Prenatal exposure to perfluoroalkyl substances and birth outcomes in a Spanish birth cohort

Cyntia B. Manzano-Salgado,[a,b,c] Maribel Casas,[a,b,c] Maria-Jose Lopez-Espinosa,[c,d] Ferran Ballester,[c,d] Carmen Iñiguez,[c,d] David Martinez,[a,b,c,] Olga Costa[d], Loreto Santa-Marina,[c,e,f] Eva Pereda-Pereda,[f,g] Thomas Schettgen,[h] Jordi Sunyer,[a,b,c] Martine Vrijheid[a,b,c]

[a] *ISGlobal, Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain*

[b] *Universitat Pompeu Fabra (UPF), Barcelona, Spain*

[c] *CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain*

[d] *Epidemiology and Environmental Health Joint Research Unit, FISABIO–Universitat Jaume I–Universitat de València, Valencia, Spain*

[e] *Subdirección de Salud Pública y Adicciones de Gipuzkoa, Donostia-San Sebastián, Spain*

[f] *Instituto de Investigación Sanitaria BIODONOSTIA, Donostia-San Sebastián, Spain*

[g] *Facultad de Psicología, Universidad del País Vasco (UPV/EHU). Donostia-San Sebastián, Spain*

[h] *Institute for Occupational Medicine, RWTH Aachen University, Aachen, Germany*

Corresponding author

Cyntia B. Manzano-Salgado, ISGlobal–Centre for Research in Environmental Epidemiology (CREAL), Doctor Aiguader, 88|08003 Barcelona, Catalonia, Spain. Tel.: + 34 932 147 314; Fax: + 34 932 045 904.

Email: cyntia.manzano@isglobal.org

Abstract

Background: Prenatal perfluorooctanoate (PFOA) exposure has been associated with reduced birth weight but maternal glomerular filtration rate (GFR) may attenuate this association. Further, this association remains unclear for other perfluoroalkyl substances (PFAS), such as perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS) and perfluorononanoate (PFNA). We estimated associations between prenatal PFAS exposure and birth outcomes, and the influence of GFR, in a Spanish birth cohort.

Methods: We measured PFHxS, PFOS, PFOA, and PFNA in 1st-trimester maternal plasma (years: 2003-2008) in 1,202 mother-child pairs. Continuous birth outcomes included standardized weight, length, head circumference, and gestational age. Binary outcomes included low birth weight (LBW), small-for-gestational-age, and preterm birth. We calculated maternal GFR from plasma-creatinine measurements in the 1st-trimester of pregnancy (n=765) using the Cockcroft-Gault formula. We used mixed-effects linear and logistic models with region of residence as random effect and adjustment for maternal age, parity, pre-pregnancy BMI, and fish intake during pregnancy.

Results: Newborns in this study weighted on average 3263g and had a median gestational age of 39.8 weeks. The most abundant PFAS were PFOS and PFOA (median: 6.05 and 2.35 ng/mL, respectively). Overall, PFAS concentrations were not significantly associated to birth outcomes. PFOA, PFHxS and PFNA showed weak, non-statistically significant associations with reduced birth weights ranging from 8.6 g to 10.3 g per doubling of exposure. Higher PFOS exposure was associated with an OR of 1.90 (95% CI: 0.98, 3.68) for LBW (in births-at-term) in boys. Maternal GFR did not confound the associations.

Conclusions: In this study, PFAS showed little association with birth outcomes. Higher PFHxS, PFOA, and PFNA concentrations were non-significantly associated with reduced birth weight. The association between PFOS and LBW seemed to be sex-specific. Finally, maternal GFR measured early during pregnancy had little influence on the estimated associations.

Keywords: INMA birth cohort; perfluoroalkyl substances; perfluorooctanoate (PFOA); perfluorooctane sulfonate (PFOS); fetal growth; glomerular filtration rate; mother-child pairs

Abbreviations

BMI	Body mass index
CI	Confidence interval
CRL	Crown-rump-length
GAM	Generalized additive model
GFR	Glomerular filtration rate
GM	Geometric mean
HPLC-MS/MS	High performance liquid chromatography–tandem mass spectrometry
INMA	Environment and Childhood Project (*INfancia y Medio Ambiente)*
LBW	Low birth weight
LOQ	Limit of quantification
OR	Odds ratio
PBPK	Physiologically-based pharmacokinetic
PFAS	Perfluoroalkyl substances
PFHxS	Perfluorohexane sulfonate
PFOS	Perfluorooctane sulfonate
PFOA	Perfluorooctanoate
PFNA	Perfluorononanoate
SD	Standard deviation
SGA	Small-for-gestational-age

1. Introduction

Birth outcomes are commonly used as indicators of fetal growth during pregnancy. Throughout pregnancy there is a constant interplay between the internal and the external maternal environment leading to better or worse health status of the offspring. This interplay can be influenced by many factors including exposure to environmental chemical pollutants such as perfluoroalkyl substances (PFAS). PFAS are synthetic chemicals that have been industrially and commercially used since the 1950's (Casals-Casas and Desvergne, 2011; Prevedouros et al., 2006). A number of PFAS - including perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid PFOA, and perfluorononanoate (PFNA) - have been detected in maternal serum and cord blood samples suggesting that PFAS can cross the placental barrier exposing the fetus to PFAS (Fei et al., 2007; Inoue et al., 2004; Manzano-Salgado et al., 2015).

PFOA is one of the most abundant and studied PFAS (as reviewed by Vrijheid et al., 2016). Prenatal PFOA exposure has been associated with reduced birth weight in animal and human studies, whereas for PFOS the evidence is less consistent (reviewed by Bach et al., 2015a; Johnson et al., 2014; Lam et al., 2014). Large prospective studies assessing other PFAS, besides PFOA and PFOS, are scarce (Rappazzo et al., 2017). One of the most comprehensive study to date with more than 1500 mother-child pairs and an assessment of 11 different PFAS - including PFOS and PFOA - only observed a small association between PFNA and reduced birth weight in girls but not in boys (Bach et al., 2015). However, this and previous studies have not consider maternal glomerular filtration rate (GFR) during pregnancy, which may influence the association between PFAS and fetal growth (Verner et al., 2015). Maternal GFR indicates the speed at which the mother can clear chemicals from her body, and increases during the first half of pregnancy and then declines during the second half (Gibson, 1973; Verner et al., 2015). Lower GFR has been associated with higher PFAS blood levels (Verner et al., 2015; Watkins et al., 2013) and smaller babies (Gibson, 1973; Verner et al., 2015). Indeed, a recent study found that a large proportion of the association between PFOS and PFOA and LBW, if there is any, may be attributable to confounding by maternal GFR (Verner et al., 2015); this study used a physiologically-based pharmacokinetic (PBPK) model to generate pairs of predictions for PFAS level and birth weight. Only one epidemiological study has considered the role of GFR on the association between PFOA and birth weight in a sub-analysis in a Norwegian birth cohort concluding that maternal GFR attenuated by 66% the association between PFOA and LBW (Morken et al., 2014).

We evaluated the association between prenatal exposure to four different PFAS and birth outcomes including weight, length, head circumference, and gestational age in a Spanish birth cohort. We also assessed the influence of maternal GFR during pregnancy on the association between PFAS and birth outcomes.

2. Methods

2.1. Study population

In this study we used data from the INMA (Environment and Childhood - *INfancia y Medio Ambiente*) birth cohort. From 2003-2008, a total of 2,150 pregnant women from the regions of Gipuzkoa, Sabadell, and Valencia were recruited during their 1st-trimester of pregnancy. The inclusion criteria were: being at least 16 years old, singleton pregnancy, no communication barrier, no reproductive assistance and giving birth in the reference hospital (Guxens et al., 2012). We had 1,242 mother-child pairs (58% from the full sample) with data on PFAS concentration and at least one birth outcome. From these, 40 mother-child pairs did not have complete information on the covariates of interest (i.e. 3.3% of the sample). For the purpose of this study, we only included the 1,202 mother-child pairs with data on prenatal PFAS exposure and at least one birth outcome, and also complete data on the covariates of interest (Supplementary Material Figure S1).

2.2. Birth Outcomes

Birth weight (grams) was measured by trained midwives at delivery. Birth length (cm) and head circumference (cm) were measured within the first 12 hours-of-life by a nurse when the newborn arrived at the hospital ward. Gestational age was calculated using the self-reported last menstrual period (LMP). An early crown-rump-length (CRL) was also available and was used to estimate gestational age when the LMP differed by $\geqslant$7 days from the ultrasound date (Westerway et al., 2000). Because gestational age has a large influence on weight, length and head circumference at birth we standardized these outcomes to week 40 of gestation using the Box–Cox power exponential method (Rigby and Stasinopoulos, 2004). These outcomes were further adjusted by sex and region of residence (Casas et al., 2015; Estarlich et al., 2011). We defined small-for-gestational-age (SGA) if newborns weights were below 10th percentile for gestational age and sex according to national references (Carrascosa et al., 2004). We considered a birth preterm if delivery was before 37 weeks of gestation. Newborns with

weight < 2500 grams were defined as LBW. Newborns with LBW born greater than or equal to 37 weeks were defined as LBW-at-term.

2.3. PFAS determination

Maternal blood samples were collected during the first trimester of pregnancy [mean: 12.3 weeks; standard deviation (SD): 5.6 weeks]. Plasma was aliquoted in 1.5mL cryotubes and stored at -80°C until their analysis at the Institute for Occupational Medicine, RWTH Aachen University (Aachen, Germany), as previously described (Manzano-Salgado et al., 2015). Briefly, plasma concentrations of PFHxS, PFOS, PFOA, and PFNA 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). The limit of quantification (LOQ) was 0.20 ng/mL for PFHxS, PFOS, and PFOA and 0.10 ng/mL for PFNA (Manzano-Salgado et al., 2015). The between day imprecision ranged from 8.7% for PFHxS (0.7 ng/mL) to 11.1% for PFNA (0.7 ng/mL).

2.4. Maternal and newborn covariates

Maternal socio-demographic and dietary information was collected by questionnaires administered during the 1^{st} and 3^{rd} - trimesters of pregnancy. Data regarding the maternal health status during pregnancy and delivery (e.g. gestational weight gain, preeclampsia, and gestational diabetes) was collected from clinical records. Plasma creatinine levels in the 1^{st}-trimester of pregnancy were determined using non-compensated kinetic alkaline picrate method (ABX-Pentra 400) in 800 pregnant women that had available blood in the same sample used to measure PFAS concentrations. The creatinine levels were used to calculate maternal GFR using the Cockcroft-Gault formula [GFR = (140-maternal age) × weight (kg) × 1.04/serum creatinine (μmol/L)]. For this study, we only analyzed GFR in mothers that had available PFAS, birth outcomes, and the other covariates (n = 765). In this same subsample of women, we determined serum albumin using Bromocresol green assay (ABX-Pentra 400). Mothers completed a 100-item food frequency questionnaire (FFQ) that was administered by trained interviewers and was used to assess the usual food and nutrient intake during the first trimester of pregnancy. The response to each food item was converted to an average daily intake for each participant. Consumption of a range of food groups was assessed: dairy products, meat, eggs, cereals, pasta, and fruit and vegetables, and fish intake. This FFQ was an adapted version of Willett's questionnaire (Willet et al., 1985) that we developed and

validated for use among adults living in Spain (Vioque et al., 2013). Further, newborn sex and type of delivery (i.e. vaginal, instrumental or cesarean) was collected from clinical records.

2.5. Statistical analyses

We replaced PFAS concentrations < LOQ with LOQ/2 (48 observations for PFHxS and 6 for PFNA). PFAS concentrations were skewed to the right so we $\log_2$-transformed our PFAS concentrations to linearize the regression models (Kleinbaum et al., 2014). We performed generalized additive models (GAMs) to assess the linear relationship between PFAS and continuous outcomes. Because GAMs did not show non-linearity (p-gain > 0.05) we used PFAS as a continuous variable in our main models and used categorized (by quartile) PFAS levels in a sensitivity analysis. We estimated associations between PFAS and birth outcomes performing linear (for continuous outcomes: weight, length, head circumference, and gestational age) and logistic (for binary outcomes: SGA, preterm, LBW, and LBW-at-term) mixed-effects models with random effects for region of residence. We first assessed the association between PFAS and each birth outcomes in a crude model (i.e. PFAS and outcome). For the fully adjusted models, we selected the covariates using directed acyclic graphs (DAGs) with all the determinants of maternal PFAS concentrations in this cohort: region of residence (Gipuzkoa, Sabadell, and Valencia), country of birth (Spain and other), age (years), previous breastfeeding (continuous number of weeks-), parity (continuous number of births), pre-pregnancy BMI (continuous in kg/m^2), and fish consumption (servings per week). For the DAG, we also considered other covariates relevant in the literature, such as maternal socio-economic-status, gestational weight gain, pregnancy complications (e.g. preeclampsia and gestational diabetes), physiology (e.g. GFR in pregnancy), and the child sex and gestational age at birth (Supplementary Material Figure S2). Based on the DAGs, the final models were adjusted for: maternal age, parity, pre-pregnancy BMI, and fish consumption during pregnancy. The model of PFAS and head circumference was further adjusted by type of delivery to account for distortions during labor (Slama et al., 2014). We compared the distribution of covariates using analysis of variance for continuous variables and chi-squared or Fisher`s-exact test for categorical variables (Supplemental Material Table S1 and S2).

We performed various sensitivity analyses to assess the robustness of our results. First, because GFR is associated with PFAS concentrations and birth weight (Morken et al., 2014) we assessed if maternal GFR during pregnancy (n = 765) changed our estimates for PFAS and

birth outcomes (Verner et al., 2015) by including the variable GFR in our models. Second, given that PFAS bind albumin in blood (Salvalaglio et al., 2010) we included maternal serum albumin as a variable in our models. Third, because PFAS concentrations differ by region of residence in our cohort (Manzano-Salgado et al., 2016) we stratified our main analysis by region of residence. Fourth, because PFAS may exhibit sex-specific effects at birth (Andersen et al., 2010; Lind et al., 2017) we evaluated whether sex modified the association between PFAS and birth outcomes by including interaction terms and stratifying our analysis. Fifth, we repeated our analysis using quartiles of PFAS exposure expressed in ng/mL to consider potential non-linear associations between PFAS and birth outcomes. Sixth, because PFAS are correlated in our cohort (Spearman's correlation coefficients ranged between 0.43 and 0.68; Manzano-Salgado et al., 2015, 2016) we included all PFAS into a single multipollutant model. Seventh, we excluded SGA, preterm, and LBW births (n = 159) from the models studying the association between PFAS and continuous birth outcomes (i.e. weight, length, head circumference and gestational age) to evaluate PFAS effects in term-, and normal-weight births. Finally, as smoking is related to impaired fetal growth (Iñiguez et al., 2013) we tested whether including the variable *smoking during pregnancy* changed our results.

We interpreted our model´s estimates as the unit change in the outcome per doubling of maternal PFAS concentrations. We used the STATA 14.1 statistical software (Stata Corporation, College Station, Texas) for our regression analysis. We considered a p-value < 0.05 to be statistically significant. We drew our DAGs using the DAGitty version 3.0 (Textor, 2011).

3. Results

Participating women were on average 31 years-old, nulliparous, Spanish, and normal-weight (Table 1). Women included in this study were generally younger and with higher education than those excluded ($p < 0.001$) (Supplementary Material Table S1). Newborns weighed on average 3263 g and had a median gestational age of 39.8 weeks (Table 1). PFAS were detected in every maternal sample, with PFOS (median concentration: 6.05 ng/mL) and PFOA (median concentration: 2.35 ng/mL) being the most abundant PFAS (Table 1). In this study, the estimated associations between maternal PFAS concentration and birth outcomes were either null or non-significant, thus we will only describe the main patterns of associations observed.

Table 1. Maternal and newborn characteristics in the present study (n=1,202) from the INMA birth cohort (2003-2008).

Characteristics	Mean (SD), or n (%)
Maternal	
PFAS (ng/mL)- median (SD)	
PFHxS	0.58 (0.37)
PFOS	6.05 (2.74)
PFOA	2.35 (1.25)
PFNA	0.66 (0.36)
Age (years)	30.7 (4.0)
Pre-pregnancy BMI (kg/m^2)	23.6 (4.3)
Region of residence	
Gipuzkoa	306 (25)
Sabadell	400 (33)
Valencia	496 (41)
Parity	
None	670 (56)
One	452 (38)
Two or more	80 (7)
Educational level	
Primary or without education	278 (23)
Secondary	503 (42)
University	418 (35)
Country of birth	
Spain	1117 (93)
Other	83 (7)
Previous breastfeeding	
Never	726 (60)
Short-term (<4 months)	129 (11)
Long-term (4–6 months)	104 (9)
Very-long-term (>6 months)	243 (20)
Type of delivery	
Vaginal	734 (61)

Instrumental	255 (21)
Cesarean section	213 (18)
Fish intake (servings / week)	4.98 (2.57)
Glomerular filtration rate (mL/min/1.73 m^2)	122.4 (37.6)
Newborn	
Sex	
Girl	584 (49)
Boy	618 (51)
Weight (g)	3263 (461)
Length (cm)	49.6 (2.1)
Head circumference (cm)	34.3 (1.4)
Gestational age (weeks) – median (SD)	39.8 (1.5)
Small for gestational age	
No	1081 (90)
Yes	121 (10)
Preterm birth	
No	1157 (96)
Yes	45 (4)
Low birth weight	
No	1144 (95)
Yes	58 (5)
Low birth weight- at term	
No	1169 (97)
Yes	33 (3)

Abbreviations: SD: standard deviation; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctanesulfonic acid; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; BMI: body mass index.

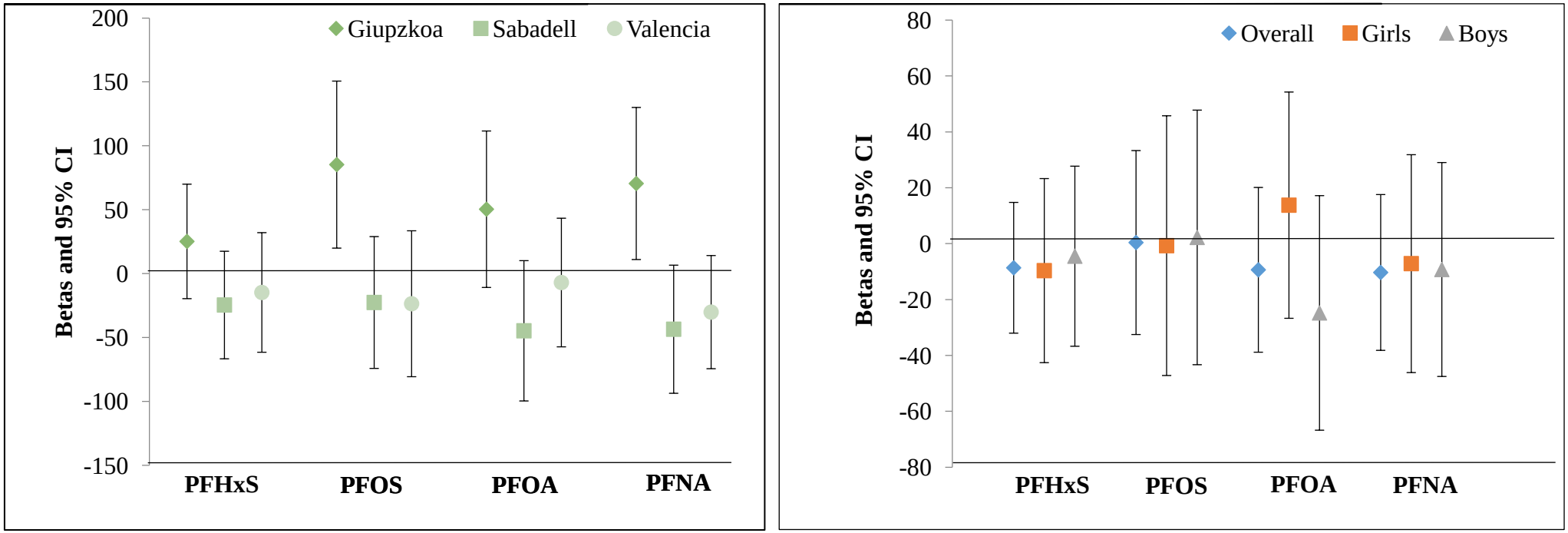
All PFAS, except PFOS, showed patterns of inverse associations (although not statistically significant) with birth weight that ranged from 8.6 g to 10.3 g per doubling of exposure (Table 2). For PFOS, this pattern was observed in the crude model [β = -12.84 (95% CI: -45.81, 20.12)] but not after adjustment for potential confounding factors. For all PFAS, increasing concentrations were associated with reductions in birth weight in Sabadell and Valencia regions and increases in birth weight in Gipuzkoa (Table S3 and Figure 1). For length at birth, we did not observe any statistically significant association; however PFHxS tended to be non-significantly associated with reduced length at birth (Table 2) both in the crude and adjusted models. Finally, no clear association was observed between PFAS and gestational age (Table 2).

Table 2. Associations between maternal PFAS concentrations (log_2-transformed, ng/mL) and continuous birth outcomes in the INMA birth cohort (2003-2008).

PFAS	Weight (g)		Length (cm)		Head circumference (cm) [§]		Gestational age (weeks)	
	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)
PFHxS								
Crude	1185	-18.07 (-41.22, 5.07)	1164	-0.09 (-0.20, 0.02)	1164	-0.03 (-0.11, 0.05)	1202	0.01 (-0.08, 0.10)
Adjusted	1185	-8.60 (-32.00, 14.80)	1164	-0.06 (-0.17, 0.06)	1164	-0.01 (-0.09, 0.07)	1202	-0.01 (-0.10, 0.09)
Not adjusted by GFR	751	20.64 (-10.01, 51.29)	733	-0.01 (-0.15, 0.13)	734	0.01 (-0.09, 0.10)	765	-0.02 (-0.14, 0.10)
Adjusted by GFR [¥]	751	21.77 (-8.95, 52.50)	733	-0.01 (-0.15, 0.14)	734	0.01 (-0.08, 0.11)	765	-0.02 (-0.14, 0.10)
PFOS								
Crude	1185	-12.84 (-45.81, 20.12)	1164	-0.02 (-0.16, 0.13)	1164	-0.02 (-0.13, 0.08)	1202	-0.06 (-0.19, 0.07)
Adjusted	1185	0.44 (-32.48, 33.36)	1164	0.03 (-0.12, 0.17)	1164	-0.00 (-0.10, 0.10)	1202	-0.06 (-0.19, 0.06)
Not adjusted by GFR	751	38.03 (-3.13, 79.18)	733	0.07 (-0.11, 0.26)	734	0.01 (-0.12, 0.13)	765	-0.10 (-0.27, 0.06)
Adjusted by GFR [¥]	751	39.30 (-1.91, 80.50)	733	0.08 (-0.11, 0.27)	734	0.01 (-0.12, 0.14)	765	-0.10 (-0.27, 0.06)
PFOA								
Crude	1185	-32.68 (-61.69, -3.66)[*]	1164	-0.08 (-0.21, 0.06)	1164	-0.13 (-0.22, -0.03)[**]	1202	0.00 (-0.11, 0.11)
Adjusted	1185	-9.33 (-38.81, 20.16)	1164	-0.01 (-0.15, 0.14)	1164	-0.07 (-0.17, 0.03)	1202	-0.05 (-0.16, 0.07)
Not adjusted by GFR	751	29.40 (-10.22, 69.01)	733	0.05 (-0.13, 0.24)	734	-0.08 (-0.20, 0.04)	765	-0.07 (-0.23, 0.08)
Adjusted by GFR [¥]	751	30.77 (-8.93, 70.46)	733	0.06 (-0.12, 0.24)	734	-0.07 (-0.20, 0.05)	765	-0.07 (-0.23, 0.08)
PFNA								
Crude	1185	-19.54 (-47.68, 8.61)	1164	-0.03 (-0.16, 0.10)	1164	-0.06 (-0.15, 0.03)	1202	0.01 (-0.10, 0.12)
Adjusted	1185	-10.27 (-38.14, 17.61)	1164	-0.00 (-0.13, 0.13)	1164	-0.04 (-0.13, 0.05)	1202	-0.00 (-0.11, 0.11)
Not adjusted by GFR	751	33.69 (-6.53, 73.92)	733	-0.01 (-0.19, 0.18)	734	-0.12 (-0.24, 0.01)	765	0.04 (-0.12, 0.20)
Adjusted by GFR [¥]	751	35.82 (-4.56, 76.20)	733	0.00 (-0.19, 0.18)	734	-0.12 (-0.24, 0.01)	765	0.04 (-0.12, 0.20)

Abbreviations: CI: confidence interval; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctanesulfonic acid; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; GFR: maternal glomerular filtration rate in pregnancy. All models were adjusted for maternal age, parity, pre-pregnancy BMI, and fish intake during pregnancy. [¥] Model further adjusted for maternal GFR during pregnancy. [§] Models of head circumference were also adjusted for type of delivery. [*] p-value<0.05; [**] p-value<0.01.

Figure 1. Associations between maternal PFAS concentrations (log_2-transformed, ng/mL) and birth weight by region of residence and sex of the child in the INMA birth cohort (2003-2008).



Abbreviations: CI: confidence interval; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctanesulfonic acid; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid. All models were adjusted for maternal age, parity, pre-pregnancy BMI, and fish intake during pregnancy.

The estimated associations between PFAS and continuous birth outcomes generally did not differ by sex of the child (Table S4 and Figure 1). We observed, however, that PFOA was inversely associated with birth weight in boys [β = -24.75 (-66.71, 17.22)] but positively associated in girls [β = 13.81 (-26.67, 54.30), p-value for sex-interaction= 0.25]. Similar results were observed for the association between PFNA and head circumference in boys [β = -0.10 (-0.22, 0.02)] compared to girls [β = 0.03 (-0.10, 0.15), p-value for sex-interaction = 0.19] (Table S4). For the binary outcomes we observed some evidence for sex-interactions (Table 3). Specifically, a doubling of PFOA concentration was associated with an increased OR of being SGA in boys [OR = 1.18 (95% CI: 0.82, 1.69)] but with a decreased OR in girls [OR = 0.84 (95% CI: 0.56, 1.24)] (p-value for sex-interaction = 0.08). Similarly, in boys, every doubling of PFOS was associated with an increased OR of being LBW [OR = 1.90 (95% CI: 0.98, 3.68)] whereas in girls the OR was 0.73 (95% CI: 0.46, 1.19) (p-value for sex interaction = 0.01) (Table 3).

In this study, maternal GFR was available only in 765 mother-child pairs. Models including maternal GFR did not yield different results from models not including maternal GFR in this subpopulation (n = 765, Table 2).

Table 3. Associations between maternal PFAS concentration (log_2-transformed, ng/mL) and binary birth outcomes in the INMA birth cohort (2003-2008).

	SGA, yes			Preterm, yes			LBW, yes			LBW-at term, yes		
PFAS	**n cases**	**OR (95% CI) ¥**	**p-sex**	**n cases**	**OR (95% CI) ¥**	**p-sex**	**n cases**	**OR (95% CI) ¥**	**p-sex**	**n cases**	**OR (95% CI) ¥**	**p-sex**
PFHxS												
Crude	121/1202	1.03 (0.85, 1.25)		45/1202	0.85 (0.64, 1.11)		58/1202	1.00 (0.76, 1.30)		33/1202	1.04 (0.72, 1.49)	
Adjusted	121/1202	0.98 (0.80, 1.19)		45/1202	0.85 (0.63, 1.13)		58/1202	0.94 (0.71, 1.23)		33/1202	0.97 (0.68, 1.41)	
Girls	59/ 584	0.87 (0.66, 1.14)	0.27	16/ 584	0.69 (0.46, 1.04)	0.31	33/ 584	0.85 (0.60, 1.20)	0.31	23/ 584	0.88 (0.58, 1.34)	0.41
Boys	62/ 618	1.09 (0.80, 1.48)		29/ 618	0.98 (0.64, 1.51)		25/ 618	1.06 (0.67, 1.68)		10/ 618	1.22 (0.59, 2.53)	
PFOS												
Crude	121/1202	1.00 (0.76, 1.31)		45/1202	1.10 (0.71, 1.72)		58/1202	1.20 (0.80, 1.79)		33/1202	1.02 (0.62, 1.70)	
Adjusted	121/1202	0.92 (0.70, 1.22)		45/1202	1.10 (0.70, 1.74)		58/1202	1.06 (0.71, 1.58)		33/1202	0.91 (0.55, 1.50)	
Girls	59/ 584	0.84 (0.56, 1.24)	0.57	16/ 584	0.79 (0.40, 1.57)	0.35	33/ 584	0.73 (0.46, 1.19)	0.01	23/ 584	0.73 (0.42, 1.29)	0.15
Boys	62/ 618	1.01 (0.69, 1.48)		29/ 618	1.34 (0.74, 2.43)		25/ 618	1.90 (0.98, 3.68)		10/ 618	1.68 (0.62, 4.54)	
PFOA												
Crude	121/1202	1.04 (0.82, 1.33)		45/1202	0.87 (0.59, 1.27)		58/1202	0.97 (0.69, 1.36)		33/1202	0.91 (0.59, 1.42)	
Adjusted	121/1202	0.92 (0.72, 1.19)		45/1202	0.90 (0.60, 1.35)		58/1202	0.90 (0.63, 1.29)		33/1202	0.85 (0.53, 1.34)	
Girls	59/ 584	0.72 (0.50, 1.04)	0.08	16/ 584	1.19 (0.62, 2.31)	0.19	33/ 584	0.76 (0.48, 1.21)	0.19	23/ 584	0.62 (0.36, 1.06)	0.05
Boys	62/ 618	1.18 (0.82, 1.69)		29/ 618	0.74 (0.43, 1.25)		25/ 618	1.13 (0.64, 1.99)		10/ 618	1.67 (0.72, 3.86)	
PFNA												
Crude	121/1202	0.90 (0.72, 1.12)		45/1202	0.86 (0.61, 1.21)		58/1202	0.90 (0.66, 1.23)		33/1202	0.96 (0.63, 1.46)	
Adjusted	121/1202	0.85 (0.68, 1.07)		45/1202	0.87 (0.62, 1.22)		58/1202	0.86 (0.63, 1.17)		33/1202	0.91 (0.60, 1.38)	
Girls	59/ 584	0.80 (0.57, 1.12)	0.61	16/ 584	1.24 (0.64, 2.40)	0.19	33/ 584	0.77 (0.50, 1.16)	0.39	23/ 584	0.72 (0.45, 1.17)	0.13
Boys	62/ 618	0.91 (0.67, 1.24)		29/ 618	0.82 (0.55, 1.23)		25/ 618	1.01 (0.61, 1.68)		10/ 618	1.58 (0.64, 3.93)	

Abbreviations: SGA: small for gestational age at birth; LBW: low birth weight; OR: odds ratio; CI: confidence interval; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctanesulfonic acid; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid. Crude: PFAS and outcome. Models were adjusted for maternal age, parity, pre-pregnancy BMI, and fish intake during pregnancy. ¥ Reference groups: SGA (Girls n=525; Boys n=556); Preterm (Girls n=568; Boys n=589); LBW (Girls n=551; Boys n=593); LBW-at term (Girls n=561; Boys n=608).

Regarding the rest of the sensitivity analyses, using quartiles of PFAS exposure (Supplementary Table S5) suggested a pattern of inverse associations between PFHxS (β for Q2, Q3, and Q4:-0.08, -0.14, and -0.16) and PFNA (β for Q2, Q3, and Q4: -0.01, -0.06, and -0.16) and head circumference. A similar pattern was observed between PFOS (β for Q2, Q3, and Q4: -0.09, -0.02 and -0.31) and gestational age that reached statistical significance in the upper quartile of PFOS exposure [β = - 0.31 (95% CI: -0.55, -0.06)]. Further, after including all PFAS in a multipollutant model the estimated betas (for the continuous outcomes) were close to the null (Supplementary material Table S6). Finally, we did not observe any substantial difference from our main results in the other sensitivity analyses (data not shown) after excluding SGA, LBW, and preterm births, or after including the variables *serum albumin* or *smoking during pregnancy* in our models.

4. Discussion

In this study, overall, we observed that PFAS were not statistically significantly associated with birth outcomes and that maternal GFR did not seem to influence these associations. However, PFHxS, PFOA, and PFNA showed weak, non-statistically significant associations with reduced birth weight ranging from 8.6 g to 10.3 g per doubling of exposure. Sex-differences were observed in the association between PFOS exposure and LBW and term LBW, with increased ORs observed in boys and decreased ORs in girls.

In this Spanish birth cohort, all mothers had quantifiable plasma levels of at least one PFAS during the years 2003-2008, and 96% of mothers had quantifiable plasma levels of all four. PFOS and PFOA were the most abundant of all PFAS. PFAS concentrations in our cohort were lower than those reported in other studies that used maternal blood samples collected before the PFOS phase-out period in the year 2002 (Fei et al., 2007; Midasch et al., 2007). However, studies using maternal samples more recently have detected lower PFAS concentrations than ours (Ashley-Martin et al., 2017; Fromme et al., 2010; Hanssen et al., 2010; Porpora et al., 2013).

Maternal PFAS concentrations, except PFOS, showed weak, non-statistically significant associations with reduced birth weight. Our findings are in line with the most recent meta-analysis on PFOA including 4,149 births (Johnson et al., 2014), which concluded that higher prenatal PFOA concentrations are associated with LBW; this was also the main conclusion from other reviews (Bach et al., 2015a; Olsen et al., 2009). Similarly, a recent large study

from Canada (n = 1705) – that was not included in the mentioned meta-analysis and reviews – also reported that higher PFOA concentrations were associated with reduced birth weight (Ashley-Martin et al., 2017). These findings were also supported by studies in animals showing that PFOA is associated with impaired fetal growth (Johnson et al., 2014). For the other PFAS the evidence is scarce and with most studies showing no associations (Ashley-Martin et al., 2017; Bach et al., 2015b; Callan et al., 2016; Shi et al., 2017). Given that in our study PFHxS and PFNA were inversely associated with birth weight (although they showed non-statistically significant associations) and that the use of these PFAS is increasing in other countries (Glynn et al., 2012), a future meta-analysis could be useful in order to elucidate if they are associated with birth weight.

The association between maternal PFAS and birth weight was not influenced by maternal GFR during pregnancy. Contrary to our results, in a sub-analysis done in a Norwegian birth cohort maternal GFR attenuated by at least 66% the association between maternal PFOA and lower birth weight (Morken et al., 2014). Our study considered maternal GFR during pregnancy for the first time in a cohort study assessing more than PFOA, observing that adjusting for GFR did not influence the association between maternal PFAS and birth weight. Assessment of PFAS using PBPK models suggest that GFR is less likely to be a confounder in studies using maternal blood sample collected early in pregnancy (Verner et al., 2015) because physiological changes (such as plasma volume expansion) associated with pregnancy have not fully occurred at this point. According to the Verner et al. study, GFR had a bigger influence with increasing number of gestational weeks at the time of maternal blood sampling. In present study we measured PFAS in the 1st trimester of pregnancy that may explain why maternal GFR did not confound our results.

In this study, higher prenatal PFOS exposure was associated with boys having increased OR of being born LBW, whereas girls had decreased odds ratios. Sex-specific associations were observed in other studies (Bach et al., 2015b; Kishi et al., 2015; Washino et al., 2009), however in those studies girls seemed to be more vulnerable to PFAS exposure than boys. In the recent PFOA meta-analysis by Johnson et al. (2014) sex was only considered as a confounder and differences by sexes were not described. Previous studies differed in the timing (pregnancy vs. birth) and matrix of sampling (maternal or cord blood) and in the PFAS concentrations.

The main strengths of the present study were its prospective design and large sample size. In addition, in this study we assessed four different PFAS and evaluated confounding by maternal GFR. However, we should consider several methodological limitations. First, we used anthropometric outcomes measured at birth as markers of fetal growth but direct ultrasound measurements may be preferable to study the direct effect of PFAS on fetal growth (Zheng et al., 2016). Second, calculating maternal GFR with a single measurement of serum creatinine might be imprecise (Aras et al., 2012). Still we adjusted for maternal GFR during pregnancy, which is novel in this type of study. Future studies should evaluate GFR at different time-points during pregnancy. Third, we estimated GFR using the Cockcroft-Gault formula, however inulin clearance is the gold standard measurement for estimating GFR (Morken et al., 2014) but this method seems to be impractical in settings like ours because the technique is time consuming and has a high price . Fourth, mothers in INMA have been exposed not only to PFAS but to a multitude of environmental pollutants at the same time including, persistent organic pollutants, bisphenol A and phenols, phthalates, and others (Guxens et al., 2012). In the present study we did not assess exposure to a mixture of these chemicals. However, the INMA birth cohort is part of the Human Early-life Exposome (HELIX) project (http://www.projecthelix.eu/) that aims to understand the health effects of multiple environmental exposures during early-life (Vrijheid et al., 2014). HELIX has now finished the data collection and may provide fruitful information on this issue. Finally, due to multiple comparisons we cannot rule out the probability of chance finding in our results, but given that most of our associations are non-significant we consider that type 1 errors are less likely.

5. Conclusion

In this study, prenatal PFAS exposure showed little association with birth outcomes. Higher PFHxS, PFOA, and PFNA concentrations were non-significantly associated with reduced birth weight. The association between PFOS and LBW seemed to be sex-specific. Finally, maternal GFR measured early in pregnancy had little influence on the estimated associations.

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Conflict of interest statement

The authors declare no conflict of interest.

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Supplementary Material

1. Figure S1. Flow chart of study population.
2. Figure S2. Directed acyclic graph for selection of covariates.
3. Table S1. Maternal and newborn characteristics in the excluded and included sample of our study.
4. Table S2. Maternal and newborn characteristics in the sample included and excluded from the maternal GFR analysis.
5. Table S3. Associations between maternal PFAS concentrations (log_2-transformed, ng/mL) and continuous birth outcomes by region of residence in the INMA birth cohort (2003-2008).
6. Table S4. Associations between maternal PFAS concentrations (log_2-transformed, ng/mL) and continuous birth outcomes by sex of the child in the INMA birth cohort (2003-2008).
7. Table S5. Associations between quartiles of maternal PFAS concentrations (in ng/mL) and continuous birth outcomes in the INMA birth cohort (2003-2008).
8. Table S6. Multipollutant models for the associations between maternal PFAS concentrations (log_2-transformed, ng/mL) and continuous birth outcomes in the INMA birth cohort (2003-2008).

Figure S1. Flowchart of study population.

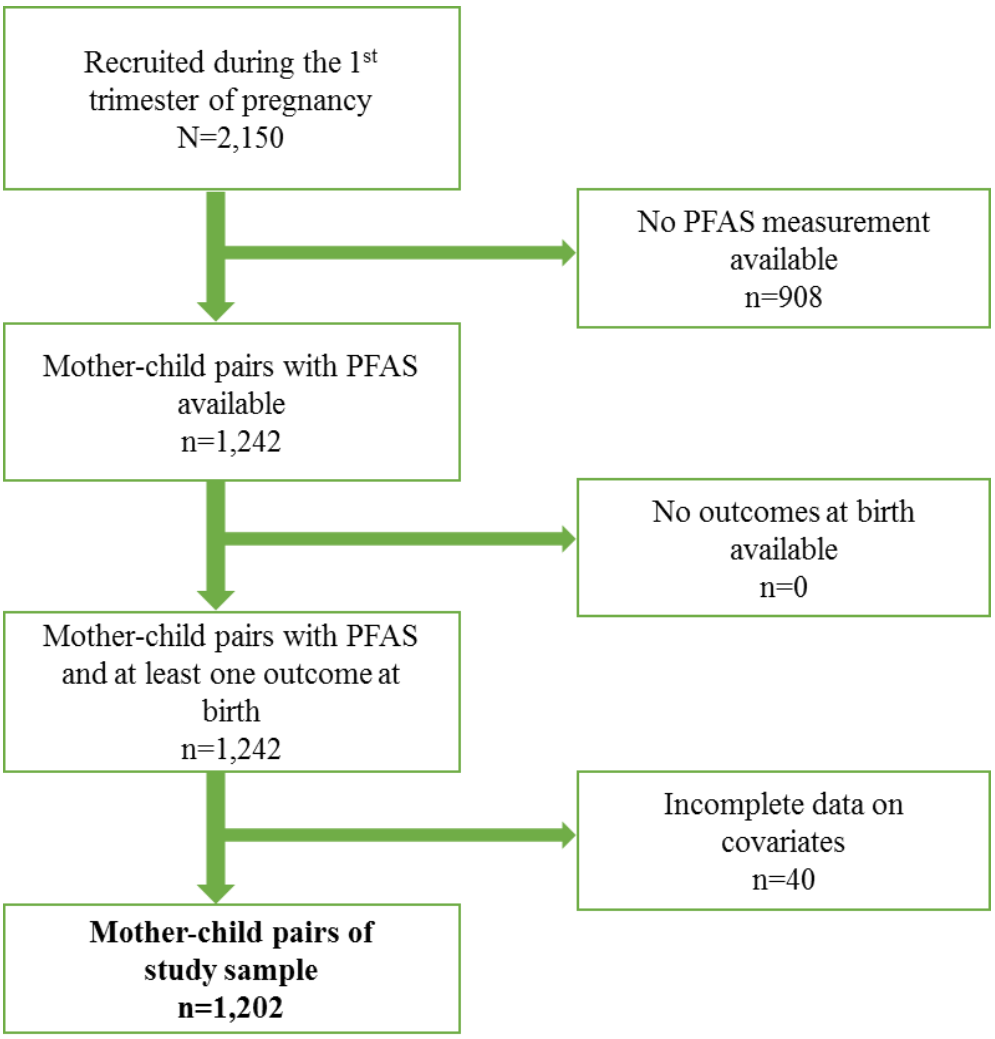
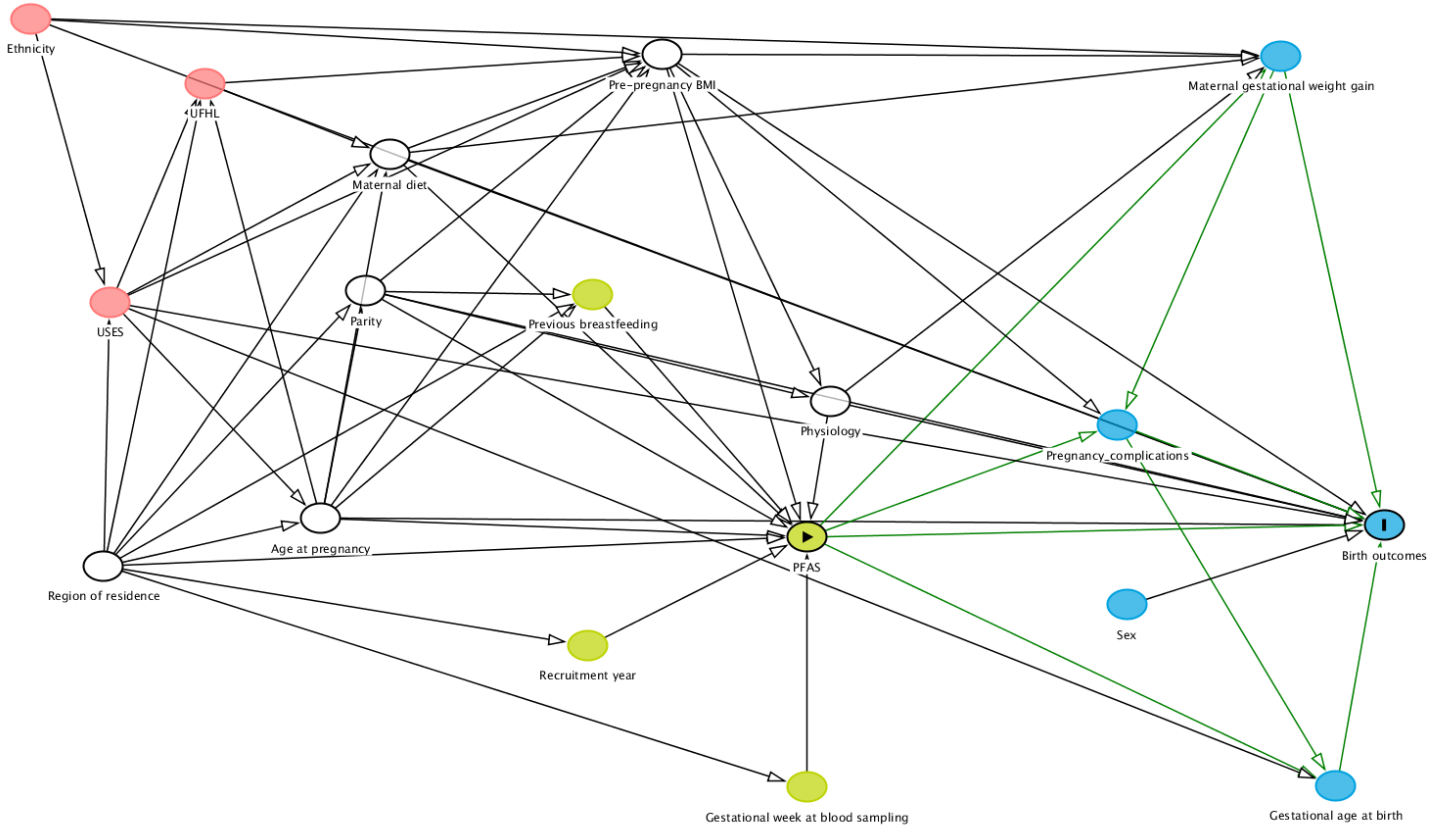


Figure S2. Directed acyclic graph for selection of covariates.



Color legend: White: covariates included in the multivariate models; Red: common ancestors of exposure and outcome; Green: ancestors of exposure; Blue: ancestors of outcome. Region of residence was used as random effects in the mixed models.

Table S1. Maternal and newborn characteristics in the excluded and included participants of our study.

Characteristic	Study population – Mean (SD), or n (%)			
	Excluded (n=948)	Included (n=1,202)	Total (N=2,150)	p-value
Maternal				
Age (years)	31.4 (4.4)	30.7 (4.0)	30.9 (4.2)	< 0.001
Pre-pregnancy BMI (kg/m^2)	23.3 (4.3)	23.6 (4.3)	23.5 (4.3)	0.16
Region of residence				
Gipuzkoa	332 (35)	306 (25)	638 (30)	< 0.001
Sabadell	257 (27)	400 (33)	657 (31)	
Valencia	359 (38)	496 (41)	855 (40)	
Parity				
None	498 (54)	670 (56)	1168 (55)	0.30
One	341 (37)	452 (38)	793 (37)	
Two or more	77 (8)	80 (7)	157 (7)	
Educational level				
Primary or without education	285 (31)	278 (23)	563 (27)	< 0.001
Secondary	359 (39)	503 (42)	862 (41)	
University	273 (30)	418 (35)	691 (33)	
Country of birth				
Spain	798 (87)	1117 (93)	1915 (90)	< 0.001
Other	119 (13)	83 (7)	202 (10)	
Previous breastfeeding				
Never	556 (61)	726 (60)	1282 (61)	0.41
Short-term (<4 months)	112 (12)	129 (11)	241 (11)	
Long-term (4–6 months)	64 (7)	104 (9)	168 (8)	
Very-long-term (>6 months)	184 (20)	243 (20)	427 (20)	
Glomerular filtration rate (mL/min/1.73 m^2)	118.8 (35.2)	122.4 (37.6)	122.2 (37.5)	0.56
Newborn				
Sex				
Girl	399 (49)	584 (49)	983 (49)	0.89
Boy	417 (51)	618 (51)	1035 (51)	
Weight (g)	3234(512)	3263 (461)	3251 (483)	0.18
Length (cm)	49.3 (2.4)	49.6 (2.1)	49.5 (2.2)	< 0.001
Head circumference (cm)	34.2 (1.6)	34.3 (1.4)	34.3 (1.5)	0.15
Gestational age (weeks) – median (SD)	39.8 (1.9)	39.8 (1.5)	39.8 (1.7)	0.06
Small for gestational age				
No	712 (89)	1081 (90)	1793 (89)	0.36
Yes	91 (11)	121 (10)	212 (11)	

Preterm birth				
No	771 (95)	1157 (96)	1928 (96)	0.10
Yes	43 (5)	45 (4)	88 (4)	
Low birth weight				
No	759 (95)	1144 (95)	1903 (95)	0.51
Yes	44 (5)	58 (5)	102 (5)	

Abbreviations: SD: standard deviation; BMI: body mass index.

P-values correspond to the following: Analysis of variance (ANOVA) for continuous variables, and chi-squared or Fisher´s-exact test for categorical variables.

Table S2. Maternal and newborn characteristics in the sample included and excluded from the maternal GFR analysis.

Characteristic	Maternal GFR analysis – Mean (SD), or n (%)		p-value
	Excluded	Included	
Maternal			
PFAS (ng/mL)- median (SD)			
PFHxS	0.58 (0.36)	0.58 (0.56)	0.48
PFOS	6.02 (2.95)	6.07 (2.99)	0.61
PFOA	2.50 (1.56)	2.29 (1.76)	0.05
PFNA	0.64 (0.47)	0.67 (0.38)	0.96
Age (years)	30.6 (4.1)	30.7 (3.9)	0.57
Pre-pregnancy BMI (kg/m^2)	23.9 (4.3)	23.4 (4.3)	0.04
Region of residence			
Gipuzkoa	30 (7)	276 (36)	< 0.001
Sabadell	98 (22)	302 (39)	
Valencia	309 (71)	187 (24)	
Parity			
None	244 (56)	426 (56)	0.88
One	162 (37)	290 (38)	
Two or more	31 (7)	49 (6)	
Educational level			
Primary or without education	124 (28)	154 (20)	< 0.001
Secondary	187 (43)	316 (41)	
University	125 (29)	293 (38)	
Country of birth			
Spain	407 (93)	710 (93)	0.78
Other	29 (7)	54 (7)	
Previous breastfeeding			
Never	271 (62)	455 (59)	0.17
Short-term (<4 months)	51 (12)	78 (10)	
Long-term (4–6 months)	41 (9)	63 (8)	
Very-long-term (>6 months)	74 (17)	169 (22)	
Newborn			
Sex			
Girl	200 (46)	384 (50)	0.13
Boy	237 (54)	381 (50)	
Weight (g)	3285 (475)	3250 (453)	0.20
Length (cm)	50.1 (2.1)	49.4 (2.0)	< 0.001
Head circumference (cm)	34.2 (1.4)	34.4 (1.4)	0.03
Gestational age (weeks) – median (SD)	39.7 (1.4)	39.6 (1.5)	0.30
Small for gestational age			
No	396 (91)	685 (90)	0.55
Yes	41 (9)	80 (10)	
Preterm birth			

No	419 (96)	738 (96)	0.60
Yes	18 (4)	27 (4)	
Low birth weight			
No	419 (96)	725 (95)	0.38
Yes	18 (4)	40 (5)	

Abbreviations: GFR: glomerular filtration rate; SD: standard deviation; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctanesulfonic acid; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; BMI: body mass index.

P-values correspond to the following: Analysis of variance (ANOVA) for continuous variables and chi-squared or Fisher´s-exact test for categorical variables.

Table S3. Associations between maternal PFAS concentrations (log_2-transformed, ng/mL) and continuous birth outcomes by region of residence in the INMA birth cohort (2003-2008).

PFAS	Weight (g)		Length (cm)		Head circumference (cm) [§]		Gestational age (weeks)	
	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)
PFHxS								
Gipuzkoa	302	25.22 (-19.58, 70.02)	289	-0.02 (-0.22, 0.19)	289	0.07 (-0.07, 0.21)	306	0.09 (-0.08, 0.25)
Sabadell	388	-24.53 (-66.61, 17.56)	380	-0.05 (-0.24, 0.13)	381	-0.02 (-0.15, 0.10)	400	-0.02 (-0.17, 0.13)
Valencia	495	-14.71 (-61.49, 32.07)	495	-0.12 (-0.32, 0.09)	494	-0.05 (-0.20, 0.10)	496	-0.04 (-0.23, 0.15)
PFOS								
Gipuzkoa	302	85.36 (20.01, 150.70)*	289	0.21 (-0.09, 0.52)	289	0.23 (0.02, 0.43)*	306	0.03 (-0.22, 0.27)
Sabadell	388	-22.56 (-74.14, 29.03)	380	-0.03 (-0.26, 0.19)	381	-0.07 (-0.22, 0.09)	400	-0.14 (-0.33, 0.05)
Valencia	495	-23.54 (-80.64, 33.56)	495	-0.04 (-0.30, 0.21)	494	-0.05 (-0.24, 0.13)	496	-0.03 (-0.26, 0.21)
PFOA								
Gipuzkoa	302	50.44 (-10.78, 111.65)	289	0.13 (-0.15, 0.41)	289	-0.02 (-0.21, 0.17)	306	0.02 (-0.21, 0.25)
Sabadell	388	-44.70 (-99.59, 10.19)	380	-0.08 (-0.32, 0.16)	381	-0.13 (-0.29, 0.04)	400	-0.03 (-0.23, 0.17)
Valencia	495	-6.93 (-57.26, 43.40)	495	-0.05 (-0.28, 0.17)	494	-0.04 (-0.20, 0.13)	496	-0.05 (-0.26, 0.16)
PFNA								
Gipuzkoa	302	70.55 (11.05, 130.05)	289	0.14 (-0.14, 0.41)	289	0.00 (-0.19, 0.19)	306	0.11 (-0.12, 0.33)
Sabadell	388	-43.47 (-93.59, 6.65)	380	-0.06 (-0.28, 0.17)	381	-0.11 (-0.25, 0.04)	400	-0.02 (-0.21, 0.16)
Valencia	495	-30.09 (-74.36, 14.18)	495	-0.04 (-0.23, 0.16)	494	-0.02 (-0.16, 0.12)	496	-0.04 (-0.22, 0.15)

Abbreviations: CI: confidence interval; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctanesulfonic acid; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; Crude: PFAS and outcome. Adjusted: All models were adjusted for maternal age, parity, pre-pregnancy BMI, and fish intake during pregnancy. [§] Models further adjusted for type of delivery. * p-value<0.05.

Table S4. Associations between maternal PFAS concentrations (log_2-transformed, ng/mL) and continuous birth outcomes by sex of the child in the INMA birth cohort (2003-2008).

	Weight (g)			Length (cm)			Head circumference (cm) [§]			Gestational age (weeks)		
PFAS	**n**	**β (95% CI)**	**p-sex**	**n**	**β (95% CI)**	**p-sex**	**n**	**β (95% CI)**	**p-sex**	**n**	**β (95% CI)**	**p-sex**
PFHxS												
Girls	576	-9.61 (-42.54, 23.33)	0.77	564	-0.06 (-0.23, 0.10)	0.82	564	-0.04 (-0.16, 0.07)	0.44	584	0.04 (-0.10, 0.18)	0.38
Boys	609	-4.45 (-36.68, 27.77)		600	-0.02 (-0.18, 0.13)		600	0.04 (-0.07, 0.14)		618	-0.06 (-0.18, 0.06)	
PFOS												
Girls	576	-0.68 (-47.15, 45.80)	0.75	564	0.04 (-0.17, 0.25)	0.98	564	-0.05 (-0.19, 0.10)	0.53	584	-0.01 (-0.19, 0.18)	0.38
Boys	609	2.27 (-43.29, 47.83)		600	0.02 (-0.18, 0.22)		600	0.03 (-0.11, 0.17)		618	-0.12 (-0.30, 0.05)	
PFOA												
Girls	576	13.81 (-26.67, 54.30)	0.25	564	0.04 (-0.16, 0.24)	0.82	564	0.03 (-0.10, 0.17)	0.66	584	-0.08 (-0.24, 0.08)	0.53
Boys	609	-24.75 (-66.71, 17.22)		600	0.01 (-0.18, 0.21)		600	-0.13 (-0.27, 0.00)		618	-0.04 (-0.20, 0.13)	
PFNA												
Girls	576	-7.10 (-46.09, 31.88)	0.94	564	-0.01 (-0.19, 0.18)	0.73	564	0.03 (-0.10, 0.15)	0.19	584	-0.02 (-0.17, 0.14)	0.77
Boys	609	-9.20 (-47.48, 29.07)		600	0.02 (-0.16, 0.20)		600	-0.10 (-0.22, 0.02)		618	0.01 (-0.13, 0.16)	

Abbreviations: CI: confidence interval; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctanesulfonic acid; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid. All models were adjusted for maternal age, parity, pre-pregnancy BMI, and fish intake during pregnancy. [§] Models further adjusted for type of delivery.

Table S5. Associations between quartiles of maternal PFAS concentrations (in ng/mL) and continuous birth outcomes in the INMA birth cohort (2003-2008).

		β (95% CI)			
PFAS, quartiles (ng/mL)	**n**	**Weight (g)**	**Length (cm)**	**Head circumference (cm)**	**Gestational age (weeks)**
PFHxS					
Q1 (0.05 – 0.41)	299	Reference	Reference	Reference	Reference
Q2 (0.42 – 0.58)	299	-30.15 (-92.83, 32.54)	-0.33 (-0.61, -0.05)*	-0.08 (-0.27, 0.12)	-0.08 (-0.32, 0.17)
Q3 (0.59 – 0.82)	301	-64.85 (-127.90, -1.80)*	-0.32 (-0.61, -0.02)*	-0.14 (-0.35, 0.07)	-0.02 (-0.27, 0.23)
Q4 (0.82 – 11.01)	303	-40.25 (-104.63, 24.13)	-0.31 (-0.64, 0.02)	-0.16 (-0.39, 0.07)	-0.16 (-0.43, 0.10)
PFOS					
Q1 (0.28 – 4.52)	301	Reference	Reference	Reference	Reference
Q2 (4.53 – 6.06)	301	23.65 (-38.99, 86.28)	0.04 (-0.24, 0.32)	0.00 (-0.19, 0.20)	-0.09 (-0.33, 0.16)
Q3 (6.07 – 7.82)	301	38.70 (-24.12, 101.51)	0.08 (-0.20, 0.37)	-0.00 (-0.20, 0.20)	-0.02 (-0.26, 0.23)
Q4 (7.84 – 38.58)	299	8.23 (-55.31, 71.78)	0.00 (-0.28, 0.29)	0.02 (-0.18, 0.22)	-0.31 (-0.55, -0.06)*
PFOA					
Q1 (0.28 – 1.63)	300	Reference	Reference	Reference	Reference
Q2 (1.64 – 2.35)	301	-29.60 (-92.82, 33.63)	0.01 (-0.28, 0.29)	-0.01 (-0.22, 0.19)	-0.05 (-0.29, 0.20)
Q3 (2.36 – 3.30)	299	-32.99 (-97.08, 31.09)	-0.06 (-0.36, 0.24)	0.04 (-0.17, 0.25)	0.03 (-0.23, 0.28)
Q4 (3.31 – 31.64)	302	-32.77 (-97.65, 32.11)	-0.03 (-0.34, 0.28)	-0.16 (-0.38, 0.06)	-0.12 (-0.37, 0.14)
PFNA					
Q1 (0.03 – 0.49)	298	Reference	Reference	Reference	Reference
Q2 (0.50 – 0.65)	301	-4.75 (-67.62, 58.13)	0.00 (-0.28, 0.29)	-0.01 (-0.20, 0.19)	0.01 (-0.23, 0.26)
Q3 (0.66 – 0.90)	301	3.56 (-60.34, 67.46)	0.05 (-0.25, 0.34)	-0.06 (-0.27, 0.15)	0.13 (-0.12, 0.38)
Q4 (0.90 – 5.51)	302	-5.34 (-69.00, 58.32)	-0.06 (-0.36, 0.24)	-0.16 (-0.37, 0.05)	-0.07 (-0.33, 0.18)

Abbreviations: CI: confidence interval; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctanesulfonic acid; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid. PFAS concentrations are presented as ng/mL. All models were adjusted for maternal age, parity, pre-pregnancy BMI, and fish intake during pregnancy. * p-value<0.05.

Table S6. Multipollutant models for the association between maternal PFAS concentrations (log_2-transformed, ng/mL) and continuous birth outcomes in the INMA birth cohort (2003-2008).

	β (95% CI)			
PFAS	**Weight (g)**	**Length (cm)**	**Head circumference (cm) [§]**	**Gestational age (weeks)**
PFHxS	-11.38 (-42.31, 19.54)	-0.11 (-0.25, 0.04)	0.00 (-0.10, 0.11)	0.03 (-0.09, 0.15)
PFOS	18.64 (-26.08, 63.36)	0.12 (-0.09, 0.32)	0.05 (-0.09, 0.19)	-0.10 (-0.27, 0.08)
PFOA	1.02 (-42.73, 44.77)	0.01 (-0.20, 0.21)	-0.08 (-0.22, 0.06)	-0.08 (-0.25, 0.09)
PFNA	-14.10 (-54.92, 26.72)	-0.01 (-0.21, 0.18)	-0.02 (-0.15, 0.11)	0.08 (-0.08, 0.24)

Abbreviations: CI: confidence interval; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctanesulfonic acid; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid. All models were adjusted for maternal PFAS concentration, age, parity, pre-pregnancy BMI, and fish intake during pregnancy. [§] Models of head circumference were also adjusted for type of delivery.