



Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants[☆]

Emiko Okada^a, Seiko Sasaki^a, Yasuaki Saijo^b, Noriaki Washino^a, Chihiro Miyashita^a, Sumitaka Kobayashi^a, Kanae Konishi^a, Yoichi M. Ito^c, Rie Ito^d, Ayako Nakata^d, Yusuke Iwasaki^d, Koichi Saito^d, Hiroyuki Nakazawa^d, Reiko Kishi^{e,*}

^a Department of Public Health, Hokkaido University Graduate School of Medicine, Sapporo, Japan

^b Department of Health Science, Asahikawa Medical University, Asahikawa, Japan

^c Department of Biostatistics, Division of Advanced Medical Sciences, Hokkaido University Graduate School of Medicine, Sapporo, Japan

^d Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo, Japan

^e Center for Environmental and Health Sciences, Hokkaido University, North 12 West 7 Kita-ku, Sapporo 060-0812, Japan

ARTICLE INFO

Article history:

Received 26 October 2010

Received in revised form

27 September 2011

Accepted 7 October 2011

Keywords:

Perfluorooctane sulfonate

Perfluorooctanoate

Cord blood IgE

Allergy

Infectious disease

ABSTRACT

Background: Recent studies have shown effects of prenatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) on infants in the general environmental levels. Laboratory animal studies have shown that exposure to PFOS and PFOA is associated with immunotoxic effects. **Objectives:** To investigate the relationship between maternal PFOS and PFOA levels and infant allergies and infectious diseases during the first 18 months of life. Cord blood immunoglobulin (Ig) E levels were also evaluated.

Methods: We conducted a prospective cohort study of pregnant women from 2002 to 2005 in Sapporo, Japan. Maternal PFOS and PFOA levels were measured in relation to cord blood IgE concentrations ($n=231$) and infant allergies and infectious diseases ($n=343$). Characteristics of mothers and their infants were obtained from self-administered questionnaires and medical records. Development of infant allergies and infectious diseases was determined from self-administered questionnaires at 18 months of age. Concentrations of PFOS and PFOA in maternal serum and concentrations of IgE in umbilical cord serum at birth were measured.

Results: Cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. However, there were no significant associations among maternal PFOS and PFOA levels and food allergy, eczema, wheezing, or otitis media in the 18 month-old infants (adjusted for confounders).

Conclusions: Although cord blood IgE level decreased significantly with high maternal PFOA levels among female infants, no relationship was found between maternal PFOS and PFOA levels and infant allergies and infectious diseases at age in 18 months.

© 2011 Elsevier Inc. All rights reserved.

Abbreviations: ATS—DLD, American Thoracic Society—Division of Lung Diseases; BMI, body mass index; CI, confidence interval; IgA, immunoglobulin A; ISAAC, International Study of Asthma and Allergies in Childhood; ND, non-detectable; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; PCBs, polychlorinated biphenyls; PFC, perfluorinated chemical; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate

☆ Role of the funding source: This study was supported by Grants-in-Aid for Health Scientific Research from the Japanese Ministry of Health, Labour and Welfare; and by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Ethics approval: This study was conducted with written informed consent from all patients and was approved by the institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

* Corresponding author. Fax: +81 11 706 4725.

E-mail address: rkishi@med.hokudai.ac.jp (R. Kishi).

1. Introduction

Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are end metabolites of perfluorinated chemicals (PFCs). PFOS and PFOA are persistent organic pollutants and are widely used in consumer products (e.g., surface-active agents, flame retardants, adhesives, and pesticides). PFOS and PFOA have recently been found to be widespread contaminants in the environment, wildlife, and humans (Lau et al., 2007). The half-life of PFOS in humans is 3.8 years, and that of PFOA is 5.4 years (Olsen et al., 2007). Possible health effects of PFOS and PFOA exposure in humans are a concern because of bioaccumulation and persistence in the environment, in animals, and in humans. It has been reported that PFC concentrations in human blood

increase with age (Harada et al., 2004, 2007). The main sources of human exposure are drinking water (Hölzer et al., 2008), foods such as red meat and animal fat (Halldorsson et al., 2008), contamination from food packaging (Begley et al., 2005; Tittlemier et al., 2007), and indoor dust (Björklund et al., 2009; Shoeib et al., 2005).

PFOS and PFOA pass the placental barrier and are transferred to the fetus in humans (Midasch et al., 2007; Monroy et al., 2008). Some studies have reported a negative association between prenatal PFOS and PFOA exposure and birth weight (Apelberg et al., 2007; Fei et al., 2007). We previously observed a strong positive correlation between PFOS concentrations in maternal blood and cord blood (Inoue et al., 2004a), and a negative correlation between relatively low levels of maternal blood PFOS concentration and birth weight among female infants (Washino et al., 2009).

The prevalence of allergies has recently increased in children (Zöllner et al., 2005). The main environmental risk factors associated with asthma and other wheezy disorders are genetics, viruses, bacteria, tobacco exposure, and allergic sensitization, as determined by long-term studies (Bisgaard and Bønnelykke, 2010). Certain oxidants, airborne particulate matter, diesel exhaust particles, and polycyclic aromatic hydrocarbons have also been suggested as risk factors for asthma (Ho, 2010). It has been suggested that susceptibility to the effects of environmental chemicals may be higher during prenatal development when many physiological systems are developing, including the immune system (Luster et al., 2003). In an epidemiological study, the Danish National Birth Cohort showed that prenatal exposure to PFOA or PFOS was not associated with risk of hospitalization for infectious diseases in early childhood (Fei et al., 2010). In the C8 Health Project that investigated residents in the vicinity of a PFOA plant, it was shown that immunoglobulin (Ig) A and C reactive protein levels had a significant decreasing trend with increasing PFOA levels in blood samples; this pattern was also indicated for IgE, but only in females (Fletcher et al., 2009). Effects of PFOS and PFOA exposure on immunity and allergy in laboratory animals include immunosuppression, suppression of IgM antibody production (Dewitt et al., 2008; Keil et al., 2008; Peden-Adams et al., 2007), and increased total IgE response in ovalbumin-sensitized mice (Fairley et al., 2007). However, reports that have examined effects of PFC on immunity and allergy in a prospective study are few. It is therefore necessary to epidemiologically evaluate the effects of PFC exposure on immunity and allergy in humans, and to determine relationships between PFC exposure and immunity and allergy in humans.

The aim of this study was to ascertain possible relationships between maternal PFOS and PFOA levels and allergies and infectious diseases in their infants during the first 18 months of life using a prospective cohort study. IgE concentrations in cord blood were also evaluated.

2. Materials and methods

2.1. Study population

This prospective birth cohort study was based on infants delivered at the Sapporo Toho Hospital in Sapporo, Hokkaido, Japan, which is an obstetrics and gynecology hospital (Hokkaido Study on Environment and Children's Health). Details regarding the study population, data collection, and the content of the questionnaires have been previously described (Kishi et al., 2011). In brief, between July 2002 and October 2005, participants were native Japanese women who enrolled at 23–35 weeks of gestation and were residents of Sapporo City or surrounding areas. Of 1796 women asked to participate, 514 agreed (participation rate of 28.6%). Among the 514 women, 10 were excluded due to miscarriage, stillbirth, relocation, or voluntary withdrawal from the study before follow-up. Thirteen women were excluded due to death of the infant, relocation, or voluntary withdrawal for the follow-up period from delivery to 18 months.

2.2. Data collection

Participants completed a self-administered questionnaire during the second trimester of pregnancy. The questionnaire included information related to previous medical history, educational level, household income, smoking status, alcohol intake, caffeine intake, and food intake frequency during pregnancy. Medical information including maternal age, maternal height, maternal pre-pregnancy weight, pregnancy complications, parity, gestational age, infant gender, birth weight, and birth length were obtained from medical records. At 18 months post-delivery, participants completed another self-administered questionnaire. Of the 491 women to whom the questionnaire was mailed, 390 responded (recovery rate of 79.4%). The questionnaire included information related to breast-feeding, infant weight and length at 18 months, smoking status of parents, environmental tobacco smoke exposure at 18 months, pets in the home, living environment, day care attendance at 18 months, infant vaccination, and previous or current medical history of infant allergies and infectious diseases (food allergy, eczema, asthma, febrile convulsion, respiratory syncytial virus (RSV) disease, otitis media, and other diseases). For this study, all participating women provided written informed consent, and the study protocol was approved by the institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

2.3. Assessment of infant allergies and infections

Infant allergies and infectious diseases that developed during the first 18 months of life were assessed based on mothers' self-administered questionnaire at 18 months post-delivery. Food allergy was defined as a positive response to the following question: "Has your child ever had symptoms such as hives, swelling of the lip, emesis, diarrhea, or respiratory distress when they ate food allergens including milk, egg rice gruel, egg-drop, shrimp, or other foods?" Eczema was defined using a modified part of the Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC) phase-I questionnaire (ISAAC Steering Committee, 1998). The part contained six questions: "(1) Has your child ever had an eczema in the past? If yes; (2) Has your child ever had an itchy rash, which was coming and going for at least 6 months? If yes; (3) Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes? (4) Has your child ever had dry skin in the past? (5) Has your child ever had a doctor's diagnosis or diagnostic possibility for an eczema in the past? (6) Does an itchy rash at any time affect any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes at present?" Wheezing was defined using a modified part of the Japanese version of the American Thoracic Society—Division of Lung Diseases (ATS—DLD) questionnaire (Nishima et al., 2009). The part contained five questions: "(1) Has your child ever had an attack of wheezing and/or shortness of breath in the past? (2) Has your child ever had twice or more attacks in the past? (3) Has your child ever had a doctor's diagnosis possibility for a bronchial asthma, asthmatic, or pediatric asthma in the past? (4) Could wheezing be heard during an attack? (5) Has your child ever had shortness of breath and wheezing during an attack?" To estimate the proportion of allergies or infectious diseases, we defined an outcome based on the following criteria: if infants had a positive response to the following medical question: "Has your child ever had a doctor's diagnosis, hospitalization, or medical treatment for the following diseases: asthma, eczema, other allergic diseases, otitis media, febrile convulsion, RSV diseases or other diseases, including chicken pox, bronchitis, rhinitis, pneumonia, skin infection and other viral infections?"

2.4. Measurement of PFOS and PFOA concentrations in maternal serum

Detailed sampling and laboratory methods for measuring PFOS and PFOA have been previously described (Washino et al., 2009). In brief, a 40 mL blood sample was taken from the maternal peripheral vein after the second trimester of pregnancy. When this was not possible because of anemia of the mother, a blood sample was taken after delivery. All samples were stored at -80°C until analysis. The analytical detection method used was a variation of a method published previously (Nakata et al., 2005a, 2005b), and the methods for developing this variation of the analytical approach have also been described elsewhere (Inoue et al., 2004a, 2004b). Human serum samples (0.1 mL) were mixed with 0.2 mL internal standard solution containing acetonitrile, centrifuged at 1450g 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. As a result of loss to follow-up, lack of serum specimen, and laboratory capacity, the concentrations of PFOS and PFOA were measured in 447 maternal serum samples. For participants with a concentration below the detection limit, a value equal to half of the detection limit was used for calculation purposes.

To examine possible effects of other environmental chemicals as confounding factors, we measured the concentrations of 7 polychlorinated dibenzo-*p*-dioxins

(PCDDs), 10 polychlorinated dibenzofurans (PCDFs), 4 non-*ortho*-polychlorinated biphenyl (PCB) congeners, 8 mono-*ortho*-PCB congeners, and 58 non-dioxin-like PCB congeners in maternal blood ($n=426$) using high-resolution gas chromatography/high resolution mass spectrometry at the Fukuoka Institute of Health and Environmental Sciences (Todaka et al., 2007, 2008).

2.5. Measurement of IgE in cord serum

At the time of delivery, a blood sample (10–30 mL) was collected from the umbilical cord. All samples were stored at -80°C until analysis. Concentrations of total IgE in cord serum were measured using an enzyme-linked immunosorbent assay (IMx[®] analyzer, ABBOTT JAPAN CO., LTD., Tokyo, Japan). IgE concentrations were measured in 268 cord serum samples at SRL, Inc. (Tokyo, Japan).

2.6. Statistical analysis

First, we analyzed a possible association between maternal PFOS and PFOA levels and cord blood IgE levels. For analysis of correlations between cord blood IgE levels and characteristics of mothers and infants, we used the Spearman correlation test, the Mann–Whitney *U*-test, and the Kruskal–Wallis test. Because data did not fall into a normal distribution, PFOS, PFOA, and IgE concentrations were converted to a $\log_{10}$ scale. We calculated the residual of potential confounding variables to $\log_{10}$ -transformed cord blood IgE levels. Polynomial regression analysis was performed as the residual of potential confounding variables for dependent variables and $\log_{10}$ -transformed maternal PFOS or PFOA levels for the independent variable, because the cubic polynomial regression model was better fitted than the linear regression model. Confounding variables were selected based on covariates that influenced cord blood IgE levels in univariate analyses and possible risk factors reported in previous studies, and also based on the change in estimate criterion. Variables considered in the analysis are: maternal age, maternal allergic history, infant gender, birth season, distance from home to highway, and sampling period. Parity was introduced into the final models because maternal PFOS and PFOA concentrations varied significantly primiparous and multiparous. Deep sea fish intake during pregnancy was also considered because IgE levels varied significantly, but since the results did not change, the variable was not introduced into the final models. For the fully adjusted model, polynomial regression analysis was applied adjusted for maternal age, maternal allergic history (yes/no), parity (primiparous/multiparous), infant gender, birth season, distance from home to highway ($<100\text{ m}$ or $\geq 100\text{ m}$), and blood sampling period (during pregnancy/after delivery). We further performed stratified analysis by infant gender.

Second, we analyzed possible associations between maternal PFOS and PFOA concentrations and the development of infant allergies and infectious diseases during the first 18 months of life. For analysis of correlations between maternal PFOS and PFOA concentrations and characteristics of parents and infants, the Spearman correlation test, the Mann–Whitney *U*-test, and the Kruskal–Wallis test were used. For analysis of correlations between allergies and infectious diseases during the first 18 months of life and characteristics of parents and infants, we used the Student's *t*-test and the Chi-square test. To assess risk factors or protective factors for infant illnesses, the characteristics of parents and infants were introduced as explanatory variables in binominal logistic regression analyses. Crude and adjusted logistic regression analyses were performed to evaluate associations between PFOS and PFOA concentrations and the risk of allergies and infections among all infants, male infants, and female infants. In logistic models, we evaluated odds ratios (ORs) for the risk of allergies and infection with $\log_{10}$ -transformed maternal PFOS and PFOA levels. Multivariate analyses were adjusted for confounding variables that influenced allergies or infections in univariate analyses, possible risk factors reported in previous studies, and the sampling period. The fully adjusted model used logistic regression analysis of allergic disease adjusted for maternal age, maternal educational level (≤ 9 years, 10–12 years, 13–16 years, and ≥ 17 years), pre-pregnancy body mass index (BMI), maternal allergic history (yes/no), paternal allergic history (yes/no), parity (primiparous/multiparous), infant gender, breast-feeding period (<4 months or ≥ 4 months), environmental tobacco smoke exposure at 18 months (yes/no),

day care attendance at 18 months (yes/no), and blood sampling period (during pregnancy/after delivery) and logistic regression analysis of infectious disease adjusted for maternal age, maternal educational level, parity, infant gender, breast-feeding period, environmental tobacco smoke exposure at 18 months, day care attendance at 18 months, and blood sampling period. Results were considered statistically significant when $p < 0.05$.

3. Results

Concentrations of PFOS and PFOA (ng/mL) in maternal serum ($n=343$) and total IgE (IU/mL) in cord serum ($n=231$) were measured (Table 1). Detection limits for both PFOS and PFOA concentrations were 0.5 ng/mL. PFOS was detected in all samples, and PFOA was below the detection limit in 22 samples (6.4%); PFOS concentrations ranged from 1.3 to 16.2 ng/mL and the median value was 5.2 ng/mL. PFOA concentrations ranged from below the detection limit to 5.3 ng/mL and the median value was 1.3 ng/mL. The detection limit of IgE concentration was 0.05 IU/mL, and 39 samples (16.9%) were below the detection limit. Concentrations ranged from below the detection limit to 10.9 IU/mL and the median value was 0.21 IU/mL.

Possible associations between maternal serum PFOS and PFOA concentrations and various characteristics of parents and infants ($n=343$) were measured (Table 2). Univariate analyses indicated that increasing age of the mother was significantly associated with lower PFOS and PFOA concentrations. Concentrations in serum from multiparous women were significantly lower than from primipara women and concentrations in samples taken after delivery were significantly lower than in those taken during pregnancy.

Cord blood IgE concentrations were also measured in relation to several characteristics of the parents and infants ($n=231$). Statistically significant differences in IgE levels by maternal allergic history, infant gender, and deep sea fish intake during pregnancy ($p < 0.05$) were observed (data not shown).

Infant allergies and infectious diseases during the first 18 months of life ($n=343$) were also determined (Table 3). The numbers of infants who developed allergies or infections up to age 18 months were as follows: food allergy, 57 (16.6%); eczema, 37 (10.8%); wheezing, 33 (9.6%); otitis media, 61 (17.8%); chicken pox, 16 (4.7%); bronchitis, 9 (2.6%); RSV diseases, 7 (2.0%); rhinitis, 6 (1.7%); pneumonia, 6 (1.7%); skin infection, 5 (1.5%); other virus infections (rotavirus, adenovirus, and cytomegalovirus), 15 (4.4%). Thus, we did not include chicken pox, bronchitis, RSV diseases, rhinitis, pneumonia, skin infection, and other viral infections in subsequent analyses because the numbers of cases of infection were very low except for otitis media, and sufficient statistical power could not be ensured in the multivariate analysis. Possible associations between characteristics of participants and infant allergies and infectious diseases were analyzed. There was no significant difference between allergies and infectious diseases with regard to gender.

We observed statistically significant differences ($p < 0.05$) for eczema by paternal allergic history, for wheezing by paternal

Table 1

Concentrations of PFOS and PFOA in maternal serum ($n=343$) and concentrations of IgE in cord serum ($n=231$).

	Detection limit	ND ^a , no. (%)	Mean	Minimum	25th	Median	75th	Maximum	Geometric mean
Maternal serum PFOS (ng/mL) ^b	0.5	0 (0)	5.6	1.3	3.4	5.2	7.2	16.2	5.0
Maternal serum PFOA (ng/mL) ^c	0.5	22 (6.4)	1.4	ND	0.8	1.3	1.7	5.3	1.2
Cord serum IgE (IU/mL) ^d	0.05	39 (16.9)	0.62	ND	0.08	0.21	0.58	10.9	0.22

^a ND: not detected.

^b PFOS: perfluorooctane sulfonate.

^c PFOA: perfluorooctanoate.

^d IgE: immunoglobulin E.

Table 2
Maternal PFOS and PFOA concentrations in relation to characteristics of parents and infants ($n=343$).

	No.	(%)	PFOS (ng/ml) ^a		PFOA (ng/ml) ^b	
			Median	(25th–75th)	Median	(25th–75th)
Parental characteristics						
Maternal age (years)	31.3 ± 4.4 ^c		<i>r</i> = −0.149 ^e		<i>r</i> = −0.114 ^e	
Maternal pre-pregnancy BMI (kg/m ²) ^d	21.2 ± 3.5 ^c		<i>r</i> = −0.077 ^e		<i>r</i> = −0.053 ^e	
Annual household income (million yen) ^f						
< 5	224	(65.3)	5.2	(3.4–7.1)	1.3	(0.8–1.7)
≥ 5	118	(34.4)	5.5	(3.3–7.2)	1.3	(0.9–1.8)
Maternal educational level (years)						
≤ 9	4	(1.1)	6.7	(3.4–8.2)	0.75	(0.3–1.1)
10–12	139	(40.5)	4.8	(3.3–6.4)	1.3	(0.8–1.8)
13–16	195	(56.9)	5.6	(3.5–7.6)	1.3	(0.8–1.7)
≥ 17	5	(1.5)	3.0	(2.8–6.4)	0.8	(0.4–1.5)
Maternal smoking status during pregnancy						
Nonsmoker	292	(85.1)	5.3	(3.5–7.4)	1.3	(0.8–1.8)
Smoker	51	(14.9)	4.6	(2.8–6.6)	1.2	(0.9–1.6)
Parity ^f						
Primiparous	163	(47.5)	5.7	(3.9–8.0)	1.5	(1.2–2.2)
Multiparous	179	(52.2)	4.8	(3.0–6.7)	0.9	(0.6–1.4)
Blood sampling period						
During pregnancy	246	(71.7)	5.6	(4.1–7.6)	1.4	(0.9–1.8)
After delivery	97	(28.3)	3.6	(2.5–6.1)	1.1	(0.7–1.6)
Maternal allergic history						
No	251	(73.2)	5.3	(3.3–7.4)	1.3	(0.8–1.7)
Yes	92	(26.8)	4.6	(3.4–6.6)	1.3	(0.8–1.8)
Paternal allergic history						
No	280	(81.6)	–	–	–	–
Yes	63	(18.4)	–	–	–	–
Infant characteristics						
Gender						
Male	169	(49.3)	–	–	–	–
Female	174	(50.7)	–	–	–	–
Birth season						
Spring (March–May)	103	(30.0)	–	–	–	–
Summer (June–August)	70	(20.4)	–	–	–	–
Autumn (September–November)	70	(20.4)	–	–	–	–
Winter (December–February)	100	(29.2)	–	–	–	–
Breast-feeding period (months)						
< 4	70	(16.5)	–	–	–	–
≥ 4	273	(83.5)	–	–	–	–
Environmental tobacco smoke exposure at 18 months						
No	210	(61.0)	–	–	–	–
Yes	133	(39.0)	–	–	–	–
Day care attendance at 18 months ^f						
No	269	(78.4)	–	–	–	–
Yes	72	(21.0)	–	–	–	–
Distance from home to highway ^f						
< 100 m	183	(53.3)	–	–	–	–
≥ 100 m	159	(46.4)	–	–	–	–

^a PFOS: perfluorooctane sulfonate.

^b PFOA: perfluorooctanoate.

^c Mean ± SD.

^d BMI: body mass index.

^e r : Spearman's correlation coefficient.

^f Missing data; annual household income (1), parity (1), day care attendance at 18 months (2), distance from home to highway (1).

allergic history, pre-pregnancy BMI, and day care attendance, and for otitis media by parity and by day care attendance (data not shown).

Next, possible associations between maternal serum PFOS and PFOA concentrations and immune system parameters were examined. Two samples with IgA levels > 10 mg/dL were considered to be contaminated by maternal blood and were excluded. Ultimately, 231 mother-infant pairs were included in the analysis, for whom PFOS, PFOA, and IgE had been measured. Table 4 shows the results of cubic polynomial regression analysis between

log₁₀-transformed maternal serum PFOS or PFOA level and residual of potential confounding variables to log₁₀-transformed cord blood IgE levels ($n=231$). In analyses stratified by infant gender, cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. However, no significant associations were observed between maternal PFOS or PFOA level and cord blood IgE levels among male infants.

Fig. 1 shows scatterplots and the linear regression model, and the cubic polynomial regression model for the log₁₀-transformed

Table 3
Number and proportion of infants who developed allergies or infections during the first 18 months of life ($n=343$).

	No.	(%)
Allergic symptoms		
Food allergy	57	(16.6)
Eczema	37	(10.8)
Wheezing	33	(9.6)
Infection		
Otitis media	61	(17.8)
Chicken pox	16	(4.7)
Bronchitis	9	(2.6)
RSV disease ^a	7	(2.0)
Rhinitis	6	(1.7)
Pneumonia	6	(1.7)
Skin infection	5	(1.5)
Other viral infections ^b	15	(4.4)

^a RSV disease; respiratory syncytial virus disease.

^b Rotavirus, adenovirus, or cytomegalovirus.

Table 4
Cubic polynomial regression between maternal PFOS or PFOA level (ng/mL) and residual of potential confounding variables to cord blood IgE levels (IU/mL).

	Estimate	95% CI
Overall ($n=231$) ^c		
$\log_{10}$ PFOS ^a (linear)	−0.240	(−0.891, 0.412)
$\log_{10}$ PFOS ^a (quadratic)	0.145	(−1.150, 1.440)
$\log_{10}$ PFOS ^a (cubic)	0.851	(−3.729, 5.432)
$\log_{10}$ PFOA ^b (linear)	0.282	(−0.229, 0.792)
$\log_{10}$ PFOA ^b (quadratic)	−1.009	(−1.918, −0.101)
$\log_{10}$ PFOA ^b (cubic)	−1.430	(−3.384, 0.524)
Male infants ($n=103$) ^d		
$\log_{10}$ PFOS ^a (linear)	−0.047	(−1.051, 0.957)
$\log_{10}$ PFOS ^a (quadratic)	0.911	(−1.101, 2.922)
$\log_{10}$ PFOS ^a (cubic)	−0.101	(−6.625, 6.422)
$\log_{10}$ PFOA ^b (linear)	−0.315	(−1.114, 0.485)
$\log_{10}$ PFOA ^b (quadratic)	0.227	(−1.584, 2.037)
$\log_{10}$ PFOA ^b (cubic)	1.277	(−2.191, 4.744)
Female infants ($n=128$) ^d		
$\log_{10}$ PFOS ^a (linear)	−0.342	(−1.230, 0.546)
$\log_{10}$ PFOS ^a (quadratic)	−0.681	(−2.500, 1.137)
$\log_{10}$ PFOS ^a (cubic)	1.464	(−5.354, 8.282)
$\log_{10}$ PFOA ^b (linear)	0.766	(0.104, 1.428)
$\log_{10}$ PFOA ^b (quadratic)	−1.429	(−2.416, −0.422)
$\log_{10}$ PFOA ^b (cubic)	−3.078	(−5.431, −0.726)

^a PFOS: perfluorooctane sulfonate.

^b PFOA: perfluorooctanoate.

^c Adjusted models included maternal age, maternal allergic history, distance from home to highway, infant gender, parity, birth season, and blood sampling period.

^d Adjusted models included maternal age, maternal allergic history, distance from home to highway, parity, birth season, and blood sampling period.

maternal PFOS or PFOA level and residual of potential confounding variables to $\log_{10}$ -transformed cord blood IgE levels ($n=231$). We found a curvilinear relationship between maternal PFOA levels and cord blood IgE levels. In female infants, when $\log_{10}$ -transformed maternal PFOA levels changed from -0.6 ng/mL to -0.4 ng/mL, $\log_{10}$ -transformed cord blood IgE levels decreased by -0.150 IU/mL, and when $\log_{10}$ -transformed maternal PFOA levels changed from -0.4 ng/mL to 0.3 ng/mL, $\log_{10}$ -transformed cord blood IgE levels increased by 0.433 IU/mL, and when $\log_{10}$ -transformed maternal PFOA levels changed from 0.3 ng/mL to 0.7 ng/mL, $\log_{10}$ -transformed cord blood IgE levels greatly decreased by -0.863 IU/mL (Fig. 1F).

Table 5 shows the results of logistic regression analyses between $\log_{10}$ -transformed maternal serum PFOS and PFOA levels

and infant allergies and infectious diseases during the first 18 months of life. We excluded 4 women who did not answer the questions related to allergies and infections at 18 months post-delivery. Therefore, we included 343 mother-infant pairs in the analysis for whom both PFOS and PFOA had been measured and from which questionnaire data at 18 months post-delivery had been obtained. No significant associations were observed between maternal PFOS or PFOA levels and food allergy, eczema, wheezing, and otitis media in the infants in both the crude and adjusted models [adjusted ORs for a 10-fold increase in maternal PFOS or PFOA concentrations; food allergy PFOS=3.72 (95% CI, 0.81–17.10) and PFOA=1.67 (95% CI, 0.52–5.37); eczema PFOS=0.87 (95% CI, 0.15–5.08) and PFOA=0.96 (95% CI, 0.23–4.02); wheezing PFOS=2.68 (95% CI, 0.39–18.30) and PFOA=1.27 (95% CI, 0.27–6.05); otitis media PFOS=1.40 (95% CI, 0.33–6.00) and PFOA=1.51 (95% CI, 0.45–5.12)]. We performed stratified analysis by blood sampling period, but no significant associations were revealed (data not shown).

Possible associations between dioxin and PCB concentrations in maternal serum with PFOS and PFOA concentrations were evaluated. Dioxin concentrations, defined as the sum of 7 PCDDs, 10 PCDFs, and 12 dioxin-like PCBs (4 non-ortho PCBs and 8 mono-ortho PCBs) were positively correlated with PFOS and PFOA levels (Spearman rank correlation coefficients: 0.126, $p=0.023$; and 0.133, $p=0.017$, respectively). Furthermore, total PCB concentrations, defined as the sum of 70 PCB congeners (58 non-dioxin-like PCB congeners and 12 dioxin-like PCBs) were positively correlated with PFOA levels (Spearman rank correlation coefficient, 0.110; $p=0.049$) but not with PFOS levels (Spearman rank correlation coefficient, 0.062; $p=0.267$). When total dioxins and total PCBs were evaluated as potential confounders, no significant association remained between PFC concentrations and infant allergies and infectious diseases during the first 18 months of life (data not shown).

4. Discussion

In this study, cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. However, no association was observed between maternal serum PFOS and PFOA concentrations and occurrence of food allergy, eczema, wheezing, and otitis media in their infants during the first 18 months of life. To our knowledge, this is the first report that has examined possible effects of PFC prenatal exposure on infant allergy including cord blood IgE level as an immune system biomarker.

Inoue et al. (2004a) examined the correlation of PFC concentrations in maternal blood and cord blood in same sample population as this study. These PFOS in maternal and cord blood had a significant correlation. Midasch et al. (2007) and Monroy et al. (2008) reported a significant correlation of PFOS and PFOA levels in maternal and cord blood, and both studies suggested that PFOS and PFOA cross the placental barrier during pregnancy, resulting in fetal exposure to PFOS and PFOA. In this study we explored possible associations between maternal serum PFOS and PFOA concentrations with infant allergies and infectious diseases, and found no significant associations. Our results are thus consistent with results of the Danish National Birth Cohort (Fei et al., 2010), in which the mean concentrations of PFOS and PFOA were 35.3 ng/mL and 5.6 ng/mL, respectively; these levels were higher than those measured in our study. Cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. The results of the C8 Health Project showed a significant decreasing trend in IgE levels with increasing PFOA levels in blood samples among females (Fletcher et al., 2009). Our results are consistent with those of that study, even though the

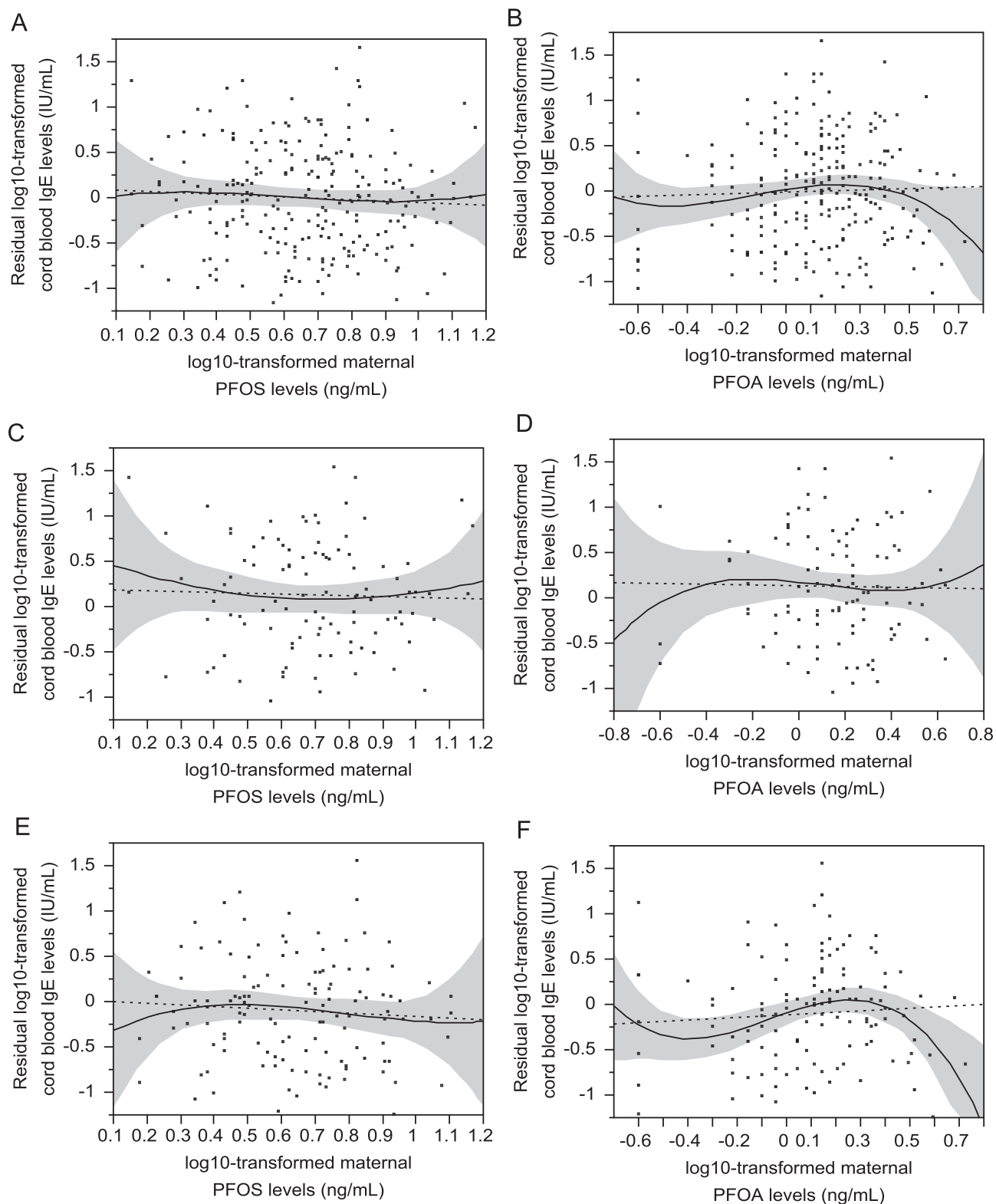


Fig. 1. Association between maternal log₁₀-transformed PFOS and PFOA levels (ng/mL) and residual of potential confounding variables to log₁₀-transformed cord blood IgE levels (IU/mL). The dashed lines denote the predicted fit from a linear regression model. The solid lines denote the predicted fit from the cubic polynomial regression model and 95% confidence interval (CI). Corresponding estimates are presented in Table 4. (A, B) Among overall. (C, D) Among male infants. (E, F) Among female infants.

concentration of maternal PFOA was lower than that measured in other studies, including the C8 Health Project (Fletcher et al., 2009; Harada et al., 2007; Jensen and Leffers, 2008; Kannan et al., 2004); we note, however, that the PFOA level in any case was not

associated with the development of allergies and infectious diseases up to age 18 months. It may be necessary to perform follow-up studies to investigate whether prenatal exposure to PFCs affects the immune system of offspring (and address

Table 5

Adjusted odds ratio (95% CI) between PFOS or PFOA concentrations in maternal serum and allergies and infectious diseases during the first 18 months of life.

	Overall (n=343)				Male infants (n=169)				Female infants (n=174)			
	Crude		Adjusted		Crude		Adjusted		Crude		Adjusted	
	OR ^a	(95% CI)	OR ^a	(95% CI)	OR ^a	(95% CI)	OR ^a	(95% CI)	OR ^a	(95% CI)	OR ^a	(95% CI)
log ₁₀ PFOS ^b												
Food allergy ^c	2.76	(0.72, 10.54)	3.72	(0.81, 17.10)	3.58	(0.54, 23.64)	5.42	(0.62, 47.20)	2.05	(0.30, 13.82)	2.75	(0.31, 24.80)
Eczema ^c	1.03	(0.22, 4.91)	0.87	(0.15, 5.08)	0.78	(0.09, 6.63)	0.62	(0.06, 6.67)	1.36	(0.14, 13.55)	1.24	(0.08, 19.30)
Wheezing ^c	1.81	(0.34, 9.60)	2.68	(0.39, 18.30)	6.32	(0.51, 79.08)	12.98	(0.80, 212.00)	0.62	(0.06, 5.93)	0.61	(0.03, 11.50)
Otitis media ^d	0.75	(0.22, 2.65)	1.40	(0.33, 6.00)	0.65	(0.12, 3.53)	1.38	(0.18, 10.60)	0.81	(0.12, 5.55)	1.43	(0.17, 12.30)
log ₁₀ PFOA ^e												
Food allergy ^c	1.25	(0.45, 3.52)	1.67	(0.52, 5.37)	0.85	(0.21, 3.49)	0.87	(0.16, 4.89)	1.87	(0.41, 8.40)	2.37	(0.50, 17.10)
Eczema ^c	0.97	(0.29, 3.30)	0.96	(0.23, 4.02)	1.51	(0.26, 8.67)	1.12	(0.15, 8.42)	0.59	(0.10, 3.40)	0.88	(0.09, 7.70)
Wheezing ^c	0.85	(0.24, 3.02)	1.27	(0.27, 6.05)	2.43	(0.32, 18.36)	2.72	(0.25, 29.90)	0.36	(0.06, 2.00)	1.31	(0.10, 18.00)
Otitis media ^d	1.41	(0.28, 2.02)	1.51	(0.45, 5.12)	1.04	(0.27, 3.97)	1.92	(0.35, 10.40)	0.47	(0.11, 2.05)	0.95	(0.16, 5.69)

^a OR for a 10-fold increase in maternal PFOS or PFOA concentrations.^b PFOS: perfluorooctane sulfonate.^c Logistic regression model adjusted for maternal age, maternal educational level, pre-pregnancy BMI, allergy of parents, parity, infant gender, breast-feeding period, environmental tobacco exposure, day care attendance and blood sampling period.^d Logistic regression model adjusted for maternal age, maternal educational level, parity, infant gender, breast-feeding period, environmental tobacco exposure, day care attendance and blood sampling period.^e PFOA: perfluorooctanoate.

potential gender-specific differences) from infancy to school-age, because it is difficult to obtain definitive diagnoses for infants.

Moreover, using univariate analyses of maternal PFOS and PFOA concentrations in relation to parent and infant characteristics, statistically significant differences with respect to maternal age, parity, and blood sampling period were identified in our study. In other studies, maternal PFOS and PFOA concentrations were significantly related to parity, with higher levels found in primipara (Fei et al., 2007), and significantly lower levels reported after delivery than during pregnancy (Monroy et al., 2008). In our study, we performed stratified analysis by blood sampling period, but the results of multiple analyses did not change.

In laboratory animals, immunosuppression and reduced IgM antibody production along with increased IgE levels were reported with high-dose PFOS and PFOA exposure (Dewitt et al., 2008; Fairley et al., 2007; Keil et al., 2008; Peden-Adams et al., 2007). Also, in these studies used the mouse strain known to be the most sensitive strain for immunomodulatory effects of PFOA and PFOS (Dewitt et al., 2009). On the other hand, sensitivity of the human fetus to PFCs may be higher than in animals because in our cohort study, maternal serum PFOS concentrations below those reported in the animal studies were negatively correlated with birth weight (Washino et al., 2009). It has been suggested that some effects of PFOS and PFOA on the immune system are independent of peroxisome proliferator-activated receptor alpha (PPAR α) activation, but PPAR α expression is lower in humans than in rodents (Dewitt et al., 2009). Because of these uncertainties, we cannot rule out the possibility that PFC exposure may be immunotoxic, and further experiments are necessary to clarify the possible effects of PFC exposure.

Sources of postnatal exposure to PFCs from birth to 18 months of age include breast milk, food, drinking water, indoor dust in living environments, and products in which PFCs are used (e.g., baby bottle and carpet). Previous studies have suggested that breast milk or indoor dust is a considerable source of PFC exposure for children (Björklund et al., 2009; Kärman et al., 2007). In the same sample population as was used in our present study, we previously measured PFOS and PFOA concentrations in 40 breast milk samples and found that their concentrations were very low (Nakata et al., 2009). Therefore, we suggest that the effects of PFOS and PFOA in breast milk might be lower than the effects of these compounds in maternal blood. Postnatal exposure from intake of food and drinking water or from indoor dust from birth to 18 months of age was not investigated

in our study. In future studies, it will be necessary to examine not only prenatal exposure but also postnatal exposure, because the latter may have affected the exposure assessment.

The present study has some limitations. First, the sample size was relatively small and was insufficient for identification of significant relationships between PFC exposure and allergies and infectious diseases, given the low number of cases in the cohort with these conditions. Second, selection bias may have occurred because the study population included only pregnant women that attended one local maternity hospital and the participation rate was low (29%), partly because we excluded pregnant women who had decided to enroll in the Japanese cord blood bank (22% of those approached) or who delivered their baby at another hospital (3% of those approached). These exclusions may limit the extrapolation of our results to the general population. Third, diagnosis of allergic disease such as wheezing in infants from the birth to 18 months might not have been accurate because of difficulties in obtaining diagnoses. The current prevalence of asthma in Japan is the highest it has been in six years, and tends to be decreasing with advancing age (Ministry of Education, Culture, Sports, Science and Technology, 2010). Because it is difficult to obtain a definitive diagnosis of allergy in infants, a follow-up study in which exposure is monitored from birth to school-age may be necessary to further examine possible effects of PFOS and PFOA on the immune system.

5. Conclusions

Although cord blood IgE levels decreased significantly with high maternal PFOA levels among female infants, no relationship was found between maternal PFOS and PFOA levels and allergies and infectious diseases in infants at age 18 months. Future studies with a larger sample size are warranted to investigate the relationship between maternal PFC exposure and child allergies and infections from infancy to school-age.

Acknowledgments

We thank the participants of all mothers and their children and the medical staff at Sapporo Toho Hospital, and the Fukuoka Institute of Health and Environmental Sciences.

References

- Apelberg, B.J., Witter, F.R., Herbstman, J.B., Calafat, A.M., Halden, R.U., Needham, L.L., et al., 2007. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environ. Health Perspect.* 115, 1670–1676.
- Begley, T.H., White, K., Honigfort, P., Twaroski, M.L., Neches, R., Walker, R.A., 2005. Perfluorochemicals: potential sources of and migration from food packaging. *Food Addit. Contam.* 22, 1023–1031.
- Bisgaard, H., Bønnelykke, K., 2010. Long-term studies of the natural history of asthma in childhood. *J. Allergy Clin. Immunol.* 126, 187–197.
- Björklund, J.A., Thuresson, K., De Wit, C.A., 2009. Perfluoroalkyl compounds (PFCs) in indoor dust: concentrations, human exposure estimates, and sources. *Environ. Sci. Technol.* 43, 2276–2281.
- Dewitt, J.C., Copeland, C.B., Strynar, M.J., Luebke, R.W., 2008. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. *Environ. Health Perspect.* 116, 644–650.
- Dewitt, J.C., Shnyra, A., Badr, M.Z., Loveless, S.E., Hoban, D., Frame, S.R., et al., 2009. Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. *Crit. Rev. Toxicol.* 39, 76–94.
- Fairley, K.J., Purdy, R., Kearns, S., Anderson, S.E., Meade, B.J., 2007. Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. *Toxicol. Sci.* 97, 375–383.
- Fei, C., McLaughlin, J.K., Tarone, R.E., Olsen, J., 2007. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. *Environ. Health Perspect.* 115, 1677–1682.
- Fei, C., McLaughlin, J.K., Lipworth, L., Olsen, J., 2010. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. *Environ. Res.* 110, 773–777.
- Fletcher, T., Steenland, K., Savitz, D., 2009. Status Report: PFOA and Immune Biomarkers in Adults Exposed to PFOA in Drinking Water in the Mid Ohio Valley: <http://www.c8sciencepanel.org/pdfs/Status_Report_C8_and_Immune_markers_March2009.pdf> (accessed 27 September 2011).
- Halldorsson, T.L., Fei, C., Olsen, J., Lipworth, L., McLaughlin, J.K., Olsen, S.F., 2008. Dietary predictors of perfluorinated chemicals: a study from the Danish National Birth Cohort. *Environ. Sci. Technol.* 42, 8971–8977.
- Harada, K., Saito, N., Inoue, K., Yoshinaga, T., Watanabe, T., Sasaki, S., et al., 2004. The influence of time, sex and geographic factors on levels of perfluorooctane sulfonate and perfluorooctanoate in human serum over the last 25 years. *J. Occup. Health* 46, 141–147.
- Harada, K., Koizumi, A., Saito, N., Inoue, K., Yoshinaga, T., Date, C., et al., 2007. Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan. *Chemosphere* 66, 293–301.
- Ho, S.-M., 2010. Environmental epigenetics of asthma: an update. *J. Allergy Clin. Immunol.* 126, 453–465.
- Hölzer, J., Midasch, O., Rauchfuss, K., Kraft, M., Reupert, R., Angerer, J., et al., 2008. Biomonitoring of perfluorinated compounds in children and adults exposed to perfluorooctanoate-contaminated drinking water. *Environ. Health Perspect.* 116, 651–657.
- Inoue, K., Okada, F., Ito, R., Kato, S., Sasaki, S., Nakajima, S., et al., 2004a. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. *Environ. Health Perspect.* 112, 1204–1207.
- Inoue, K., Okada, F., Ito, R., Kawaguchi, M., Okanouchi, N., Nakazawa, H., 2004b. Determination of perfluorooctane sulfonate, perfluorooctanoate and perfluorooctane sulfonylamide in human plasma by column-switching liquid chromatography–electrospray mass spectrometry coupled with solid-phase extraction. *J. Chromatogr. B. Anal. Technol. Biomed. Life Sci.* 810, 49–56.
- ISAAC Steering Committee, 1998. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* 351, 1225–1232.
- Jensen, A.A., Leffers, H., 2008. Emerging endocrine disruptors: perfluoroalkylated substances. *Int. J. Androl.* 31, 161–169.
- Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K.S., Loganathan, B.G., et al., 2004. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* 38, 4489–4495.
- Kärman, A., Ericson, I., van Bavel, B., Darnerud, P.O., Aune, M., Glynn, A., et al., 2007. Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996–2004, in Sweden. *Environ. Health Perspect.* 115, 226–230.
- Keil, D.E., Mehlmann, T., Butterworth, L., Peden-Adams, M.M., 2008. Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. *Toxicol. Sci.* 103, 77–85.
- Kishi, R., Sasaki, S., Yoshioka, E., Yuasa, M., Sata, F., Saijo, Y., et al., 2011. Cohort profile: the Hokkaido study on environment and children's health in Japan. *Int. J. Epidemiol.* 40, 611–618.
- Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol. Sci.* 99, 366–394.
- Luster, M.I., Dean, J.H., Germolec, D.R., 2003. Consensus workshop on methods to evaluate developmental immunotoxicity. *Environ. Health Perspect.* 111, 579–583.
- Midasch, O., Drexler, H., Hart, N., Beckmann, M.W., Angerer, J., 2007. Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. *Int. Arch. Occup. Environ. Health* 80, 643–648.
- Ministry of Education, Culture, Sports, Science and Technology, Japan. School Health Examination Survey 2010: <http://www.mext.go.jp/b_menu/toukei/chousa05/hoken/kekka/k_detail/_icsFiles/afieldfile/2011/03/25/1303380_2.pdf> (accessed 27 September 2011).
- Monroy, R., Morrison, K., Teo, K., Atkinson, S., Kubwabo, C., Stewart, B., et al., 2008. Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. *Environ. Res.* 108, 56–62.
- Nakata, H., Nakata, A., Kawaguchi, M., Iwasaki, Y., Ito, R., Saito, K., et al., 2005a. Development of an analytical method for perfluorochemicals in human plasma and blood by liquid chromatography–tandem mass spectrometry coupled with solid-phase extraction using a column-switching technique. *Organohalogen Compd.* 67, 219–221.
- Nakata, H., Nakata, A., Okada, F., Ito, R., Inoue, K., Saito, K., et al., 2005b. Development of online solid-phase extraction–HPLC/MS/MS method for the determination of perfluorochemicals in human plasma. *Bunseki Kagaku* 9, 877–884 (in Japanese).
- Nakata, A., Saito, K., Iwasaki, Y., Ito, R., Kishi, R., Nakazawa, H., 2009. Determination of perfluorinated compounds in human milk and evaluation of their transition from maternal plasma. *Bunseki Kagaku* 58, 653–659 (in Japanese).
- Nishima, S., Chisaka, H., Fujiwara, T., Furusho, K., Hayashi, S., Hiraba, K., et al., 2009. Surveys on the prevalence of pediatric bronchial asthma in Japan: a comparison between the 1982, 1992, and 2002 surveys conducted in the same region using the same methodology. *Allergol. Int.* 58, 37–53.
- Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., et al., 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ. Health Perspect.* 115, 1298–1305.
- Peden-Adams, M.M., EuDaly, J.G., Dabra, S., EuDaly, A., Heesemann, L., Smythe, J., et al., 2007. Suppression of humoral immunity following exposure to the perfluorinated insecticide sulfluramid. *J. Toxicol. Environ. Health A* 70, 1130–1141.
- Shoeib, M., Harner, T., Wilford, B.H., Jones, K.C., Zhu, J., 2005. Perfluorinated sulfonamides in indoor and outdoor air and indoor dust: occurrence, partitioning, and human exposure. *Environ. Sci. Technol.* 39, 6599–6606.
- Tittlemier, S.A., Pepper, K., Seymour, C., Moisey, J., Bronson, R., Cao, X.L., et al., 2007. Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. *J. Agric. Food Chem.* 55, 3203–3210.
- Todaka, T., Hirakawa, H., Kajiwar, J., Hori, T., Tobiishi, K., Onozuka, D., et al., 2007. Concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in blood collected from 195 pregnant women in Sapporo City, Japan. *Chemosphere* 69, 1228–1237.
- Todaka, T., Hori, T., Hirakawa, H., Kajiwar, J., Yasutake, D., Onozuka, D., et al., 2008. Congener-specific analysis of non-dioxin-like polychlorinated biphenyls in blood collected from 195 pregnant women in Sapporo City, Japan. *Chemosphere* 73, 923–931.
- Washino, N., Saijo, Y., Sasaki, S., Kato, S., Ban, S., Konishi, K., et al., 2009. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. *Environ. Health Perspect.* 117, 660–667.
- Zöllner, I.K., Weiland, S.K., Piechotowski, I., Gabrio, T., von Mutius, E., Link, B., et al., 2005. No increase in the prevalence of asthma, allergies, and atopic sensitization among children in Germany: 1992–2001. *Thorax* 60, 545–548.