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前向きコーホート研究に基づく先天異常、免疫アレルギーおよび小児発達障害のリスク評価と環境化学物質に対する遺伝的感受性の解明

平成 27 年度　総括・分担研究報告書

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Prenatal exposure to perfluorinated chemicals and neurodevelopment in early infancy: The Hokkaido Study

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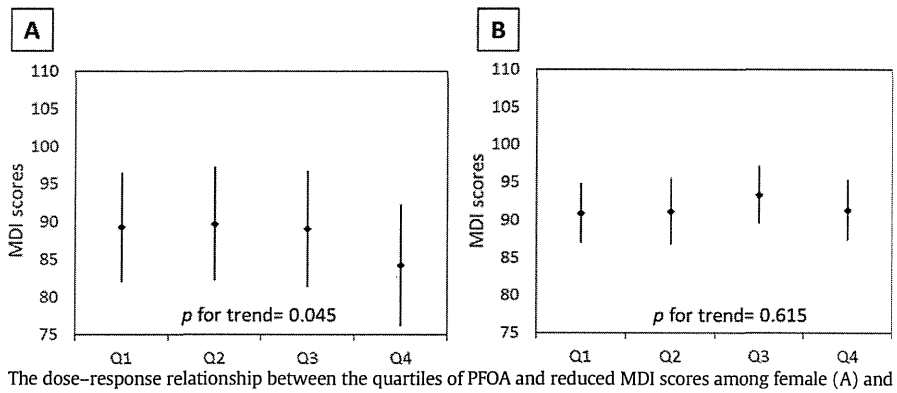
HIGHLIGHTS

- The association of prenatal exposure to PFCs with neurodevelopment were assessed.
- Neurodevelopment was evaluated at 6 and 18 months of age using the Bayley Scales of Infant Development.
- PFOA was negatively associated with mental developmental indices among 6-month female infants.

GRAPHICAL ABSTRACT



The dose–response relationship between the quartiles of PFOA and reduced MDI scores among female (A) and male (B) infants at 6 months of age.

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ABSTRACT

Perfluorinated chemicals (PFCs) are ubiquitous and persistent pollutants widely detected in blood samples of animals and humans across the globe. Although animal studies have shown the potential neurotoxicity of PFCs, there are few epidemiological studies regarding neurological effects of PFCs in humans, and those studies have had inconclusive results. In this study, we conducted a hospital-based prospective birth cohort study between 2002 and 2005 (n = 514) to examine the associations between prenatal perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) exposures and the neurodevelopment of infants at 6 (n = 173) and 18 (n = 133) months of age. Using the second edition of the Bayley Scales of Infant Development (BSID II), the Mental and Psychomotor Developmental Indices (MDI and PDI, respectively) were assessed. PFOS and PFOA were measured in maternal serum samples by liquid chromatography–tandem mass spectrometry. After controlling for confounders, prenatal PFOA concentrations were associated with the MDI of female (but not male) infants at 6 months of age (β = −0.296; 95% confidence interval (CI): −11.96, −0.682). Furthermore, females born to mothers with prenatal concentrations of PFOA in the fourth quartile had MDI scores −5.05 (95% CI: −10.66 to 0.55) lower than females born to mothers with concentrations of PFOA in the first quartile (p for trend = 0.045). However, PFOA concentrations were not significantly associated with neurodevelopmental indices at

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18 months of age. In addition, we did not observe any significant association between PFOS concentrations and neurodevelopmental outcomes in early infancy. In conclusion, our results suggest that prenatal PFOA exposure may affect female mental scales of neurodevelopment at 6 months of age. Further studies with larger sample sizes and longer observation periods are required to clarify sex difference of the neurodevelopmental effects.

1. Introduction

Perfluorinated chemicals (PFCs) are persistent and ubiquitous chemicals widely used in several industrial applications and consumer products (Lau et al., 2007). The most common route of exposure to PFCs is dietary via the consumption of contaminated food and drinking water; indoor air and dust are other potential source of exposure to PFCs (Fromme et al., 2009; Kato et al., 2009; Beesoon et al., 2011). The most well-detected PFCs in humans and biota are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). While PFOS and PFOA are being voluntarily phased out by several industries, they are still present in older products. Additionally, PFCs are resistant to metabolism, and PFOS and PFOA are slowly eliminated from the human body with mean half-lives of 5.4 and 3.8 years, respectively (Olsen et al., 2007).

In rats, neonatal exposure to PFCs can inhibit synaptogenesis in the developing brain (Liao et al. 2008; Johansson et al., 2009, Johansson et al., 2008), and exposure to PFOS and PFOA in neonatal mice results in spontaneous deranged behavior, including irreversibly reduced habituation or hyperactivity in adult mice (Johansson et al., 2008). In vitro and in vivo studies have suggested that PFCs exert toxic effects on developing brains via the disruption of thyroid hormone balances (Lau et al., 2003; Wang et al., 2011).

Fetuses are exposed to PFCs during the maternofetal passage (Inoue et al., 2004; Monroy et al., 2008). Infants and children may experience higher exposure to PFCs than adults due to crawling, inhalation and ingestion of indoor air dust. PFCs are detectable in brain tissue following oral exposure (Bogdanska et al., 2011), and the blood brain barrier is not completely formed until early infancy (Adinolfi, 1985), leaving infants more susceptible to the potential adverse effects of chemicals including PFCs.

Animals and humans are vulnerable to exposure of chemicals at the period of extensive growth and development of the brain called the brain growth sprout (BGS). In humans, BGS begins during the last trimester of pregnancy and extends throughout the first two years of life (Mariussen, 2012). A Taiwanese study showed a negative association between PFOS levels in cord blood and neurodevelopmental scales, particularly the gross-motor subdomain at 2 years of age (Chen et al., 2013). In contrast, no convincing associations were reported between prenatal PFOS/PFOA exposure and neurodevelopmental milestones at 6 and 18 months of age in the prospective Danish birth cohort study (Fei et al., 2008). In addition, no association was found between prenatal PFC levels and childhood behavioral or coordination problems at 7 years of age in the same cohort (Fei and Olsen, 2011).

There are a limited number of studies focusing on the potential neurobehavioral effects of PFCs in humans, and these studies have nonconclusive results; the potential effects of prenatal exposure to PFCs on the neurodevelopment of humans and especially in early infancy are not well understood. Therefore, in the present analysis, we explored the relationship between prenatal exposure to PFOS/PFOA and the neurodevelopment of infants at 6 and 18 months of age assessed by the Bayley Scales of Infant Development.

2. Material and methods

2.1. Study population

This study was a part of the Hokkaido Study on Environment and Children's Health performed between July 2002 and October 2005, and the details have been previously described (Kishi et al., 2011 and 2013). In this prospective birth cohort study, pregnant women were recruited between 23–35 weeks of gestation and delivered their children in one hospital in Sapporo, Japan. Of 1796 potentially eligible women, the following subjects were excluded: the women who decided to participate in the Japanese cord blood bank (22% of those approached), and the women who decided to deliver at another hospital (3% of those approached). Ultimately, 514 (28.6%) pregnant women agreed to participate in this study. All participants were natives of Japan and residents of Sapporo or the surrounding areas. For the analysis of the associations between maternal PFCs and BSID II, the following subjects were excluded: women with pregnancy-induced hypertension (n = 11), women with diabetes mellitus (n = 1), mother–infant pairs with fetal heart failure (n = 1), and twins (n = 7). After the exclusion of these subjects, 428 mother–infant pairs had available PFOS and PFOA concentrations. In addition, the eligibility criteria for the analysis of subjects included the term newborns of 37–42 weeks of gestation, Apgar score of >7 at 1 min, infants without congenital anomalies or diseases, and having completed BSID-II. To assess the association between PFCs and neurodevelopment at 6 months of age, we included infants who were examined between 5.5 and 6.5 months of age, and we excluded 4 subjects because the neurodevelopment examination was performed after 6.5 months of age.

2.2. Questionnaires and medical records

A self-administered questionnaire survey was completed after the second trimester of pregnancy (Washino et al., 2009) containing information related to previous medical history, smoking, economic status, educational levels, alcohol and caffeine intake during pregnancy, and dietary intake during pregnancy including daily fish intake. For estimation of alcohol and caffeine intake during pregnancy, a self-administered questionnaire was used as described by Nagata et al. (1998). Medical information including maternal age, maternal body mass index (BMI) before pregnancy, parity, gestational age, pregnancy complications, type of delivery, infant's sex, and birth size (weight, length, and head circumferences) was obtained from participant medical records. This study was conducted with the written informed consent of all participants, and the study protocol was approved by the institutional ethical board for epidemiological studies at the Graduate School of Medicine and Center for Environmental and Health Sciences, Hokkaido University.

2.3. Blood sampling and exposure assessment

A 40-mL blood sample was taken from the maternal peripheral vein during the perinatal period after the second trimester of pregnancy. All samples were stored at −80 °C until analysis. Detailed methods for the measurement of PFOS and PFOA have been described in our previous report (Nakata et al., 2009). In brief, serum samples (0.1 mL) were mixed with 0.2 mL internal standard ($^{13}C_4$–PFOS–Na^+ and $^{13}C_2$–PFOA) solution containing acetonitrile, centrifuged at 1450 × *g* for 10 min, and the supernatant was transferred to a polypropylene tube. An aliquot of the filtered sample solution was subjected to column-switching liquid chromatography–tandem mass spectrometry (LC/MS/MS). The detection limit for both PFOS and PFOA was 0.5 ng/mL. PFOS levels were detected in

all samples, and for samples with PFOA levels below detection limit, we used a value of half the detection limit (0.25 ng/mL). Detection rate for PFOA was 91.3% and 93.2% in maternal serum whose children examined at 6 and 18 months, respectively.

2.4. Neurodevelopmental assessment

Details of the developmental measures have been previously described by our group (Nakajima et al., 2006). Briefly, infants' neurodevelopment was assessed at 6 and 18 months of age using the Bayley Scales of Infant Development, second edition (BSID-II, 1993). This test measures the mental developmental index (MDI) and psychomotor developmental index (PDI) from 0 to 3 years of age. The BSID-II mental scale assesses the age-appropriate level of cognitive, language, and personal/social development. The motor scale examines fine and gross motor development. Mental and motor scores are based on the calibration scale from a raw score and are represented as index scores. The mean scores of MDI and PDI (±SD) are 100 (±15). All children were examined by one examiner in a quiet, private room in the presence of the parent(s). The indicators of neurodevelopment were evaluated by three occupational therapists with clinical experience in the field of developmental disabilities. The examiners were unaware of the participants' PFC levels. First, the scoring was performed by the examiner who conducted the assessment of all of the children, and two other examiners then verified and confirmed the scores by reviewing videotaped evaluations. In addition, we assessed the index of the childcare environment of the subjects using the questionnaire (Anme et al., 1997).

2.5. Data analysis

We analyzed the correlations between PFOS and PFOA concentrations and the characteristics of the mothers and infants using the Spearman rank correlation coefficient (rs), the Mann–Whitney *U*-test, and the Kruskal–Wallis test. The same statistical analyses were performed to find correlations between infants' BSID II scores and the participants' characteristics. We performed multiple-regression analysis to examine the association between BSID-II scores (MDI, PDI) and the levels of PFCs in maternal serum samples. The levels of PFCs in maternal blood were $\log_{10}$ transformed, and potential confounders were selected according to the previous literature and current results in this paper. The analysis was adjusted for maternal age (year), parity (0/≥1), maternal educational levels (categorical), alcohol consumption and smoking during pregnancy (yes/no), caffeine intake during pregnancy (milligrams/day), blood sampling period (before and after delivery), breastfeeding (less or more than 3 months). Previously, our group reported that prenatal exposure to some dioxin congeners is negatively associated with MDI and PDI scores at 6 months of age (Nakajima et al., 2006). Therefore, prenatal dioxin levels were added to the aforementioned confounders in an additional adjustment model (fully adjusted model). Moreover, the associations between the PFCs and BSID II scores were assessed after stratifying by the infants' sex. For the assessment of dose–response, the prenatal PFC concentrations in the maternal blood samples were divided into quartiles, and least square means (LSMs) and 95% confidence interval (CI) were calculated. For calculation of *p* for trend, we used the linear contrast coefficient −3, −1, +1, +3 assigned to quartile 1, 2, 3, and 4, respectively. The LSM of the BSID II for each quartile was compared using the Hsu–Dunnet method to accommodate for multiple comparisons. We performed all of the statistical analyses using JMP pro 11 (SAS Institute Inc., NC, USA). Results were considered significant if $p < 0.05$.

3. Results

The basic characteristics of the study population are presented in Table 1. The mean (SD) scores were 90.5 (5.7) for MDI and 90.1 (10.1) for PDI in infants at 6 months of age, whereas the mean (SD) for MDI and PDI at 18 months of age was 84.2 (12.0) and 86.3 (10.9), respectively. The median value of prenatal PFOS and PFOA levels in mothers whose infants were examined at 6 months of age was 5.7 ng/mL (25–75 percentile: 4.4–7.4) and 1.2 ng/mL (25–75 percentile: 0.8–1.7), respectively (Table 2). Tables 2 and 3 show maternal PFOA and PFOS levels according to characteristics of the mothers and the infants at 6 and 18 months of age, respectively. Prenatal PFOS levels were significantly correlated with maternal age, parity, blood sampling period in mother–infant pairs at 6 and 18 months of age. PFOA levels were negatively associated with parity (Tables 2 and 3).

Birth weight was associated with the infants' MDI at 6 months of age ($rs = 0.182$; $p = 0.016$). At 6 months of age, PDI scores were negatively correlated with caffeine intake during pregnancy and positively correlated with gestational age, birth weight and length (Table 4). At 18 months, MDI was correlated positively with higher annual income during pregnancy, female gender, and longer gestational age, whereas PDI was correlated positively with higher annual income during pregnancy and female gender and negatively correlated with pre-pregnancy BMI (Table 5).

After adjusting for appropriate confounders, PFOS and PFOA did not show any significant association with MDI nor PDI at 6 months of age in total infants (Table 6). After sex stratification, we found a significant negative association between prenatal PFOA exposure and MDI scores only in female infants ($\beta = -0.296$; 95% confidence interval: −11.96, −0.682) in the model 2. We also examined the association between the serum PFOA quartiles and MDI at 6 months of age. In the adjusted model, female infants whose mothers were in the highest quartile of PFOA concentration had MDI scores −5.05 [95% CI: −10.66 to 0.55]

Table 1
Characteristics of mother–infant pairs.

Characteristics	6-month postpartum assessment (n = 173)	18-month postpartum assessment (n = 133)
	No. (%)/mean ± SD	No. (%)/mean ± SD
Maternal characteristics		
Age (years)[a]	30.8 ± 4.6	31.0 ± 4.4
Prepregnancy BMI (kg/m^2)[a]	21.1 ± 2.9	21.2 ± 2.8
Primipara	83 (48.0)	65 (48.9)
Educational ≤ 12 years	66 (38.1)	48 (36.1)
Annual income during pregnancy < 5 million yen	112 (64.7)	85 (64.0)
Smoking during pregnancy (yes)	20 (11.6)	11 (8.3)
Alcohol intake during pregnancy (yes)	51 (29.5)	40 (30.1)
Child characteristics		
Male infant	83 (48.0)	66 (49.6)
Gestational age (days)[a]	276.3 ± 8.3	276.3 ± 8.5
Birth weight (g)[a]	3111.2 ± 358.5	3096.8 ± 323.8
Birth length (cm)[a]	48.2 ± 1.7	48.2 ± 1.6
Breast-feeding < 3 months	71 (41.0)	50 (37.6)
BSID II mental index score (MDI)[a]	90.5 ± 5.7	84.0 ± 12.0
BSID II psychomotor index score (PDI)[a]	90.1 ± 10.1	86.3 ± 10.9
Index of child care environment[b]	22.2 ± 3.9	28.2 ± 3.5

[a] Mean ± SD.
[b] Perfect score is 30 points for 6 months and 38 for 18 months of age, respectively.

lower than female infants born to mothers with PFOA concentrations in the first quartile. The p-value between quartile 1 and 4 did not meet significance (p = 0.086), but the p for trend was significant (p = 0.045) (Fig. 1, and Supplementary Table 1). However, at 18 months of age, we observed no significant association of PFCs with MDI and PDI (Table 7). Moreover, we did not find any sex differences in the

Table 2
Maternal blood PFC levels (ng/mL) in relation to characteristics of mothers and 6-month old infants (n = 173).

Characteristics	N	PFOS (mean or correlation)	p-Value	PFOA (mean or correlation)	p-Value
Median (25–75 percentile)		5.7 (4.4–7.4)		1.2 (0.8–1.7)	
Maternal characteristics					
Age (years)		rs = −0.186	**0.014**	rs = −0.093	0.220
Prepregnancy BMI (kg/m^2)		rs = 0.017	0.822	rs = −0.088	0.247
Parity (times)					
0	83	6.95 ± 0.29	**0.003**	1.65 ± 0.08	**<0.001**
≥1	90	5.54 ± 0.28		1.04 ± 0.07	
Educational level (years)					
≤12	66	5.79 ± 0.33	0.056	1.30 ± 0.09	0.695
≥13	107	6.48 ± 0.26		1.35 ± 0.07	
Annual income (million yen)					
<5	112	6.27 ± 0.25	0.757	1.34 ± 0.07	0.856
≥5	61	6.12 ± 0.35		1.32 ± 0.10	
Smoking during pregnancy					
Yes	20	5.25 ± 0.61	0.092	1.21 ± 0.18	0.519
No	153	6.34 ± 0.22		1.35 ± 0.06	
Alcohol intake during pregnancy					
Yes	51	6.38 ± 0.38	0.208	1.34 ± 0.11	0.731
No	122	6.15 ± 0.24		1.33 ± 0.07	
Alcohol intake during pregnancy (g/day)		rs = 0.078	0.306	rs = 0.020	0.793
Caffeine intake during pregnancy (mg/day)		rs = −0.136	0.073	rs = −0.048	0.526
Fish intake during pregnancy					
Inshore fish					
Less than 1–2 times/month	95	6.02 ± 0.28	0.218	1.31 ± 0.08	0.482
More than 1–2 times/week	78	6.46 ± 0.31		1.36 ± 0.09	
Deep-sea fish					
Less than 1–2 times/month	81	6.02 ± 0.30	0.135	1.36 ± 0.08	0.727
More than 1–2 times/week	92	6.39 ± 0.28		1.31 ± 0.08	
Blood sampling period					
During pregnancy	131	6.55 ± 0.23	**0.002**	1.40 ± 0.06	0.135
After delivery	42	5.19 ± 0.41		1.12 ± 0.12	
Child characteristics					
Sex					
Male	83	6.23 ± 0.30	0.805	1.45 ± 0.08	0.164
Female	90	6.21 ± 0.29		1.23 ± 0.08	
Type of delivery					
Vaginal	146	6.39 ± 0.22	0.061	1.36 ± 0.06	0.272
Cesarean section	28	5.26 ± 0.52		1.17 ± 0.15	
Gestational age (days)		rs = 0.071	0.352	rs = −0.018	0.812
Birth weight (g)		rs = −0.058	0.444	rs = −0.105	0.166
Birth length (cm)		rs = 0.151	0.046	rs = −0.004	0.949
Head circumference (cm)		rs = −0.013	0.855	rs = −0.020	0.791
Feeding					
Breast-feeding	72	6.30 ± 0.32	0.684	1.36 ± 0.09	0.929
Mix	79	6.02 ± 0.30		1.29 ± 0.08	
Bottle-feeding	6	6.66 ± 1.12		1.20 ± 0.32	
Breast-feeding (month)					
<3	71	6.26 ± 0.32	0.900	1.31 ± 0.09	0.603
≥3	102	6.19 ± 0.27		1.35 ± 0.07	

Statistical analysis is performed using Spearman rank correlation coefficient (rs), and Mann–Whitney *U*-test and Kruskal–Wallis test.

Table 3
Maternal blood PFC levels (ng/mL) in relation to characteristics of mothers and 18-month old infants (n = 133).

Characteristics	N	PFOS (mean or correlation)	p-Value	PFOA (mean or correlation)	p-Value
Median (25–75 percentile)		5.8 (4.5–7.4)		1.2 (0.8–1.7)	
Maternal characteristics					
Age (years)		rs = −0.251	**0.003**	rs = −0.137	0.130
Prepregnancy BMI (kg/m^2)		rs = 0.049	0.569	rs = −0.056	0.518
Parity (times)					
0	65	6.87 ± 0.31	**0.019**	1.69 ± 0.09	**<0.001**
≥1	68	5.64 ± 0.30		1.09 ± 0.09	
Educational level (years)					
≤12	48	5.81 ± 0.37	0.083	1.45 ± 0.12	0.480
≥13	85	6.49 ± 0.28		1.34 ± 0.09	
Annual income (million yen)					
<5	85	6.08 ± 0.28	0.408	1.38 ± 0.09	0.990
≥5	48	6.53 ± 0.37		1.38 ± 0.12	
Smoking during pregnancy					
Yes	11	5.20 ± 0.78	0.164	1.23 ± 0.25	0.700
No	122	6.33 ± 0.23		1.40 ± 0.07	
Alcohol intake during pregnancy					
Yes	40	6.41 ± 0.41	0.231	1.40 ± 0.13	0.609
No	93	6.17 ± 0.27		1.37 ± 0.08	
Alcohol intake during pregnancy (g/day)		rs = 0.088	0.313	rs = 0.022	0.800
Caffeine intake during pregnancy (mg/day)		rs = −0.215	**0.012**	rs = −0.081	0.349
Fish intake during pregnancy					
Inshore fish					
Less than 1–2 times/month	73	6.10 ± 0.30	0.487	1.34 ± 0.09	0.291
More than 1–2 times/week	60	6.41 ± 0.33		1.44 ± 0.10	
Deep-sea fish					
Less than 1–2 times/month	64	5.98 ± 0.32	0.105	1.40 ± 0.10	0.872
More than 1–2 times/week	69	6.48 ± 0.31		1.37 ± 0.10	
Blood sampling period					
During pregnancy	106	6.56 ± 0.24	**0.006**	1.45 ± 0.08	**0.083**
After delivery	27	5.00 ± 0.49		1.12 ± 0.16	
Child characteristics					
Sex					
Male	66	6.17 ± 0.32	0.388	1.52 ± 0.10	0.053
Female	67	6.31 ± 0.32		1.25 ± 0.10	
Type of delivery					
Vaginal	110	6.46 ± 0.24	**0.036**	1.42 ± 0.08	0.328
Cesarean section	23	5.18 ± 0.53		1.21 ± 0.17	
Gestational age (days)		rs = 0.138	0.112	rs = −0.039	0.654
Birth weight (g)		rs = −0.062	0.474	rs = −0.067	0.440
Birth length (cm)		rs = 0.169	0.051	rs = 0.066	0.447
Head circumference (cm)		rs = −0.099	0.256	rs = 0.013	0.874
Feeding					
Breast-feeding	59	6.40 ± 0.34	0.759	1.43 ± 0.11	0.808
Mix	66	6.03 ± 0.32		1.35 ± 0.10	
Bottle-feeding	5	6.66 ± 1.10		1.16 ± 0.37	
Breast-feeding (month)					
<3	50	6.04 ± 0.37	0.403	1.40 ± 0.11	0.935
≥3	83	6.36 ± 0.28		1.37 ± 0.09	

Statistical analysis is performed using Spearman rank correlation coefficient (rs), and Mann–Whitney *U*-test and Kruskal–Wallis test.

Table 4
Characteristics of mother–infant pairs in relation to 6-month old MDI and PDI (n = 173).

Characteristics	No.	MDI		PDI	
		Mean ± SD	p-Value	Mean ± SD	p-Value
Maternal characteristics					
Age (years)		rs = −0.046	0.539	rs = −0.001	0.987
Prepregnancy BMI (kg/m^2)		rs = −0.008	0.911	rs = 0.023	0.755
Parity (times)					
0	83	91.1 ± 0.6	0.484	89.9 ± 1.1	0.681
≥1	90	90.0 ± 0.6		90.2 ± 1.0	
Educational level (years)					
≤12	66	91.0 ± 0.7	0.346	91.0 ± 1.2	0.448
≥13	107	90.2 ± 0.5		89.5 ± 0.9	
Annual income (million yen)					
<5	112	90.4 ± 0.5	0.532	89.5 ± 0.9	0.344
≥5	61	90.7 ± 0.7		91.2 ± 1.3	
Smoking during pregnancy					
Yes	20	90.1 ± 1.2	0.441	88.7 ± 2.2	0.630
No	153	90.6 ± 0.4		90.2 ± 0.8	
Alcohol intake during pregnancy					
Yes	51	91.1 ± 0.8	0.691	90.5 ± 1.4	0.981
No	122	90.3 ± 0.5		89.9 ± 0.9	
Alcohol intake during pregnancy (g/day)		rs = 0.041	0.585	rs = −0.003	0.968
Caffeine intake during pregnancy (mg/day)		rs = −0.023	0.762	rs = −0.188	**0.013**
Fish intake during pregnancy					
Inshore fish					
Less than 1–2 times/month	95	90.6 ± 0.5	0.686	90.1 ± 1.0	0.898
More than 1–2 times/week	78	90.4 ± 0.6		90.0 ± 1.1	
Deep-sea fish					
Less than 1–2 times/month	81	90.2 ± 0.6	0.575	89.8 ± 1.1	0.603
More than 1–2 times/week	92	90.8 ± 0.6		90.3 ± 1.0	
Blood sampling period					
During pregnancy	131	90.4 ± 0.5	0.381	89.3 ± 0.8	0.076
After delivery	42	90.8 ± 0.8		92.4 ± 1.5	
Child characteristics					
Sex					
Male	83	91.0 ± 0.6	0.234	89.2 ± 1.1	0.483
Female	90	90.0 ± 0.6		90.8 ± 1.0	
Type of delivery					
Vaginal	146	90.7 ± 0.4	0.313	90.6 ± 0.8	0.062
Cesarean section	27	89.2 ± 1.1		87.3 ± 1.9	
Gestational age (days)		rs = 0.144	0.058	rs = 0.245	**0.001**
Birth weight (g)		rs = 0.182	**0.016**	rs = 0.153	**0.044**
Birth length (cm)		rs = 0.142	0.061	rs = 0.157	**0.038**
Head circumference (cm)		rs = 0.101	0.182	rs = 0.051	0.503
Feeding					
Breast-feeding	72	91.7 ± 0.6	0.052	91.4 ± 1.1	0.113
Mix	79	89.4 ± 0.6		88.6 ± 1.1	
Bottle-feeding	6	92.8 ± 2.3		93.1 ± 4.0	
Breast-feeding (month)					
<3	71	90.4 ± 0.6	0.688	89.5 ± 1.2	0.317
≥3	102	90.5 ± 0.5		90.5 ± 1.0	
Index of child care environment		rs = −0.004	0.951	rs = −0.107	0.158

Statistical analysis is performed using Spearman rank correlation coefficient (rs), and Mann–Whitney *U*-test and Kruskal–Wallis test.
Bold values indicate significance at p-value.

neurodevelopmental effects of PFCs at 18 months of age (data not shown).

4. Discussion

This study is one of few reports examining the effects of prenatal exposure to PFCs on neurodevelopment in early life. Median concentrations for maternal PFOS and PFOA in the current study were 5.7 and 1.2 ng/mL, respectively, which are one of the lowest levels reported among pregnant women compared to the median of those in the US (PFOS: 8.2, PFOA: 2.9 ng/mL) (Stein et al., 2012), Canada (PFOS: 16.6, PFOA: 2.1 ng/mL) (Monroy et al., 2008), Denmark (PFOS: 21.5, PFOA: 3.7 ng/mL) (Halldorsson et al., 2012), and Korea (PFOS: 9.3, PFOA: 2.6 ng/mL) (Lee et al., 2013). In this study, we examined the association between prenatal low exposure levels of PFOS/PFOA and neurodevelopment at 6 and 18 months of age using the BSID-II. We found an inverse association between prenatal exposure to PFOA and MDI scores only among female infants at 6 months of age; also quartile PFOA dose–response trend analysis showed a significant decrease in MDI scores at 6 months in female infants. We did not find an association between prenatal PFOA exposure and BSID II scores at 18 months of age. Prenatal exposure to PFOS was not associated with any of the measured neurodevelopmental scores at 6 and 18 months of age. Our study suggests that low levels of in utero exposure to PFOA may affect neurodevelopment in early infancy by sex differences.

The effects of PFCs on neurodevelopment in infancy and early childhood are not well understood. A group in Taiwan examined the association of PFC levels in cord blood plasma and neurodevelopment using a Taiwanese questionnaire (the Comprehensive Developmental Inventory for Infants and Toddlers) at 2 years of age (n = 239). The whole test contained 5 domains: motor (gross and fine), cognitive, language, social and self-help. Each item of these domains was scored 0 or 1, where indicates success in evaluation through direct testing, observation and self-reporting. In contrast to our results, PFOS but not PFOA levels in cord blood plasma were inversely associated with the overall test results and especially the gross-motor domain (Chen et al., 2013). In this

Table 5
Characteristics of mother–infant pairs in relation to 18-month old MDI and PDI (n = 133).

Characteristics	No.	MDI		PDI	
		Mean ± SD	p-Value	Mean ± SD	p-Value
Maternal characteristics					
Age (years)		rs = −0.068	0.436	rs = −0.090	0.301
Prepregnancy BMI (kg/m^2)		rs = −0.120	0.166	rs = −0.223	**0.009**
Parity (times)					
0	65	83.0 ± 1.4	0.550	84.8 ± 1.3	0.115
≥1	68	85.0 ± 1.4		87.7 ± 1.3	
Educational level (years)					
≤12	48	82.6 ± 1.7	0.540	85.9 ± 1.5	0.966
≥13	85	84.8 ± 1.3		86.5 ± 1.1	
Annual income (million yen)					
<5	85	81.7 ± 1.2	**0.012**	84.4 ± 1.1	**0.007**
≥5	48	88.1 ± 1.6		89.7 ± 1.5	
Smoking during pregnancy					
Yes	11	83.7 ± 3.6	0.964	86.4 ± 3.3	0.937
No	122	84.1 ± 1.0		86.3 ± 0.9	
Alcohol intake during pregnancy					
Yes	40	84.3 ± 1.9	0.945	87.2 ± 1.7	0.652
No	93	83.9 ± 1.2		86.0 ± 1.1	
Alcohol intake during pregnancy (g/day)		rs = 0.010	0.902	rs = 0.046	0.596
Caffeine intake during pregnancy (mg/day)		rs = −0.053	0.544	rs = 0.008	0.922
Fish intake during pregnancy					
Inshore fish					
Less than 1–2 times/month	73	83.6 ± 1.4	0.608	87.9 ± 1.2	0.091
More than 1–2 times/week	60	84.5 ± 1.5		84.4 ± 1.3	
Deep-sea fish					
Less than 1–2 times/month	64	83.5 ± 1.5	0.745	87.2 ± 1.3	0.418
More than 1–2 times/week	69	84.5 ± 1.4		85.5 ± 1.3	
Blood sampling period					
During pregnancy	106	84.3 ± 1.1	0.383	86.2 ± 1.0	0.813
After delivery	27	83.0 ± 2.3		86.8 ± 2.1	
Child characteristics					
Sex					
Male	66	81.2 ± 1.4	**0.007**	83.3 ± 1.2	**0.001**
Female	67	86.8 ± 1.4		89.2 ± 1.2	
Type of delivery					
Vaginal	110	85.1 ± 1.1	0.058	86.5 ± 1.0	0.652
Cesarean section	23	79.1 ± 2.4		85.4 ± 2.2	
Gestational age (days)		rs = 0.181	**0.036**	rs = 0.092	0.289
Birth weight (g)		rs = 0.125	0.151	rs = 0.050	0.567
Birth length (cm)		rs = 0.087	0.315	rs = 0.052	0.547
Head circumference (cm)		rs = −0.061	0.482	rs = 0.005	0.951
Feeding					
Breast-feeding	59	83.4 ± 1.5	0.852	86.7 ± 1.4	0.776
Mix	66	84.1 ± 1.4		85.4 ± 1.3	
Bottle-feeding	5	83.8 ± 5.4		86.4 ± 4.8	
Breast-feeding (month)					
<3	50	84.2 ± 1.7	0.881	86.6 ± 1.5	0.788
≥3	83	83.9 ± 1.3		86.1 ± 1.2	
Index of child care environment[a]		rs = 0.155	0.134	rs = −0.040	0.695

Statistical analysis is performed using Spearman rank correlation coefficient (rs), and Mann–Whitney *U*-test and Kruskal–Wallis test.
Bold values indicate significance at p-value.
[a] Not available for 39 subjects.

Taiwanese report, PFCs were examined in cord blood samples, not maternal blood samples, with medians of 7.0 and 2.5 ng/mL for PFOS and PFOA, respectively. No negative effects of low PFOS levels on infant neurodevelopment in our study may be due to the small sample size and low power. Inoue et al. (2004) reported that the mean PFOS concentration ratio in maternal blood to cord blood is 0.32, which indicates higher exposure levels of PFOS in the Taiwanese cohort compared to our study. Although we did not measure exposure levels of PFCs in cord blood samples, previous studies have determined that PFOA has higher transplacental transfer efficiency than PFOS (Beesoon et al., 2011; Lee et al., 2013), which may partially explain why we found a reverse association between only prenatal PFOA and developmental scores of infants in our study. In a Danish nationwide cohort study, Fei et al. (2008) investigated the associations between prenatal exposure to PFCs (PFOS and PFOA) and maternally reported developmental milestones at 6 and 18 months of age using a structured questionnaire with a large sample size (6 months, n = 1336; 18 months, n = 1255). The mothers were asked to recall at what time their infants developed motor (gross and fine motor) and mental skills. However, they did not find convincing associations between prenatal PFCs and neurodevelopmental milestones in early infancy. In addition, this group reported no association between prenatal PFC levels and behavioral or motor coordination problems in 7 year old children in the same cohort using the Strengths and Difficulties Questionnaire (SDQ) and the Developmental Coordination Disorder Questionnaire (DCDQ) (Fei and Olsen, 2011). In these two studies of the same cohort, maternal plasma PFOA and PFOS levels were 4–6 times higher than those in our study, although subtle effects of PFCs may not be detected by questionnaire-based examinations.

We selected confounders in the multiple linear regression model based on findings in this study and well-known factors important in infant neurodevelopment such as smoking and alcohol consumption during pregnancy. Additionally, our group reported the negative association between prenatal exposure to some congeners of dioxins

Table 6
The association between prenatal exposure to PFCs and 6-month old MDI and PDI (n = 173).

	MDI		PDI	
	Beta	(95% CI)	Beta	(95% CI)
Total (n = 173)				
PFOS				
Crude	0.035	(−3.32 to 5.40)	−0.007	(−8.05 to 7.29)
Model 1	0.015	(−4.33 to 5.21)	0.018	(−7.01 to 8.85)
Model 2	0.018	(−4.52 to 5.59)	0.039	(−6.38 to 10.37)
PFOA				
Crude	0.005	(−2.86 to 3.08)	−0.042	(−6.69 to 3.74)
Model 1	−0.039	(−4.15 to 2.59)	−0.013	(−6.09 to 5.13)
Model 2	−0.045	(−4.33 to 2.56)	−0.006	(−5.93 to 5.50)
Boys (n = 83)				
PFOS				
Crude	−0.084	(−8.74 to 3.88)	−0.020	(−10.70 to 8.85)
Model 1	−0.117	(−10.39 to 3.66)	0.124	(−4.44 to 15.47)
Model 2	−0.141	(−11.26 to 3.45)	0.120	(−5.24 to 15.60)
PFOA				
Crude	0.091	(−2.32 to 5.62)	−0.013	(−6.54 to 5.78)
Model 1	0.101	(−2.95 to 6.62)	0.055	(−5.27 to 8.36)
Model 2	0.110	(−3.31 to 7.14)	0.068	(−5.56 to 9.26)
Girls (n = 90)				
PFOS				
Crude	0.136	(−2.11 to 10.03)	0.002	(−11.63 to 11.90)
Model 1	0.093	(−3.93 to 9.34)	0.012	(−11.52 to 12.88)
Model 2	0.072	(−5.19 to 9.38)	0.031	(−11.66 to 15.09)
PFOA				
Crude	−0.094	(−6.56 to 2.48)	−0.055	(−10.97 to 6.34)
Model 1	−0.276	(−11.48 to −0.393)	0.068	(−7.59 to 13.25)
Model 2	−0.296	(−11.96 to −0.682)	0.055	(−8.37 to 12.93)

Model 1: adjusted for gestational age, parity, maternal age, smoking during pregnancy, alcohol consumption during pregnancy, caffeine during pregnancy, maternal education level, blood sampling period, breast feeding.
Model 2: model 1 + total dioxin levels (TEQ, WHO 2005).

and the neurodevelopment of infants at 6 months (Nakajima et al., 2006). We also found the same results in the current study. Therefore, we included dioxin levels in the fully adjusted model, although it did not change the results. In this study, PFOA and PFOS levels were modestly correlated (rs = 0.333), and mutual adjustment did not change the results in any consistent way. We have also adjusted this association for other potential confounders including the index of child care environment and birth weight, but the results did not change. Due to association of PFOS levels with type of delivery, we included type of delivery into fully adjusted models, and the results remained consistent.

At age 18 months, we did not find any association between PFCs and neurodevelopment. Fewer infants were examined at 18 months than at 6 months of age (n = 133 vs n = 173). Also, the significant correlation of birth weight and length with BSID II scores disappeared at 18 months, whereas we found a significant correlation of MDI and PDI scores at 18 months of age with higher annual income during pregnancy. Previous studies reported that socioeconomic status is associated with neurological functions including language, memory, cognition and social development (Hackman and Farah, 2009). We did not observe association between annual income and neurodevelopment at 6 months of age. However, we found a significant differences of MDI (high income vs low income: 88.1 vs 81.7, p = 0.012) and PDI (high income vs low income: 89.7 vs 84.4, p = 0.007) scores at 18 months of age according to annual income during pregnancy. Infants during first 6 months of life usually feed exclusively by breastfeeding, and they are not able to crawl, walk, talk, and interact with environment as much as 18-month

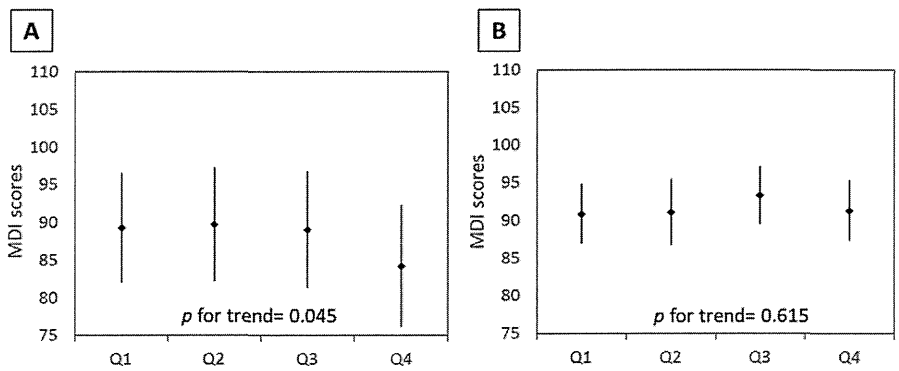

Fig. 1. The dose–response relationship between the quartiles of PFOA and reduced MDI scores among female (A) and male (B) infants at 6 months of age. For female infants (n = 90), values for the first (n = 26), second (n = 19), third (n = 27), and fourth (n = 18) quartiles, respectively, were as follows: <limit of detection (LOD) to 0.70, 0.70 to 1.15, 1.15 to 1.60, and 1.60 to 3.10 μg/mL. For male infants (n = 83), values for the first (n = 21), second (n = 17), third (n = 23), and fourth (n = 22) quartiles, respectively, were: <LOD to 0. 0.80, 0.80 to1.30, 1.30 to 1.80, 1.80 to 4.3. LSMs were adjusted for gestational age, parity, maternal age, smoking and alcohol consumption during pregnancy, caffeine intake during pregnancy, maternal education level, blood sampling period, breast feeding and total dioxin levels (TEQ, WHO 2005). LSMs are indicated in black circles and the error bars depict the upper and lower 95% CI. Q: quartile.

Table 7
The association between prenatal exposure to PFCs and 18-month old MDI and PDI (n = 133).

	MDI		PDI	
	Beta	(95% CI)	Beta	(95% CI)
PFOS				
Crude	0.090	(−5.27 to 16.94)	−0.026	(−11.65 to 8.54)
Adjusted[a]	0.052	(−9.91 to 16.66)	−0.023	(−13.45 to 10.72)
PFOA				
Crude	−0.098	(−11.20 to 3.03)	−0.072	(−9.19 to 3.72)
Adjusted[a]	−0.078	(−11.74 to 5.28)	0.002	(−7.66 to 7.85)

[a] Adjusted for gestational age, parity, maternal age, smoking and alcohol consumption during pregnancy, caffeine intake during pregnancy, maternal education, blood sampling period, breast feeding, and total dioxin levels (TEQ, WHO 2005).

infants. Families with higher annual income can provide better supplementary food and nutrition, educational resources, physical and psychosocial environment especially when infants start interaction with home environment and it helps infants for better neurodevelopment. It may partly explain why annual income affect neurodevelopment of infants at 18 months significantly compared to 6 months of age. Therefore, a negative association between exposure and mental scores at 6 months of age becomes difficult to find at 18 months of age because of the strong impact of annual income on neurodevelopment at 18 months of age. Another explanation for null impact of PFCs on neurodevelopment at 18 months of age could be postnatal environmental factors.

In this study, Bayley scores at 6 months were almost similar between girls and boys; however we found higher MDI and PDI scores at 18 months of age in girls. Girls have earlier cognitive changes than boys and this change occurs between 14 and 20 months of age (Reznick et al., 1997). It may be due to more sensitivity of girls to the environment and negative association of male testosterone with language skills (Christiansen and Knussmann, 1987). Previous epidemiological studies using BSID-II reported higher MDI and PDI scores among girls after first year of life but not at 6 months of age, however boys had steadier neurodevelopment trajectory (Lung et al., 2009; Augustyniak et al., 2013). These findings are consistent with our results and indicate role of sex on neurodevelopment.

The mechanistic effects of PFCs on neurodevelopment are not well understood. Recent research suggests that the endocrine-disrupting properties of PFCs, which can perturb metabolic endpoints including glucose homeostasis, thyroid hormone and sex hormone balance in animals may be the mechanism behind the adverse effects of PFCs (Seacat et al., 2003; Thibodeaux et al., 2003). Prenatal and postnatal exposure to PFCs interfere with thyroid hormone balance in humans resulting in higher thyroid stimulating hormone (TSH), decreased thyroxine (T4), and triiodothyronine (T3) (Ji et al., 2012; Wang et al., 2014; Berg et al., 2015), which may be responsible for the effects of these chemicals on the neurodevelopment of humans. Our results suggest that an infant's sex modifies the association between in utero PFOA exposure and neurodevelopment at 6 months of age. PFCs reduce serum testosterone and increase estradiol levels in rodents (Lau et al., 2007). Epidemiological studies suggest that PFCs are positively and negatively associated with estradiol and testosterone levels, respectively (Knox et al., 2011, Vested et al., 2013). A previous study in a highly PFOA-exposed population in the US showed that childhood PFOA levels has favorable association with neurodevelopment among boys but adverse association among girls (Stein et al., 2014). This result is in line with our results in terms of adverse effects of PFOA exposure on neurodevelopment among girls. However, more studies should be conducted to elucidate the sex differences of PFCs effect on neurodevelopment.

It has been shown that PFCs are associated with an increased risk of miscarriage (Darrow et al., 2014), preeclampsia (Stein et al., 2009), pregnancy-induced hypertension (Darrow et al., 2013), premature birth (Chen et al., 2012), and birth defects (Stein et al., 2009; Liew et al., 2014). In this study, we excluded participants with pregnancy-induced hypertension (n = 11), premature infants (n = 30), and infants with malformations (n = 1); infants born with these complicated pregnancies are more susceptible to neurodevelopmental problems. Therefore, we may underestimate the effects of prenatal exposure to PFCs on the neurodevelopment of infants due to the exclusion of these susceptible groups.

In this study, we measured prenatal PFC levels in a prospective birth cohort, which provides strong causality between exposure levels and outcomes in infants. In addition, we assessed the infants' neurodevelopment through expert staff experienced in the field of developmental disabilities and therefore avoiding measurement bias and recall bias from mother-reported neurodevelopmental milestones. The limitations of this study need to be considered. The small sample size precluded estimation of the subtle effects of PFCs on infant neurodevelopment. Among subjects with available PFC levels in original cohort (n = 428), a subpopulation of those had neurodevelopment assessment at 6 and 18 months of age in the current study, it may be a potential source of selection bias. The participants in the current study had higher maternal education, higher annual income and lesser smoking rate during pregnancy compare to the original cohort. However, the characteristics of subjects in original cohort were similar to participants of the current study in terms of PFC levels, maternal age, prepregnancy BMI, parity, and gestational age. We did not assess postnatal PFC exposure, and this may introduce some uncontrolled confounders, particularly for the assessment done at 18 months of age.

Previously, our group reported temporal trends of 11 types of PFCs between 2003 and 2011 in plasma samples of pregnant women in Hokkaido (Okada et al., 2013). The results indicated that PFOS and PFOA concentrations declined, whereas long-chain PFCs (including PFNA and PFDA) levels increased. In future studies, assessment of the effects of pre- and postnatal exposure to PFCs with longer carbon chains on the neurodevelopment of infants and children with bigger sample sizes, different battery tests and longer observation periods is necessary.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.10.017.

Conflict of interest

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

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Associations of Perfluoroalkyl Substances (PFASs) with Lower Birth Weight: An Evaluation of Potential Confounding by Glomerular Filtration Rate Using a Physiologically Based Pharmacokinetic Model (PBPK)

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Abstract

Background: Prenatal exposure to perfluoroalkyl substances (PFAS) has been associated with lower birth weight in epidemiologic studies. This association could be attributable to glomerular filtration rate (GFR) which is related to PFAS concentration and birth weight.

Objectives: To use a physiologically based pharmacokinetic (PBPK) model of pregnancy to assess how much of the PFAS-birth weight association observed in epidemiologic studies might be attributable to GFR.

Methods: We modified a PBPK model to reflect the association of GFR with birth weight (estimated from three studies of GFR and birth weight) and used it to simulate PFAS concentrations in maternal and cord plasma. The model was run 250,000 times, with variation in parameters, to simulate a population. Simulated data were analyzed to evaluate the association between PFAS levels and birth weight due to GFR. We compared simulated estimates to those from a meta-analysis of epidemiologic data.

Results: The reduction in birth weight for each 1 ng/ml increase in simulated cord plasma for perfluorooctane sulfonate (PFOS) was 2.72 g (95% CI: -3.40, -2.04), and for perfluorooctanoic acid (PFOA) was 7.13 g (95% CI: -8.46, -5.80); results based on maternal plasma at term were similar. Results were sensitive to variations in PFAS level distributions and the strength of the GFR-birth weight association. In comparison, our meta-analysis of epidemiologic studies suggested that each 1 ng/ml increase in prenatal PFOS and PFOA levels was associated with 5.00 g (95% CI: -21.66, -7.78) and 14.72 g (95% CI: -8.92, -1.09) reductions in birth weight.

Conclusion: Results of our simulations suggest that a substantial proportion of the association between prenatal PFAS and birth weight may be attributable to confounding by GFR and that confounding by GFR may be more important in studies with sample collection later in pregnancy.

Introduction

Perfluoroalkyl substances (PFAS) are synthetic compounds that are resistant to degradation and have been found worldwide in environmental media and biota, including humans. The most widely studied PFAS are perfluorooctane sulfonate (PFOS) and perfluorooctanoaic acid (PFOA). PFOS was an ingredient in the Scotchgard stain repellent manufactured by 3M, but the company decided to stop producing PFOS and related compounds in 2002 after it had been found in wildlife and humans (3M 2000). PFOA is a surfactant that is used in the production of many consumer goods, including nonstick coating in cookware. The eight major companies producing or using PFOA have agreed to work toward eliminating emissions and product content of PFOA by 2015 (PFOA Stewardship Program 2006). Despite the reductions in the production and emission of PFOS and PFOA, these persistent compounds can still be detected in biological samples from the general population. For example, PFOS and PFOA have been detected in the blood of more than 98% of participants in the 2009-2010 National Health and Nutrition Examination Survey (NHANES) (CDC 2013) and 2009-2011 Canadian Health Measure Survey (CHMS) (Health Canada 2013). PFOS and PFOA have also been detected in maternal blood during pregnancy, cord blood at delivery and breast milk (SK Kim et al. 2011; Olsen et al. 2009), indicating that humans are exposed during critical prenatal and early postnatal windows of development.

Many epidemiologic studies have reported an association between maternal and cord blood PFAS levels and reductions in birth weight (Apelberg et al. 2007; Chen et al. 2012; Fei et al. 2007; Maisonet et al. 2012; Washino et al. 2009; Whitworth et al. 2012). Although these studies accounted for potential confounding by many variables, none adjusted for glomerular filtration rate (GFR). GFR, the flow rate of fluid being filtrated by the kidneys, increases by about 50%