) and around 6 to 12 years old (y.o) (child concentration in plasma). Individual exposure scenarios were established with information collected in the questionnaires (e.g., duration of breastfeeding, weight at birth, etc.) and the measured plasma concentrations during prenatal life and childhood. This framework allowed to estimate the contribution of various exposure pathways (*in utero* exposure, breastfeeding, dietary intakes) for each HELIX child at different times during childhood (from birth to 12 y.o), and to identify the target tissues of PFAS exposure.

2. Materials and methods

2.1. General workflow

The PBPK model (supplementary material, S.1) was run in two steps to reconstruct the exposure of the child from conception to the age of the child at the sampling time (between 6 and 12 y.o). First, the PBPK model was run for the pregnant woman and her foetus to reconstruct the maternal exposure, to estimate the foetal internal exposure and to predict the PFAS concentration in the mother's breast milk. These predictions at delivery were used as inputs for the newborn's burden and the breast milk uptake to run the PBPK model for the child from birth to the age of 6 to 12 y.o. The different steps of our workflow (supplementary material, S.2) were accordingly: (i) estimation of the prenatal exposure, (ii) construction of individual exposure scenarios for each child based on her/his body burden at birth, breastfeeding, and diet, and (iii) estimation of the early childhood exposure.

2.2. Study population and data

The Human Early Life Exposome (HELIX) study (<https://www.projecthelix.eu>) is a collaborative project across population-based birth cohort studies in six European countries (France, Greece, Lithuania, Norway, Spain, and the United Kingdom (UK)). The entire study population includes 31,472 mother-child pairs recruited during pregnancy (Vrijheid et al., 2014). More information is provided in the supplementary material (S.3).

A subcohort of 1,301 mother-child pairs was selected that consisted of exposure measurements from several chemicals, including PFOA and PFOS, at two times: during pregnancy and around the age of 8 y.o (Maitre et al., 2018). Extensive information was available from the questionnaires and was used to build individual prenatal and postnatal exposure scenarios for each child (Table 1). A total of 1,239 mother-child pairs were included in our study after removing pairs for which information in the questionnaires was inconsistent (e.g., missing information on the date of birth, breastfed or not, etc.). When an entry was missing, the arithmetic mean value of the children from the same birth country (i.e., same national cohort) was used. When the sibling position of the child was missing, it was set to one by default.

The chemical analyses of PFOA and PFOS plasma concentrations were previously described (Haug et al., 2018; Manzano-Salgado et al., 2015). The limit of quantification (LOQ) for PFOA and PFOS was 0.05 µg/L. All samples were above the LOQ for PFOA and PFOS, except for four PFOA maternal samples and three PFOS child samples. A summary of the PFOA and PFOS concentrations measured in maternal and child plasma and of the individual characteristics used in the model is

Table 1

Description of the HELIX subcohort (1,239 women-children) selected for this study. The summary presents the arithmetic mean values \pm sd [min–max] for each birth country as well as for all the subcohort (total). NA stands for the missing values.

Description	France (EDEN)	Greece (RHEA)	Lithuania (KANC)	Norway (MOBA)	Spain (INMA)	UK (BIB)	Total
MOTHER							
Number	198	173	195	253	223	197	1239
PFOA concentration ($\mu\text{g/L}$)	3.8 \pm 1.5 [0.7–10.6]	2.6 \pm 1.3 [0.6–12.2]	1.2 \pm 0.9 [0.3–7.7]	2.3 \pm 1.1 [0.4–6.1]	2.9 \pm 2.5 [<LOQ–31.6]	2.3 \pm 1.4 [0.2–10.6]	2.5 \pm 1.7 [<LOQ–31.6]
PFOS concentration ($\mu\text{g/L}$)	15.1 \pm 7.8 [0.5–48.0]	6.0 \pm 3.5 [1.8–35.2]	4.6 \pm 2.1 [0.6–14.9]	10.4 \pm 5.1 [1.6–31.2]	6.0 \pm 3.3 [0.3–20.0]	4.8 \pm 3.6 [0.4–10.6]	2.5 \pm 5.9 [0.02–31.6]
Year of sampling	2004 [2003–2005]	2008 [2007–2008]	2008 [2006–2009]	2006 [2005–2007]	2005 [2005–2007]	2008 [2007–2008]	2006 [2003–2009]
Maternal age at the time of prenatal sampling (years)	30.6 \pm 4.8 [20.0–43.4]	30.6 \pm 4.8 [17.0–42.3]	29.2 \pm 4.9 [19.2–43.5]	32.6 \pm 3.6 [23.0–43.0]	32.0 \pm 4.0 [19.1–40.1]	28.6 \pm 5.8 [16.0–42.0]	30.7 \pm 4.9 [16.0–43.5]
Maternal birth year	1973 [1960–1985]	1977 [1966–1990]	1978 [1964–1988]	1973 [1962–1984]	1974 [1965–1987]	1979 [1965–1992]	1976 [1960–1992]
Sampling time (week of pregnancy)	26.1 \pm 1.3 [22.9–29.1]	14.3 \pm 3.8 [6.9–33.0]	39.4 \pm 1.3 [32.0–41.0]	18.7 \pm 0.8 [14.4–22.0]	13.7 \pm 2.0 [9.0–26.7]	26.6 \pm 1.4 [22.3–34.1]	23.2 \pm 9.1 [6.9–41.0]
Duration of pregnancy (weeks)	39.7 \pm 1.8 [30.9–42.6]	38.4 \pm 1.5 [32.9–41.9]	39.4 \pm 1.3 [32.0–41.0]	40.1 \pm 1.8 [31.7–43.6]	40.0 \pm 1.4 [34.3–44.0]	39.7 \pm 1.8 [28.0–41.8]	39.6 \pm 1.7 [28.0–44.0]
Maternal body weight at the start of pregnancy (kg)	62.0 \pm 11.8 [39.0–114]	64.5 \pm 12.4 [48.0–117]	78.8 \pm 15.8 [47.0–140]	64.1 \pm 9.4 [43.0–107]	63.2 \pm 12.5 [43.0–115]	75.5 \pm 15.4 [43.9–133]	67.8 \pm 14.4 [39.0–140]
Weight gain during pregnancy (kg)	13.4 \pm 5.3 [0.0 (n = 4)–32.0]	14.5 \pm 6.3 [0.0 (n = 1)–35.0]	15.2 \pm 6.3 [0.0 (n = 5)–40.0]	13.8 \pm 5.2 [0.0 (n = 2)–35.0]	13.0 \pm 6.5 [0.0 (n = 11)–55.0]	11.9 \pm 5.2 [0.0 (n = 6)–32.0]	13.6 \pm 5.9 [0.0–55.0]
	NA = 19	NA = 3	NA = 18	NA = 15	NA = 5	NA = 82	
Description	France (EDEN)	Greece (RHEA)	Lithuania (KANC)	Norway (MOBA)	Spain (INMA)	UK (BIB)	Total
CHILD							
Sex	85 girls 113 boys	71 girls 102 boys	89 girls 106 boys	120 girls 133 boys	103 girls 120 boys	88 girls 109 boys	556 girls 683 boys
PFOA concentration ($\mu\text{g/L}$)	1.6 \pm 0.7 [0.5–6.7]	1.6 \pm 0.8 [0.6–6.2]	1.4 \pm 0.7 [0.2–5.8]	1.8 \pm 0.5 [0.6–3.8]	1.5 \pm 0.5 [0.6–4.0]	2.0 \pm 0.8 [0.7–6.6]	1.7 \pm 0.7 [0.2–6.7]
PFOS concentration ($\mu\text{g/L}$)	3.8 \pm 3.2 [1.0–28.5]	2.8 \pm 3.2 [0.4–33.8]	1.5 \pm 1.3 [< LOQ–8.2]	3.5 \pm 2.0 [0.6–23.8]	1.8 \pm 2.2 [< LOQ–30.0]	1.8 \pm 1.4 [0.1–10.4]	2.6 \pm 2.5 [< LOQ–33.8]
Birth year	2004 [2003–2005]	2008 [2007–2008]	2008 [2007–2009]	2006 [2005–2007]	2005 [2005–2007]	2008 [2007–2008]	2006 [2003–2009]
Birth weight (kg)	3.3 \pm 0.5 [1.3–5.3]	3.4 \pm 0.5 [1.7–5.1]	3.6 \pm 0.5 [1.8–4.5]	3.5 \pm 0.5 [1.6–4.9]	3.3 \pm 0.4 [2.0–4.4]	3.3 \pm 0.6 [1.1–4.7]	3.4 \pm 0.5 [1.1–5.3]
Age of the child at the sampling time (years)	10.8 \pm 0.6 [9.3–12.1]	6.5 \pm 0.3 [6.0–7.5]	6.5 \pm 0.5 [5.4–7.8]	8.5 \pm 0.5 [6.9–9.8]	8.8 \pm 0.6 [7.3–10.2]	6.6 \pm 0.2 [6.2–7.4]	8.0 \pm 1.6 [5.4–12.1]
Weight at sampling time (kg)	37.2 \pm 7.9 [23.4–71.1]	24.5 \pm 4.8 [17.1–40.7]	24.6 \pm 5.0 [14.5–43]	29.0 \pm 5.0 [20.1–57.3]	32.4 \pm 7.6 [21.2–58.4]	23.1 \pm 4.0 [16.0–41.6]	28.6 \pm 7.7 [14.5–71.1]
Sibling position	2 [1–4]	2 [1–4]	2 [1–4]	2 [1–4]	1 [1–3]	2 [1–6]	2 [1–6]
Formula milk day starts (days)	54 [0–366]	49 [0–1440]	64 [0–420]	51 [0–361]	93 [0–720]	39 [0–720]	59 [0–1440]
Exclusive breastfeeding duration (days)	55 [0–365]	96 [0–1440]	223 [0–1440]	290 [0–1440]	193 [0–1800]	107 [0–810]	167 [0–1800]
Mixed breastfeeding duration (days)	58 [1–510]	101 [1–510]	58 [1–1950]	197 [10–685]	148 [3–1620]	112 [1–1005]	134 [1–1955]
Total breastfeeding duration (mixed feeding duration and exclusive breastfeeding, days)	76 [0 (n=67)–600 (n=1)]	155 [0 (n=20)–1440 (n=1)]	260 [0 (n=20)–2040 (n=1)]	359 [0 (n=11)–1440 (n=1)]	255 [0 (n=25)–1800 (n=2)]	130 [0 (n=59)–1095 (n=1)]	215 [0 (n=202)–2040 (n=1)]
	NA = 8		NA = 8	NA = 4			

provided in Table 1.

2.3. The PBPK model

The PBPK model for men and women previously developed by Beaudouin et al. (2010) was adapted in Brochot et al. (2019) to PFOA and PFOS kinetics in women (including pregnancy and breastfeeding). In this work, the PBPK model for men was adapted to describe the HELIX boys (i.e., adding specific processes of PFAS to the model published by Beaudouin et al. (2010), and adding updates on the physiological parameters such as the growth of the child's body weight). Some physiological and compound-specific parameters for the models of both women and men were updated to account for the latest data. Details and all the new equations are provided in the supplementary material (S.4).

2.3.1. Physiological parameters

The haematocrits for the pregnant women and the foetus were updated based on the meta-analysis of Dallmann et al. (2017). Some physiological parameters for the foetus (i.e., the cardiac output, the relative foetal blood flows, and organ volumes) were also updated from the last reviews of Abduljalil et al. (2018) and Abduljalil et al. (2021). The growth of the child's body weight was fitted to two measurements: the weight at birth and the weight at the sampling time.

2.3.2. Substance-specific parameters

In our previous model (Brochot et al., 2019), the free fraction of PFOA or PFOS in plasma was assumed to be constant. Dallmann et al. (2017) have collected data on maternal plasma proteins and modelled their concentrations over time during pregnancy. Based on this study,

Codaccioni and Brochot (2020) have computed an increase in the free fraction during pregnancy for the mother and the foetus based on the equations provided by Zhang et al. (2017). The computation of the free fraction during pregnancy was then updated in our model.

New values for the half-lives of PFOA and PFOS in plasma were set based on the review published in the recent EFSA opinion on PFAS (EFSA, 2020). The PFOA half-life was set to 2.5 years and the PFOS one to 4.1 years. The half-life was assumed to be constant over the lifetime.

In a recent study, Mamsen et al. (2019) measured the concentrations of PFAS in human embryos and foetus organs with corresponding placentas and maternal serum samples obtained after selective pregnancy terminations and cases of intrauterine foetal death. From these data, the mother serum concentrations and the cord:serum ratio of PFOA and PFOS (from Brochot et al. (2019)) were used to calculate the serum concentrations in the foetus, and new values for the foetal plasma:tissue partition coefficients were calculated and used in our foetal PBPK model (supplementary material, Table S.3).

2.4. Estimation of the prenatal exposures

The methodology described in our previous work (Brochot et al., 2019) was used to estimate the prenatal exposure to PFOA and PFOS in the children of the HELIX subcohort. Briefly, the maternal exposure was first reconstructed from the maternal PFAS concentration measured during pregnancy by reverse dosimetry using the PBPK model for pregnant women to fit the measurements. Once the maternal exposure was estimated, forward dosimetry was performed to simulate the amounts and concentrations of PFOA and PFOS in the foetus. As described in Brochot et al. (2019), the PBPK model for the pregnant woman and her foetus was individualized with some characteristics reported in the questionnaires: maternal body weight before pregnancy, weight gain during pregnancy, number of pregnancies, date of start and duration of pregnancy(ies), previous breastfeeding and their duration, and the body weight of the newborn at birth.

Because human exposure to PFOA and PFOS tends to decrease since their phase out by their major producer (Gebbink et al., 2015; Glynn et al., 2012; Haug et al., 2009), the dynamic of the exposure was accounted for the exposure scenarios (Fig. 1) as described in Brochot et al. (2019). The birth year of the mother was considered to define her exposure as well as the sample collection year for the maternal plasma. More details are provided in the supplementary material S.5.

The PFOA and PFOS daily intakes (ng/kg body weight (bw)/d) were estimated for each of the 1,239 mothers of the HELIX subcohort according to their individual exposure scenario. The reverse dosimetry was performed in a Bayesian framework and non-informative uniform prior distributions were assigned to the daily intakes (between 1×10^{-5} and 1

$\times 10^3$ ng/kg bw/day). A log-normal distribution (PFOA: 1.89×10^{-5} for the mean and 74 % for the coefficient of variation; PFOS: 6.2×10^{-6} for the mean and 55 % for the coefficient of variation) was assigned to the placental transfer (L/min) to account for inter-individual variability (Brochot et al., 2019). For data likelihood, we assumed a log-normal distribution, where the mean was the predicted concentration in maternal plasma at the sampling time and allowed 15 % variations around the measured concentration in maternal plasma.

A Markov Chain Monte Carlo (MCMC) method was used to simulate a sample of parameter values for each woman. Three independent MCMC were run for 3,000 iterations. One in two of the last 2,000 iterations was recorded and used to produce the results (yielding 1,000 parameter vectors per chain). At the end of this step, a total of 3,000 combinations of these parameters (the daily intakes and the placental transfer rate) were obtained for each HELIX mother. Then, the maternal daily intake and the placental transfer rate combination obtained previously were used as inputs in the PBPK model to simulate the *in utero* internal dose in all tissues over the whole pregnancy (forward dosimetry). Monte Carlo (MC) simulations were run for each parameter combination to simulate inter-individual variability (resulting in a total of 3,000 simulations per individual). Several outputs were simulated: the median PFOA and PFOS amount in several foetal organs during the pregnancy and at delivery, as well as the median PFOA and PFOS breast milk concentrations at delivery.

2.5. Construction of individual exposure scenarios for each child

Postnatal exposure scenarios were built for all the children to account for breastfeeding (duration and levels of PFOA and PFOS in their mother's milk), and the variation of the dietary intake as the consumption of food varies with age during childhood.

2.5.1. Breastfeeding intake

When breastfeeding occurs, the child can be either exclusively breastfed or fed by breast milk and formula milk (mixed feeding). Three parameters define the PFAS intake from breastfeeding: the duration, the volume of breast milk ingested by day, and the concentration of PFAS in the breast milk. From the individual information provided in the questionnaires, the duration of exclusive breastfeeding and mixed feeding were accounted for each child. In the case of exclusive breastfeeding, the volume of milk intake by day evolves with the age of the young child, and was updated in our model with the equation for the weight-normalized human milk intake provided by Yeung et al. (2020) as follows (Eq. (1)):

$$F_{milk_{total}} = 160.39 \times \frac{0.232}{0.232 - 0.00252} \times (e^{-0.00252 \times Age} - e^{-0.232 \times Age}) \quad (1)$$

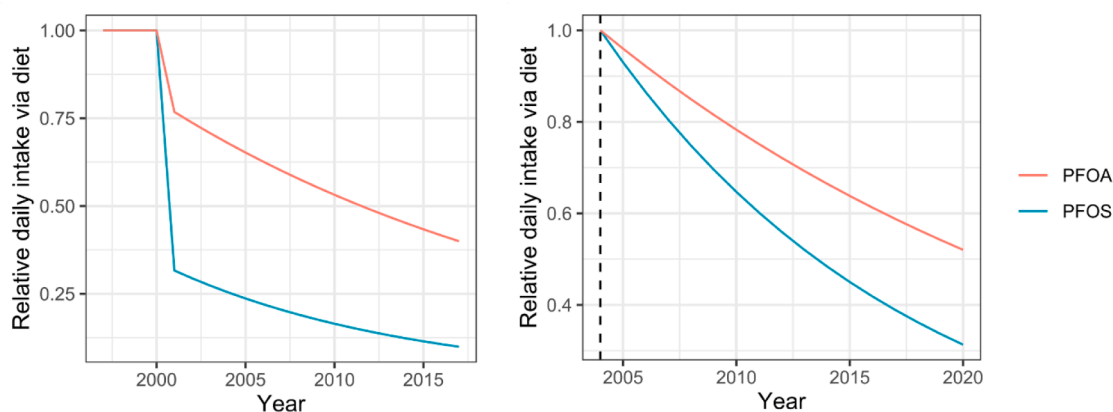


Fig. 1. PFOA and PFOS diet daily intake for (i) a mother born before the year 2000, normalized by the year 2000 diet intake (left panel) and (ii) a child born after the year 2000, normalized by the birth diet intake (right panel). The curves are the model predictions for PFOA (red) and PFOS (blue).

where $Fmilk_{total}$ is the volume of milk intake by infant per day (in mL/d) and Age is the age of the child (in days). When exclusive breastfeeding has stopped or in the case of mixed feeding, the volume of milk intake per day was set at 400 mL/d (Neville et al., 1991; Neville et al., 1988).

The PFAS breast milk concentrations decrease over the breastfeeding period as illustrated in the study of Thomsen et al. (2010). The decrease of the PFOA or PFOS concentrations in milk when the individuals are breastfed was introduced by a new variable in the PBPK model. This variable, $C_{milk_{evol}}$, is equal to the individual milk concentration estimated at delivery ($\mu\text{g/L}$) until 16 days (Eq. (2)), based on the data from Thomsen et al. (2010), and starts to decrease after 16 days (Eq. (3)):

$$\text{If } t < 16\text{days, } C_{milk_{evol}} = C_{milk_{input}} \quad (2)$$

$$\text{If } t \geq 16\text{days, } C_{milk_{evol}} = C_{milk_{input}} \times e^{-0.0024 \times t - 16} \quad (3)$$

The daily intake through breast milk ($DI_{breastfeeding}$, in ng/kg bw/d) was then obtained by multiplying the PFOA or PFOS concentration in milk by the daily body weight-normalized milk intake ($Fmilk_{total}$) (Eq. (4)):

$$DI_{breastfeeding} = Fmilk_{total} \times C_{milk_{evol}} \quad (4)$$

2.5.2. Diet intake

The dynamic exposure for the child intake was based on the changes in PFOA and PFOS exposures in the environment over the years, as for their mothers (Fig. 1), but also on the evolution of his/her consumption of the different food groups during the early years. The recent EFSA scientific opinion (EFSA, 2020) reviewed the PFOA and PFOS dietary intakes for different subgroups of the populations in several countries. Based on their data, factors that allocate the adult intake to children were computed for PFOA: 3.99 for infants (≤ 1 y.o), 4.21 for toddlers (≥ 1 y.o to < 3 y.o), 2.47 for children (≥ 3 y.o to < 10 y.o), and 1.41 for teens (≥ 10 y.o to < 18 y.o). For PFOS, the factors were: 3.65 for infants, 3.80 for toddlers, 2.75 for children, and 1.41 for teens. Given these values, children (from newborn to teenagers) are more exposed than adults to PFOA and PFOS by diet relative to body weight. For instance, infants are 3.99 more exposed than adults in the same calendar year. Only some of the HELIX countries (*i.e.*, Spain, France, and the UK) were considered to calculate these factors (supplementary material, S.5).

In our model, the dietary exposure includes exposure via food and water consumption (which are the main exposure sources according to the work of Rovira et al. (2019)), while non-dietary sources are considered negligible in our approach, and was implemented with the following equation (Eq. (5)):

$$DI_{diet} = \text{Frac}_{Intake_{age}} \times DI_{temporal} \quad (5)$$

where DI_{diet} is the daily dietary intake (ng/kg bw/d), $\text{Frac}_{Intake_{age}}$ is the factor from dietary intakes according to the age classes, and $DI_{temporal}$ (ng/kg bw/d) is the oral intake accounting for temporal trends (supplementary material, S.5).

2.5.3. Total intake

The total intake (ng/kg bw/d), DI_{total} , for children is the sum of the breastfeeding intake (if the child is breastfed) and the dietary intake (including formula milk). For all children, it was assumed that food (other than breast milk) was introduced at the age of 6 months. Three scenarios for computing the dietary intake were then possible.

In the first scenario, the infant is exclusively breastfed until an end date (but before 6 months old, Eq. (6)), then the infant is fed exclusively through the diet (Eq. (7)):

$$DI_{total} = DI_{breastfeeding}, \text{ for } 0 < \text{age} < \text{stop breastfeeding} \quad (6)$$

$$DI_{total} = DI_{diet}, \text{ for age } \geq \text{stop breastfeeding} \quad (7)$$

In the second scenario, the infant is exclusively breastfed for six months

(Eq. (8)). Then, the infant is fed both through diet and breastfeeding, also called partial breastfeeding (Eq. (9)). Finally, when the breastfeeding stops, the child is fed exclusively through the diet (Eq. (10)):

$$DI_{total} = DI_{breastfeeding}, \text{ for } 0 < \text{age} < 6 \text{ months} \quad (8)$$

$$DI_{total} = DI_{breastfeeding} + DI_{diet}, \text{ for } 6 \text{ months} < \text{age} < \text{stop breastfeeding} \quad (9)$$

$$DI_{total} = DI_{diet}, \text{ for age } > \text{stop breastfeeding} \quad (10)$$

The third scenario is that the child is not breastfed. In this case, the exposure is exclusively from diet. Thus, the total intake is defined as follows (Eq. (11)):

$$DI_{total} = DI_{diet} \quad (11)$$

2.6. Estimation of the early childhood exposure

In this last step, the PBPK model was run for the 556 girls and 683 boys of the HELIX subcohort, to estimate, by reverse dosimetry, their postnatal exposure. This was based on the measured PFOA or PFOS concentrations in plasma around the age of 8, their individual exposure scenario accounting for breastfeeding and dietary intakes, and their prenatal exposures. The PBPK model was individualized with the characteristics of the child reported in the questionnaires: year of birth, body weight at birth, and body weight at the blood draw. As described in the previous section, the breast milk intake and the dietary intake were also individualized for each child. To account for prenatal exposure, PFOA and PFOS amounts in each tissue at birth were defined as inputs in the PBPK model. The reverse dosimetry was performed in a Bayesian framework. A prior was defined for the daily intakes at the individual level as a uniform distribution (between 1×10^{-8} and 1×10^2 ng/kg bw/d). For data likelihood, we assumed a log-normal distribution, where the mean was the predicted concentration in child plasma at the sampling time and allowed 15 % variations around the measured concentration in child plasma. The PFOA and PFOS daily intakes were estimated individually for each of the 556 girls and 683 boys. To obtain a sample of parameter values for each child, 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 1,000 parameter values per chain for one child). Finally, the PFOA and PFOS internal concentrations were predicted during whole childhood using the associated postnatal daily intake estimated and individual exposure scenarios for each child.

2.7. Codes, software and statistical analyses

The PBPK model codes are available at <https://doi.org/10.5281/zenodo.7303637> (supplementary material (S.6)). All the MCMC or MC simulations were performed with the GNU MCSim software (version 6.2.0, <https://www.gnu.org/software/mcsim/>). The R criterion was used to check the convergence of the chains (Gelman et al., 1995). Convergence is assumed to be achieved when all R criterion values are below 1.2 (supplementary material, S.7). For statistical analyses and graphical visualizations, we used the R software (version 4.0.4, <https://cran.r-project.org/>).

Possible significant differences between (i) children's concentrations, (ii) children's daily intake, (iii) prenatal concentrations, and (iv) prenatal daily intake with some parameters were evaluated. First, the data were normalized in a logarithm scale. Then, Shapiro-Wilk tests were performed to check the normality of the data. If the normality was not achieved (p -value < 0.01), Kruskal Wallis tests were performed instead of 1-way ANOVA tests (supplementary material, S.7).

3. Results

3.1. Convergence checking

The convergence of the MCMC chains was checked before their analysis. For the prenatal life, the three MCMC chains have reached convergence for both PFOA and PFOS. For the postnatal life, the three MCMC chains have reached convergence for a large majority of the children. For PFOA, the chains did not converge for one boy among the 1,239 children. The same was the case for seven boys (four from Lithuania, two from Spain and one from the UK) for PFOS. Thus, the results for these children were not considered in the following. The reasons for this non-convergence were investigated (see section 4). Regarding the children included in the analysis of the result ($n = 1,238$ for PFOA and $n = 1,232$ for PFOS), their external exposures were successfully optimized to minimize the difference between the predicted and measured maternal and children plasma concentrations (between a factor of 1.2 and 2.3 difference, supplementary material, S.8), with the exception of six children for PFOS.

3.2. Evolution of PFAS internal exposure during early life

3.2.1. Plasma

The PFOA and PFOS concentrations in venous plasma were predicted for each child in the HELIX subcohort from her/his conception until 10 y.o. Fig. 2 presents the population levels with the inter-individual variability. The predicted plasma concentrations at the first and second trimesters of pregnancy were higher for PFOA than for PFOS, which is consistent with the placental transfer values and the PFAS concentrations measured in mothers. At birth, PFOA and PFOS plasma concentrations reached a similar level (0.87 [0.004–23.7] $\mu\text{g/L}$ for PFOA and 0.77 [0.012–14.7] $\mu\text{g/L}$ for PFOS).

After birth, from the age of 1 y.o, the median PFOS concentration of the subcohort was higher than the one of PFOA. Starting from 2 y.o, the

distribution of the PFOS plasma concentration is more spread than the one for PFOA, indicating a higher inter-individual variability for PFOS. The ratio between PFOS and PFOA concentrations slightly evolves over the years and is equal to 1.26 at 1 y.o, 1.36 at 2 y.o, 1.42 at 3 y.o and 1.37 at 5 y.o. For most of the HELIX children, it seems that a steady state for PFOA and PFOS plasma concentration is reached between 5 and 6 y.o. At 10 y.o, the variability of concentrations in plasma is less scattered. The population PFOA and PFOS maximum plasma concentration (C_{max}) are predicted at 4.80 $\mu\text{g/L}$ and 6.02 $\mu\text{g/L}$ respectively, and they are reached around 6 and 9 months old, respectively.

The PFOA and PFOS concentrations according to the birth country are presented in the supplementary material (S.9). Some differences are observed between the six countries: Norway, France and Spain exhibit the highest PFOA plasma concentrations, and Norway and France for PFOS. In all countries but the UK, the PFOA concentrations are lower than the PFOS concentrations. Children in Spain and Lithuania reached similar concentrations for both compounds until 10 y.o. The highest differences in PFOA and PFOS concentrations are observed in France and Norway. At the time of blood sampling for children, PFOS plasma concentrations were significantly different in the studied countries (except for two cases), whereas this pattern is not observed for PFOA (supplementary material, S.9).

For similar PFOA or PFOS measured concentrations in plasma at the same sampling times (*i.e.*, children with the same age at the test), a high inter-individual variability can be predicted during early life (Fig. 3). The main variations are predicted between birth and 3 y.o for both compounds, in particular on the maximum concentration (C_{max}) of PFOA or PFOS in plasma, mostly observed during the first two years. Between birth and 1 y.o, C_{max} is reached for 88 % (PFOA) and 77 % (PFOS) of the HELIX children. For the other children, C_{max} were predicted at 3 y.o for PFOS, and at 3, 6 and 7 y.o for PFOA. The maximum plasma concentrations increased on average by a 2.9- and 3.4-factor for PFOA and PFOS, respectively, in comparison to the birth plasma concentration, with 28 and 73 children exceeding a factor of five for PFOA

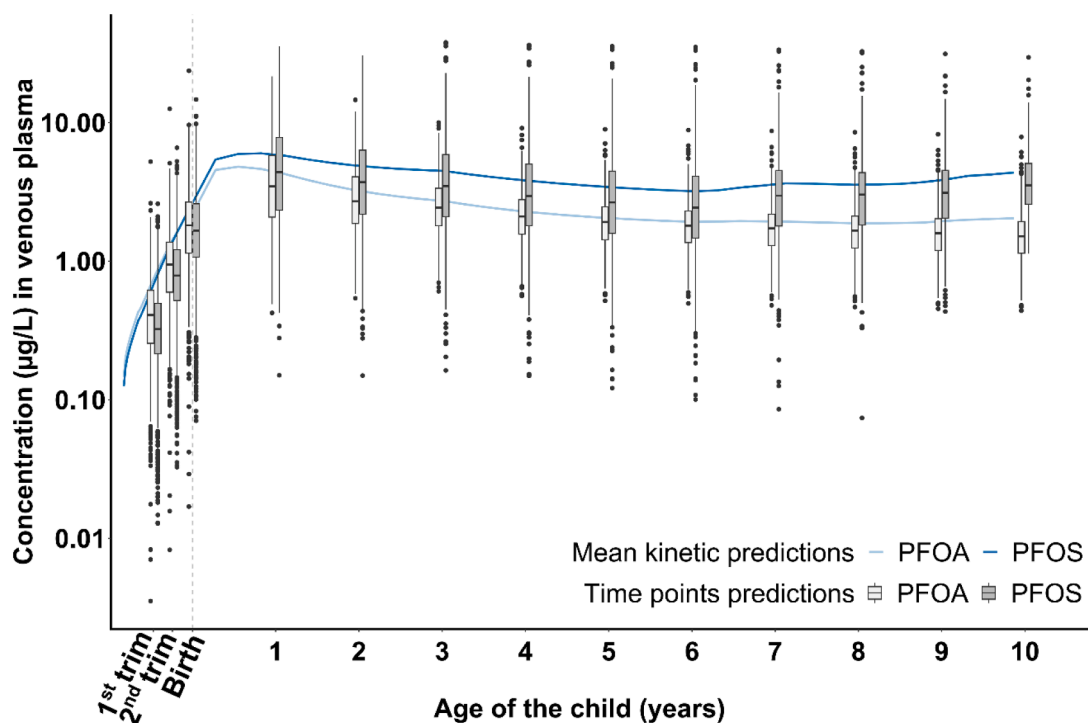


Fig. 2. PFOA (light grey, $n = 1,238$) and PFOS (dark grey, $n = 1,232$) predicted venous plasma concentrations ($\mu\text{g/L}$) for all HELIX children at three time points during pregnancy (end of the first and second trimesters, and at birth) and during childhood. The kinetics of mean estimated venous plasma kinetic concentrations ($\mu\text{g/L}$) of all children are represented by lines for both PFOA (light blue) and PFOS (dark blue). The vertical grey dashed line represents the birth of the HELIX children. The y-axis is in a decimal logarithm scale.

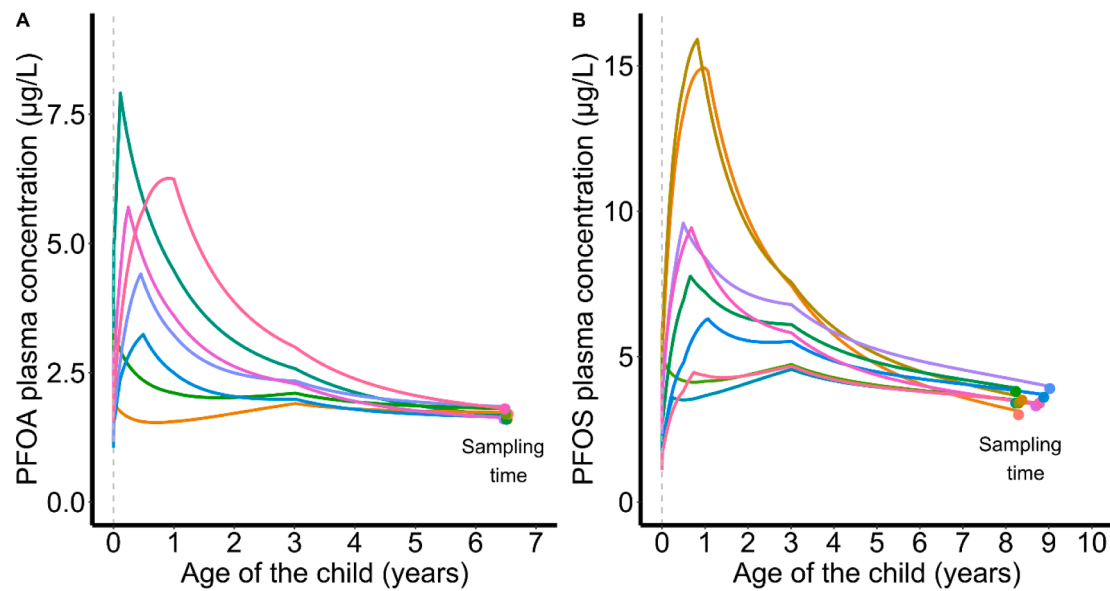


Fig. 3. Example of PFOA (A) and PFOS (B) toxicokinetic profiles during early life incorporating individual scenarios exposure. These profiles are for HELIX children with a sampling time between 6 and 7 y.o (PFOA) and 8 and 9 (PFOS) y.o. The dots represent the children’s sampling time (blood sample). The same colour in (A) and (B) does not represent the same child.

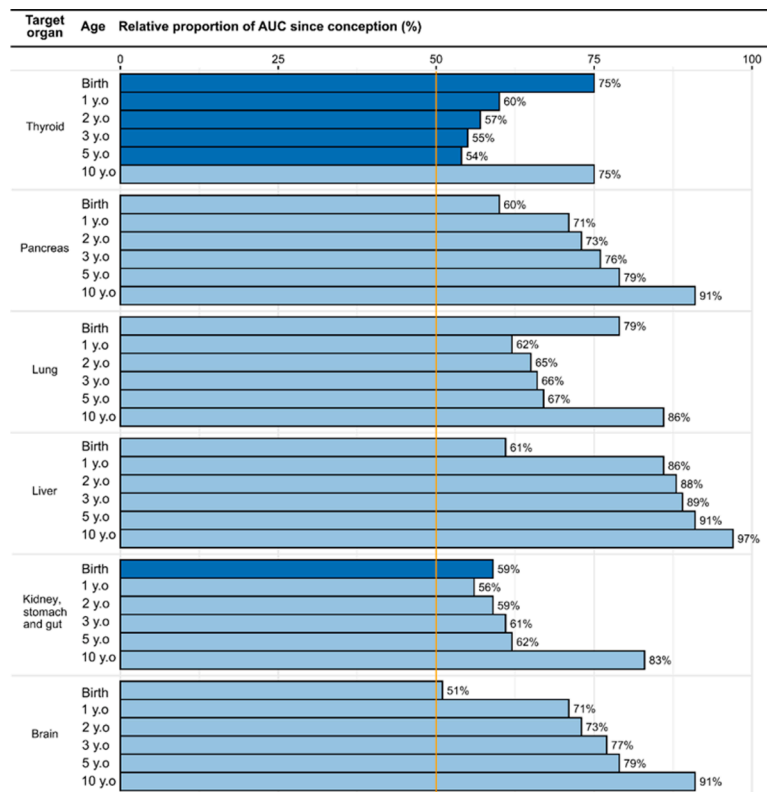
and PFOS, respectively. These differences can be explained by the exposure pathways (*in utero* exposures, breastfeeding, and diet) and are detailed in a next section (section 3.3).

3.2.2. Target organs

The PBPK model was used to predict the PFOA and PFOS amounts in several foetal and child target tissues (linked to the effects reported in the literature, e.g., by Kirk et al. (2022): kidney (Blake and Fenton, 2020; Dötsch et al., 2012; Hershkovitz et al., 2007), thyroid (Blake and Fenton,

Table 2

PFOA and PFOS ranking by the AUC indicators (µg.day/L) in each target organ for the HELIX children (n = 1,232). The results are in percentages of the individuals. The light blue cells indicate that PFOS is in the highest proportion, while the dark blue cells indicate that PFOA is in the highest proportion. The orange line symbolises the 50 % proportion, which indicates equal proportions of both compounds.



2020; Lee and Choi, 2017), lung (Zhang et al., 2021), liver (Gallo et al., 2012), pancreas (Abudayyak et al., 2021), brain (Starnes et al., 2022; Yu et al., 2016), gut and stomach (Rashid et al., 2020; Wang et al., 2021). The PFOA and PFOS area under the curve (AUC) in these target tissues were predicted over childhood. The AUC represents the cumulative exposure over a time period since conception. For each child, the PFOA and PFOS AUC (arithmetic mean value, $n = 1,238$ for PFOA and $n = 1,232$ for PFOS) in the target organs were compared at several ages, and the number of children having a higher or lower PFOA AUC than PFOS ones was computed (Table 2). The prenatal AUC in the brain is similar for both compounds, while the gut, kidney, stomach, and thyroid have a higher AUC for PFOA than PFOS. The prenatal AUC for PFOS is higher for the lungs, pancreas, and liver than for PFOA. From 1 y.o., the cumulative exposure is higher for PFOS than for PFOA in most organs, except thyroid, for which a higher partition coefficient is assigned for PFOA (0.38, against 0.26 for PFOS). Besides, during childhood, the relative PFOA over PFOS exposures do not change significantly over time.

3.3. Contribution of the exposure pathways to early life exposure

The individual factors that influence the TK profiles were investigated, as our model predictions show that children with similar levels at the same age can exhibit very different internal exposure during their

first three years (Fig. 3). Several factors can explain these inter-individual differences, such as prenatal life exposure, breastfeeding, and the child's diet.

Fig. 4 presents the relative contribution of the three exposure pathways to PFOA and PFOS plasma concentrations over childhood in the HELIX children. The relative contributions were established for each HELIX child and then averaged at the population level. Between birth and the first four months, internal exposure to PFOA and PFOS is mostly explained by the *in utero* exposure and then decreases with the child's growth. Between 5 months old and 2 (PFOA) or 5 (PFOS) y.o., breastfeeding is the main source of exposure. From 3 y.o. for PFOA or 6 y.o. for PFOS, the diet exposure contributes more than half to the internal concentration and increases as the child grows. This picture, which illustrates the population exposure contribution, masks high inter-individual variabilities (e.g., no breastfeeding for some children). Fig. 5 shows PFOA TK profiles for three individuals with different exposure scenarios, as well as their associated contributions of exposure pathways on the internal concentration that are also described in the following.

3.3.1. In utero exposure

To illustrate the influence of prenatal exposure, Fig. 6 presents the PFOA plasma profiles of two HELIX children from Greece, with different PFOA levels at birth (0.68 and 2.27 $\mu\text{g/L}$) but similar levels at blood

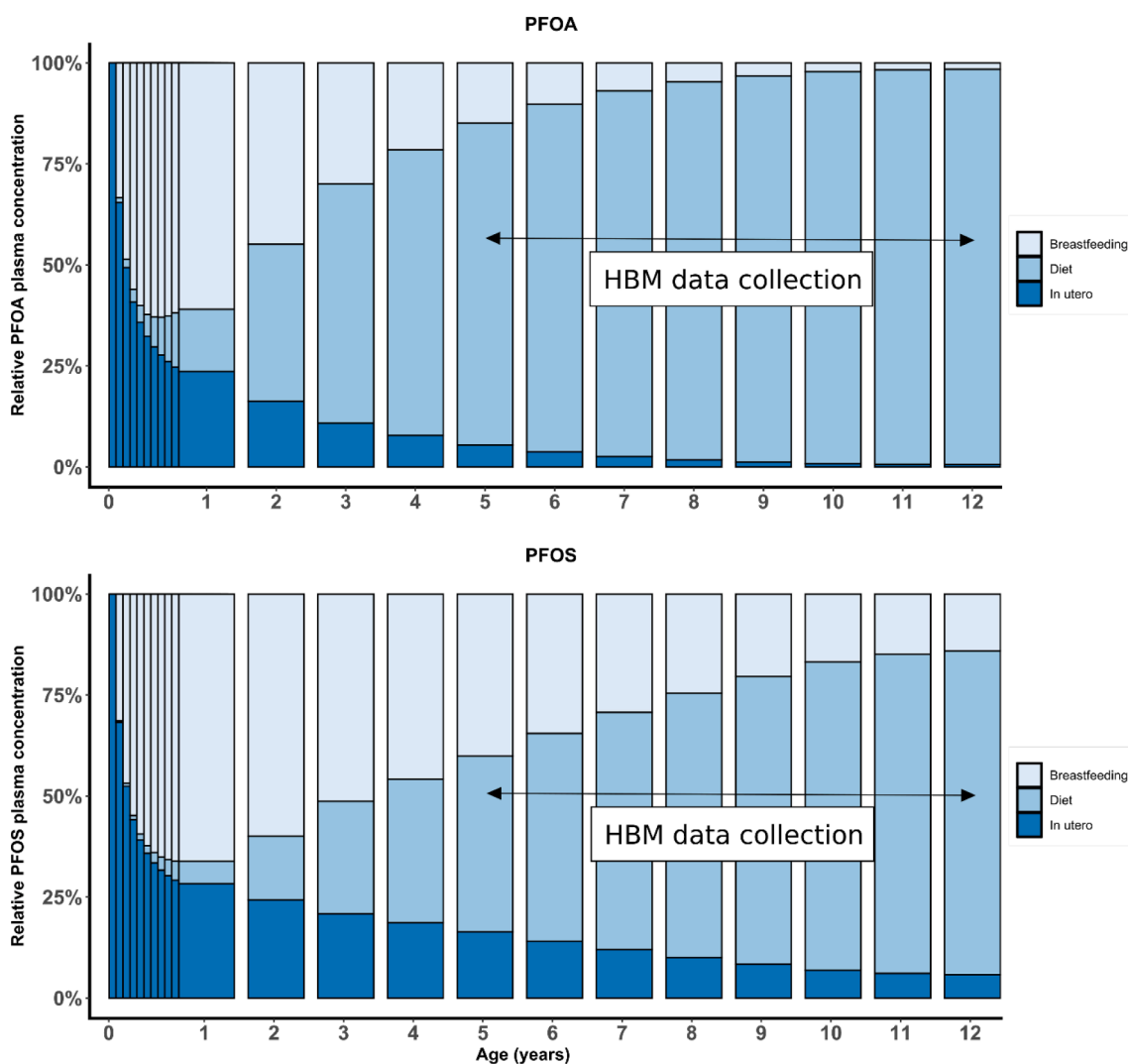


Fig. 4. Predicted relative contribution of exposure pathways (breastfeeding, diet and *in utero*) on PFOA and PFOS plasma concentration over childhood at a population level. The window of HBM data sampling for the HELIX subcohort is indicated by the arrows (between 5.4 and 12.1 y.o.).

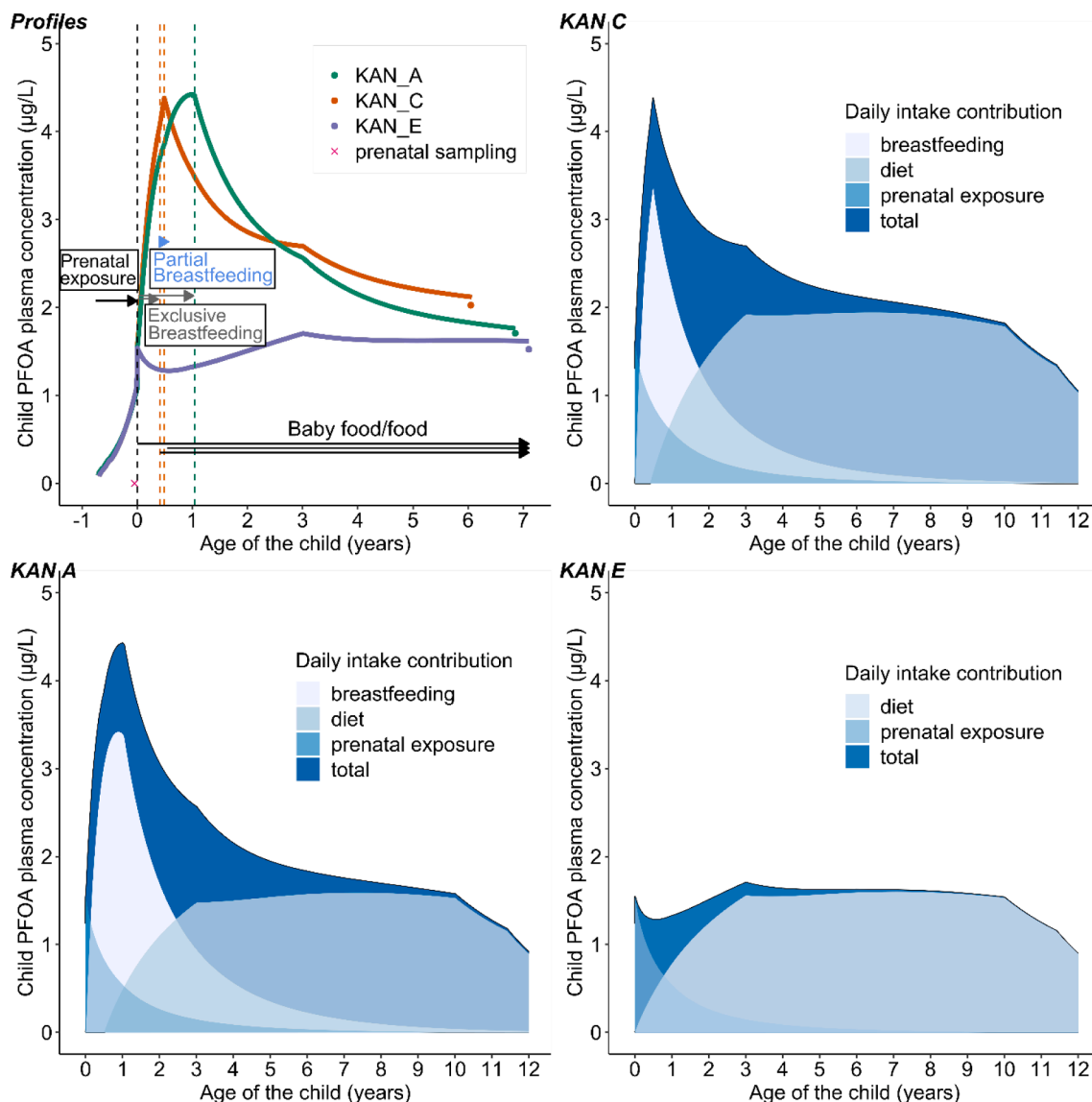


Fig. 5. Example of PFOA (a) toxicokinetic profiles for three HELIX children from Lithuania with variations of the breastfeeding duration, and (b), (c) and (d) PFOA plasma concentration ($\mu\text{g/L}$) simulated for the individuals KAN_A, KAN_C and KAN_E respectively over childhood depending on (i) only prenatal exposure, (ii) only diet exposure, (iii) only breastfeeding exposure when applicable and (iv) total exposure (sum of prenatal, diet and breastfeeding exposures). The individuals have similar levels at birth ($1.1 \mu\text{g/L}$), identical levels in breast milk ($0.05 \mu\text{g/L}$ for KAN_A and KAN_C) and the same range of daily intakes (KAN_A: 0.25 , KAN_C: 0.30 , KAN_E: $0.25 \text{ ng/kg bw/day}$). Only a difference in the breastfeeding duration is noticed: KAN_A was breastfed exclusively for 380 days (symbolized by the longest grey arrow on the top left panel (a)), KAN_C was in exclusive breastfeeding for 150 days (symbolized by the shortest grey arrow on the top left panel (a)) and then in partial breastfeeding for 30 days (symbolized by the blue arrow on the top left panel (a)), and KAN_E was not breastfed. The prenatal sampling was performed at delivery or the week before for the three children so that only one prenatal sampling is illustrated on the top left panel (a).

sampling for the children (equal to $1.17 \mu\text{g/L}$). The two children were not breastfed and had similar estimated daily intake (DI) ($0.18 \text{ ng/kg bw/day}$). After 3 y.o, the plasma concentration is similar, indicating that the *in utero* exposure has a lower influence after this age due to the half-life of PFOA. It is considered that after one half-life, 50 % of a substance is eliminated, and after five half-lives, about 97 % of a substance is eliminated. In the case of PFAS with long half-lives, it is expected that the *in utero* exposure will still influence internal exposure even after 6 y.o. For children that are not breastfed, the relative contributions from prenatal exposure are still high at 1 y.o, being 55 % for PFOA and 73 % for PFOS, compared to 23 % for PFOA and 31 % for PFOS for breastfed children. This highlights that the relative contribution of prenatal exposure is different depending on whether the children are breastfed or not and should be considered when reconstructing their early life exposure.

3.3.2. Breastfeeding

Breastfeeding is an important exposure pathway to PFAS, especially in the first months of life as illustrated in Fig. 5. The predicted median breast milk concentrations at birth for the HELIX subcohort were quite similar for PFOA ($0.076 [0.001\text{--}0.394] \mu\text{g/L}$) and for PFOS ($0.072 [0.003\text{--}0.603] \mu\text{g/L}$), leading to similar intakes via breastfeeding ($DI_{\text{breastfeeding}}$) for both compounds. The maximum $DI_{\text{breastfeeding}}$ of the children was on average $0.035 [0\text{--}0.20] \text{ ng/kg bw/day}$ for PFOA and $0.036 [0\text{--}0.31] \text{ ng/kg bw/day}$ for PFOS. At 1 month, breastfeeding contributes to 33 % (PFOA) and 31 % (PFOS) of the total exposure (Fig. 4), while at 6 months it represents 62 % (both PFOA and PFOS), and at 1 y.o it accounts for 61 % (PFOA) and 66 % (PFOS).

For breastfed children ($n = 1017$), the C_{max} of PFOA in plasma is reached at the end of breastfeeding for 64 % of children, at 3 y.o for 9 % of children, close to birth for 12 % of them, and after 6 y.o for five

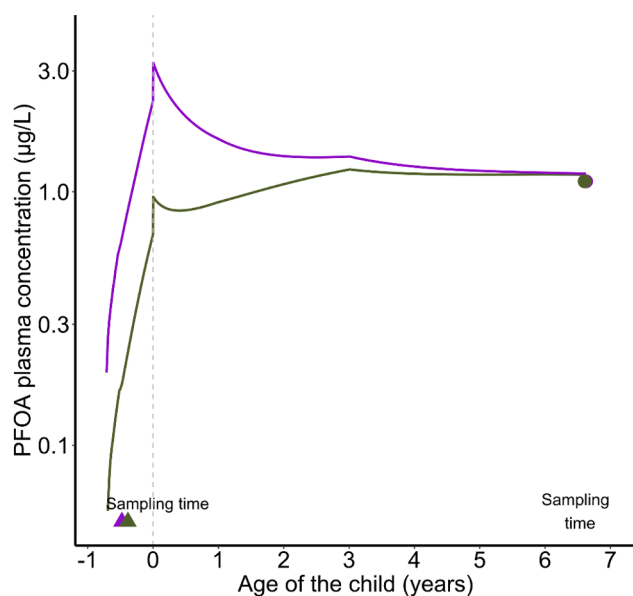


Fig. 6. PFOA TK profiles for two HELIX children (birth country: Greece). Those children were not breastfed, they are both the third child in the family and have similar PFOA plasma concentrations at the time of the second sampling (coloured circles). The triangles symbolize the sampling time for the maternal biomarker's measurement. The dashed vertical line represents the birth of the children.

children. For children that were not breastfed, the C_{max} of PFOA in plasma is reached at birth (77.5 %) or at 3 y.o (22.1 %), except for one child. For PFOS, when children were breastfed ($n = 1009$), C_{max} is reached at the end of breastfeeding for 58.2 % of children, at 3 y.o for 16.3 % of children and close to birth for 9.2 % of them. A one-way ANOVA showed that PFOA and PFOS C_{max} are significantly higher in breastfed children and that the longer the breastfeeding, the higher the maximum predicted plasma concentration (p -value ≤ 0.007) (supplementary material, S.10).

3.3.3. Dietary intakes

Diet is an important contributor to the internal exposure to PFOA and PFOS as illustrated in Fig. 4. As HELIX children get older, diet becomes the main source of exposure. In the window of HBM data collection for the HELIX subcohort, the relative contribution from diet evolves from 51 % and 57 % for PFOA and PFOS respectively at 6 y.o, to 81 % at 12 y.o for both PFOA and PFOS. Consequently, as the diet exposure increases throughout the years, the HELIX HBM data collected for children aged from 5.4 y.o will give information mainly on the diet exposure rather than the breastfeeding and the *in utero* exposure. In Fig. 5, a similar diet contributions curve shape is observed for the three individuals over the years. It follows the factors from diet intakes according to the age classes, which change at 1, 3, 10 and 18 y.o (EFSA, 2020), the highest being for infants (3.65- or 3.99-factor for PFOA and PFOS, respectively) and toddlers (3.8- or 4.21-factor for PFOA and PFOS, respectively). However, in terms of relative contributions to the total exposure pathways over time, differences can be observed between individuals. For the three children represented on Fig. 5, the exposure through diet represents 11 % (KAN_A), 20 % (KAN_C) and 59 % (KAN_E) of the total exposure pathways at 1 y.o, while at 3 y.o it represents 58 % (KAN_A), 71 % (KAN_C) and 91 % (KAN_E), at 6 y.o it accounts for 86 % (KAN_A), 91 % (KAN_C) and 98 % (KAN_E), and at 10 y.o it accounts for 97 % (KAN_A), 98 % (KAN_C) and 99 % (KAN_E). The greatest variations in the dietary exposure contribution in children are observed in early life as prenatal exposure and breastfeeding also contribute considerably to the exposure at this age.

3.4. Estimation of the total intake

The total daily intake of each HELIX child can be computed as the sum of the diet and the breastfeeding intakes. The PFOA and PFOS median DI_{total} were computed at several ages for all children (Fig. 7). The DI_{total} decreases for both PFOA and PFOS over the years whatever the country considered. The maximum DI_{total} are observed between birth and 1 y.o, equal to 2.78 and 10.7 ng/kg bw/day for PFOA and PFOS respectively. France and Norway exhibit the highest total intakes for PFOS, and France, the UK and Norway for PFOA. Besides, higher total intakes of PFOA than PFOS are predicted in the UK, Lithuania and Spain.

Statistical tests were performed and indicated that the estimated DI (independent of age) was not statistically associated with some individual characteristics. The PFOA and PFOS median DI estimated are not associated with the sibling position of the child in the family, neither the breastfeeding duration, maternal age, maternal body mass index and birth weight. However, estimated DI of both PFOA and PFOS, independent of age, were significantly associated with some groups of maternal concentrations, predicted breast milk concentrations at birth and the age of the child at the test. However, the highest maternal concentrations were not always associated with the highest children DI ($r^2 = 0.22$ for PFOA, 0.26 for PFOS), meaning that other factors than prenatal exposure influence postnatal exposure. The detailed results are reported in the supplementary material, S.7.

As mentioned in the scientific opinion of EFSA (2020), a tolerable weekly intake (TWI) of 4.4 ng/kg bw/week for four PFAS (PFOS, PFOA, PFNA, PFHxS) was recommended, corresponding to a serum concentration of 6.9 ng/mL in women of reproductive age, based on a serum concentration of 17.5 ng/mL (benchmark dose (lower confidence limit), $BMDL_{10}$) in children of 1 y.o (EFSA, 2020). In the scientific opinion (EFSA, 2020), recommendations are provided to not compare the TWI to the intake of infants, especially when it considers the intake via breastfeeding. Thus, the threshold value of the $BMDL_{10}$ (17.5 µg/L) was used for a first comparison with our approach, and as being the most relevant according to the recommendations provided in the scientific opinion (EFSA, 2020). In the HELIX subcohort, a total of 251 individuals exceeded this threshold: 102 girls and 149 boys (80 in France, 16 in Greece, 106 in Norway, 32 in Spain, and 17 in the UK) were predicted to have a higher sum of PFOS and PFOA concentrations. The concentrations were predicted to exceed the $BMDL_{10}$ between birth and 1 y.o mostly (minimum: 0.1 y.o, maximum: 3 y.o, 95th percentile: 1.08 y.o, arithmetic mean: 0.76 y.o).

4. Discussion

The framework proposed here allows to estimate the early life exposure of HELIX children to PFOA and PFOS and to assess the contribution of three exposure pathways throughout the years. HBM data were used, and two biomarker measurements were available for each child. A PBPK model for PFOA and PFOS including pregnancy, lactation and childhood, was updated and run for more than a thousand mother:child pairs while accounting for their individual characteristics.

To our best knowledge, our study is the first to link a mother-foetus-child PBPK model applied to PFAS, which includes and combines *in utero* exposure and several oral exposure pathways at the same time. A few studies have reported TK (Verner et al., 2016) or PBPK models for early postnatal life on persistent compounds (Deepika et al., 2021; Shin et al., 2018; Verner et al., 2013; Verner et al., 2010), and most of them have considered exposure pathways separately (e.g., lactation and *in utero* exposure; *in utero* exposure and diet, or only lactation). One strength of our approach is to describe mechanistically the PFAS toxicokinetics for both the prenatal and postnatal life using one PBPK model for each period. The continuity in the exposure between prenatal and postnatal life was ensured and required to update the existing PBPK models. Although the more data the better, accounting for several measurements

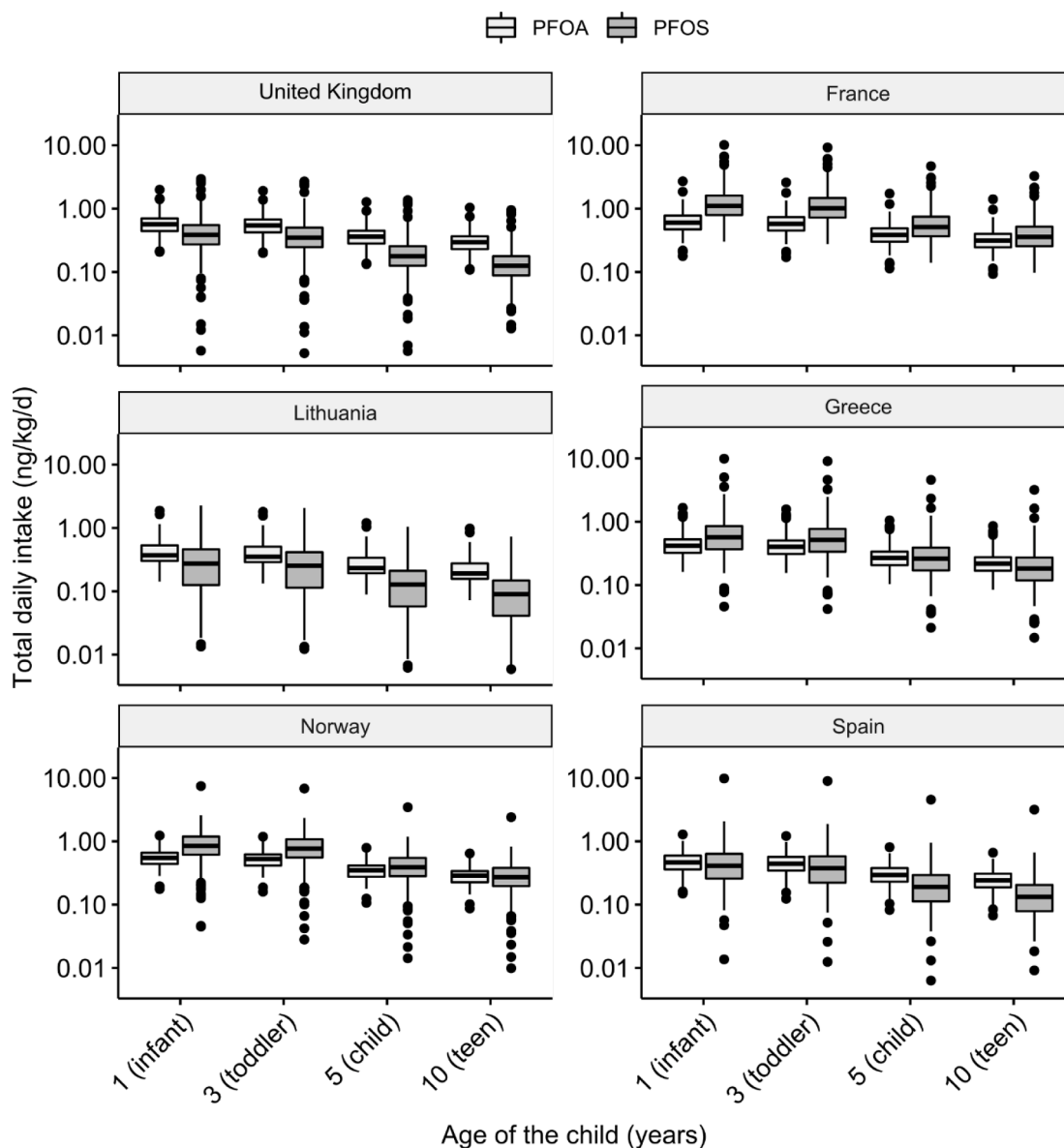


Fig. 7. PFOA and PFOS total daily intakes estimated for the HELIX children at several ages (years) in the six countries. The y-axis is in a decimal logarithm scale.

about the physiology of the child can be challenging. For instance, the body weight records at birth and at the second sampling time could not overlay the standard body weight curve and some new equations have been derived.

One of the main results of our study is that similar PFAS blood levels in children measured on two occasions can correspond to very different internal exposures between the two (*i.e.*, concentration–time profile). Our PBPK model combined with HBM data (biomarkers and questionnaires) can provide realistic predictions of the time-courses of internal PFAS levels in plasma and target organs at any time during pregnancy or childhood. It allows to compare populations of different ages from different countries and to improve the power of subsequent analyses of effects (Krauss et al., 2015; Sohn et al., 2004). Three exposure pathways were accounted for in our study: prenatal exposure, breastfeeding and diet. *In utero* exposure influences PFAS levels at birth and during the first year. That source is particularly relevant when investigating the effects of chemicals in sensitive populations such as infants, toddlers and small children, especially on specific developmental processes (Rice and Barone, 2000; Verner et al., 2013). For most of the children, the maximum concentration in plasma is predicted to occur between birth

and 2 y.o. and is linked to breastfeeding for breastfed infants. Breastfeeding being the predominant exposure pathway during this sensitive window has been reported in other studies (Koponen et al., 2018; Mogensen et al., 2015; Papadopoulou et al., 2016). As illustrated by Fig. 3, the inter-individual variability of the plasma concentration can be very high during that time. Future studies should consider blood and/or breastmilk sampling in order to provide data for assessing the PBPK model predictions and for studying their association with health effects. Even though at similar concentrations at birth, it was also observed that the blood concentration at the date of the second sampling (6–10 years old) was not significantly impacted by the breastfeeding duration. To our knowledge, no studies have shown a correlation between breastfeeding duration and PFAS blood levels in teenagers (Criswell et al., 2023; Hoadley et al., 2023; Papadopoulou et al., 2016). In our study, the estimation of that exposure was rendered possible by using information collected via the questionnaires (*e.g.*, duration of breastfeeding) and simulating the concentration in breast milk with the PBPK model for the mother. Although experimental data support the modelling of the PFAS transfer to breast milk, it could be envisioned to collect data on a small number of mother–child pairs to evaluate the model predictions, such as

breast milk and child blood samples. Ultimately, such data could help in a better understanding of the transfers to breast milk and its absorption by the breastfed child and could reduce uncertainty in the exposure estimation. However, the collection of blood samples in early life is often challenging or impractical in epidemiological studies (Verner et al., 2015).

Exposure to PFOA and PFOS via diet for the mother and the child was assumed to decrease over the study's duration, as supported by the decrease in PFOA and PFOS production (Paul et al., 2009; Wang et al., 2014). This assumption resulted in quite significant daily intakes during the critical window of 0–3 y.o, as the food intake factors provided by EFSA (2020) are high compared to other age groups (a 3- or 4-factor for early life food intake compared to adults). For now, the geographical differences among the HELIX children were not accounted for in the computation of the dietary consumption factors but the mean values of European countries involved in the HELIX cohort were used. If national or regional data becomes available on local exposure pathways and their magnitude, it could be incorporated to refine the maternal and children's exposure and deliver daily intakes accounting for geographical differences. Regarding the exposure pathways, only diet (including breast milk) and *in utero* exposure were integrated into our approach as they are the main routes of exposure to PFAS, as also mentioned in several studies (e.g., Fisher et al. (2016); Fraser et al. (2012); Haug et al. (2010); Poothong et al. (2020)), where oral ingestion represents more than 95 % of the total exposure (e.g., Rovira et al. (2019)). To a lesser extent, humans can be exposed through dermal contact by cosmetics (skin, accounting for around less than 1 % of the total exposure according to Trudel et al. (2008)) or inhalation (dust, accounting for around 1.5 % of the total exposure according to Rovira et al. (2019)), especially for young children in direct contact with dust while crawling on the floor (Haug et al., 2011; Winkens et al., 2017). While our PBPK model considers only exposure through the oral ingestion route, one improvement could be to consider the two other routes, such as performed by Rovira et al. (2019) for the ingestion and inhalation exposure routes or by Husøy et al. (2023) for exposure through diet and personal care products.

Some uncertainties in our approach could limit its applicability in risk assessment. Despite very recent improvements in the modelling of the physiology and anatomy of the foetus throughout the pregnancy (Abduljalil et al., 2018; Abduljalil et al., 2021; Dallmann et al., 2017; Kapraun et al., 2019; Thépaut et al., 2023), the PFAS TK is still poorly known due to an evident lack of data thus limiting the predictability of the foetal PBPK model that is used to assess *in utero* internal exposures and body burdens at birth. In this work, the most up-to-date parameters' values were used for the foetus. For instance, the foetal partition coefficients were updated using a recent study (Mamsen et al., 2019) that provides PFAS concentrations in several fetal organs (liver, lung, heart, central nervous system and adipose tissue) throughout the three trimesters of pregnancy. The foetal partition coefficients were obtained from these data and from the foetal serum concentrations, which were calculated from maternal serum concentrations and cord:serum ratios available for other individuals (Brochot et al., 2019). This approach may introduce additional uncertainties, particularly because the maternal samples were not collected alongside the fetal samples (Mamsen et al., 2019), leading to a potential overestimation of partition coefficients as a decrease in maternal serum concentrations was not accounted for. Thus, this approach leads to a limitation of the model concerning the fetal target tissues for PFOA and PFOS. Adding to that, the PFOA placental transfer rate is highly variable compared to PFOS. This is a key parameter for prenatal exposure, and despite its sensitivity to individuals considered in our model, it is crucial to understand the sources of this variability. In this study, adding a sampling in the cord blood at birth for a small number of mother:child pairs would have allowed to refine the knowledge of the PFOA and PFOS samples. Another alternative is to collect data on the mother-foetus exchanges during the first months of pregnancy, as it is a period of rapid changes, in order to

reduce the uncertainty and improve the quality of predictions of the foetal exposure and thus early postnatal life (Liu et al., 2022).

In our model, the half-life of PFOA and PFOS was assumed to be constant throughout childhood and was set to adult values (EFSA, 2020; Li et al., 2018; Winkens et al., 2017). The PFOA and PFOS plasma concentration is very sensitive to their half-life which drives their elimination from the body. In a reverse dosimetry approach, the half-life will then influence the dose estimation. As an illustration of PFOA for a child who is not breastfed and if the half-life is fixed at 1.2 and 8.5 years (*minimum and maximum values provided by EFSA (2020)*), the estimated dose for the mother varies between 0.087 and 0.012 ng/kg bw/d respectively. For the associated children, it corresponds to a variation of the estimated dose between 0.20 and 0.08 ng/kg bw/d, which then impacts the predicted PFOA venous plasma concentration in the child. In this example, a factor of seven between the half-lives corresponds to a factor less than two in terms of maximum concentrations for children. Our results showed that a steady state can be reached in plasma for some children after they have become 3 years old depending on their breastfeeding duration. Despite the importance of the PFAS half-life in risk assessment, there is still a disparity in the values reported by different studies that can be explained by large inter-individual variations in half-lives (Li et al., 2018). For example, half-lives between different human studies have been reported by EFSA (2020), in a wide range of 1.9–18 y.o for PFOS, and 1.2–8.5 y.o for PFOA.

Among the HELIX subcohort ($n = 1,239$), the modelling approach failed for six children. The main issue was that current known information about the PFOA and PFOS TK integrated into the PBPK models, the individual information collected in the questionnaires, and the biomarkers' measurements were not consistent. In most cases, the estimated *in utero* and breastfeeding exposures from the maternal samples were too high to be coherent with the biomarker measurement during childhood. Indeed, when fixing a diet intake to zero, the plasma concentration predicted from the *in utero* and breastfeeding exposures for a girl from Lithuania was 0.9 µg/L, and the measurement was at 0.4 µg/L, *i.e.*, more than a 2-factor difference without accounting for diet exposure. Several hypotheses could explain these discrepancies, such as an overestimation of breast milk concentrations, or missing information or an error in the reporting of breastfeeding duration in the questionnaire (supplementary material, S.11). To a lesser extent, it can also be due to misinformation in chemical analyses. As highlighted by Verner et al. (2013), epidemiologic questionnaires have always missing values (e.g., no information on nursing duration, etc.), and a reflection on criteria of exclusion is required when using the PBPK modelling to reconstruct postnatal exposure.

The recent recommended tolerable week intakes (TWI) from the last EFSA scientific opinion (EFSA, 2020) now applies to the sum of four PFAS (PFOA, PFNA, PFHxS and PFOS) to better account for combined exposures. However, for children, the EFSA guidance (EFSA, 2020) recommends to compare the predicted PFAS concentrations to the BMDL₁₀ threshold, as this value is used to establish the TWI. For several HELIX children, the predicted PFOA and PFOS concentrations were estimated above this threshold. Two scenarios are possible: (i) this prediction is the actual situation, and young children are exposed to PFOA and PFOS levels that exceed the threshold, or (ii) this is not the case, and this prediction is affected by uncertainties in the modelling approach that need to be reduced. It could be feasible to apply our modelling framework to PFNA and PFHxS to assess the risk of the sum of these four compounds on the HELIX children. Our first results (*i.e.*, only for two of the four compounds) show that the median of the combined PFOS and PFOA predicted concentrations for French and Norwegian HELIX infants is above this threshold (EFSA, 2020) and could indicate a health concern (Zheng et al., 2022).

5. Conclusions

The PBPK modelling framework presented in this paper allows to

simulate the PFOA and PFOS internal exposures during early life, including the *in utero* period, by accounting for children's individual exposure scenarios based on information collected via questionnaires (e. g., breastfeeding duration, weight at birth, etc.). This approach was applied to the European HELIX subcohort gathering six countries, where PFOA and PFOS plasma concentrations were sampled on two occasions (prenatal and postnatal). Our simulation results showed high inter-individual differences in TK profiles among the HELIX children, and that similar plasma concentrations during childhood (between 6 and 10 y.o) can lead to different internal exposures during the first years. The contribution of the exposure routes studied also evolves during early life and depends on individual behaviour, emphasising the need to account for individualising the exposure scenario. Our study then demonstrates the importance to consider inter-individual variability when interpreting biomarkers measurements and that using PBPK models when measured biomarkers are scarce, can help risk assessors in gaining insight into internal exposure during critical windows, such as early life. Our workflow is quite generic and could be applied to other persistent compounds and other PFAS compounds.

Ethics

This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), and consent was obtained for experimentation with human subjects.

Funding sources

The research leading to these results received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under Grant Agreement No 308333 – the HELIX project. This work is also supported by the European Partnership for the Assessment of Risks from Chemicals (PARC project), founded by the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014.

INERIS acknowledges support from the Ministries for Agriculture and Food Sovereignty, for an Ecological Transition and Territorial Cohesion, for Health and Prevention and of Higher Education and Research, with the financial support of the French Ministry of Ecological Transition (P-181 MIV 34, P-190 toxicology). ISGlobal acknowledges support from the Spanish Ministry of Science and Innovation through the "Centro de Excelencia Severo Ochoa 2019–2023" Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program. The Norwegian Mother, Father and Child Cohort Study (MoBa) is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research. KANC was funded by a grant from the Lithuanian Agency for Science Innovation and Technology (6-04-2014_31V-66). The Rhea project was financially supported by European projects, and the Greek Ministry of Health (Program of Prevention of Obesity and Neurodevelopmental Disorders in Preschool Children, in Heraklion district, Crete, Greece: 2011–2014; 'Rhea Plus': Primary Prevention Program of Environmental Risk Factors for Reproductive Health, and Child Health: 2012–2015). The work was also supported by MICINN (MTM2015-68140-R) and Centro Nacional de Genotipado-CEGEN-PRB2-ISCI. CW received funding from the Fondation de France.

CRedit authorship contribution statement

Aude Ratier: Writing – original draft, Visualization, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Maribel Casas:** Resources, Data curation. **Regina Grazuleviciene:** Resources, Data curation. **Remy Slama:** Resources, Data curation. **Line Småstuen Haug:** Data curation, Resources, Writing – review & editing. **Cathrine Thomsen:** Data curation, Resources, Writing – review & editing. **Marina Vafeiadi:** Data curation, Resources. **John Wright:** Data

curation, Resources. **Florence A. Zeman:** Conceptualization, Methodology, Visualization, Writing – review & editing. **Martine Vrijheid:** Writing – review & editing, Visualization, Validation, Data curation, Funding acquisition, Project administration, Resources, Supervision. **Céline Brochot:** Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

I have shared the link to the model codes and example of input files. The data that has been used is confidential.

Acknowledgements

We would particularly like to thank all the participants for their generous collaboration, including all the participating families in France, Norway, the United Kingdom, Spain, Lithuania, and Greece who take part in this on-going cohort study. We are also thankful to Cleo Bodin for helping with the statistical analyses. We acknowledge the funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under Grant Agreement No 308333 – the HELIX project, the European Partnership for the Assessment of Risks from Chemicals (PARC project) founded by the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014, and the ministries and national agencies of the six countries involved in the funding.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2024.108621>.

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