Journal of Immunotoxicology

http://informahealthcare.com/imt
ISSN: 1547-691X (print), 1547-6901 (electronic)
J Immunotoxicol, Early Online: 1–7
© 2013 Informa Healthcare USA, Inc.
DOI: 10.3109/1547691X.2012.755580

informa healthcare

RESEARCH ARTICLE

Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood

Berit Granum[1], Line S. Haug[1], Ellen Namork[1], Solvor B. Stølevik[1], Cathrine Thomsen[1], Ingeborg S. Aaberge[2], Henk van Loveren[3,4], Martinus Løvik[1,5], and Unni C. Nygaard[1]

[1]Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway, [2]Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway, [3]Maastricht University, Maastricht, the Netherlands, [4]National Institute of Public Health and the Environment, Bilthoven, the Netherlands, and [5]Norwegian University of Science and Technology, Trondheim, Norway

Abstract

Perfluoroalkyl substances (PFAS) are suggested to have immunosuppressive effects; exposure *in utero* and in the first years of life is of special concern as fetuses and small children are highly vulnerable to toxicant exposure. The objective of this study was to investigate the effect of pre-natal exposure to PFAS on responses to pediatric vaccines and immune-related health outcomes in children up to 3 years of age. In the prospective birth-cohort BraMat, a sub-cohort of the Norwegian Mother and Child Cohort Study (MoBa), pregnant women from Oslo and Akershus, Norway, were recruited during 2007–2008. Three annual questionnaire-based follow-ups were performed. Blood samples were collected from the mothers at the time of delivery and from the children at the age of 3 years. As a measure of pre-natal exposure to PFAS, the concentrations of perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) were determined in maternal blood from 99 BraMat participants. Main outcome measures were anti-vaccine antibody levels, common infectious diseases and allergy- and asthma-related health outcomes in the children up to the age of 3 years. There was an inverse association between the level of anti-rubella antibodies in the children's serum at age 3 years and the concentrations of the four PFAS. Furthermore, there was a positive association between the maternal concentrations of PFOA and PFNA and the number of episodes of common cold for the children, and between PFOA and PFHxS and the number of episodes of gastroenteritis. No associations were found between maternal PFAS concentrations and the allergy- and asthma-related health outcomes investigated. The results indicate that pre-natal exposure to PFAS may be associated with immunosuppression in early childhood.

Keywords

Childhood, infections, immunotoxicity, perfluoroalkyl substances, Pre-natal exposure, vaccination response

History

Received 19 October 2012
Revised 27 November 2012
Accepted 30 November 2012
Published online 25 January 2013

Introduction

The environmental pollutants known as perfluoroalkyl substances (PFAS) are ubiquitously distributed in wildlife and humans (Houde et al., 2006; Lau et al., 2007). PFAS are synthetic fluorinated compounds with attractive water and oil repellent characteristics that have been used in a wide variety of consumer products and industrial applications for the last 50 years (Vestergren and Cousins, 2009).

The majority of human PFAS exposures have been attributed to the diet. A wide range of PFAS has been found in food and beverages selected from the Norwegian market where fish, meat, and eggs contained the highest concentrations (Haug et al., 2010). Dust in the indoor environment may also be an important contributor to PFAS exposure (Fromme et al., 2009; Haug et al., 2011). Early life exposure occurs both via placental transfer (Apelberg et al., 2007; Gutzkow et al., 2012) and mother's milk (Thomsen et al., 2010; Haug et al., 2011). Pre-natal exposure to toxicants is of concern since the fetus may be especially vulnerable due to an extensively developing immune system (Holsapple et al., 2004; van Loveren and Piersma, 2004).

Immunotoxic effects of PFAS have been demonstrated in *in vitro* and animal studies (DeWitt et al., 2012), but, until recently, human data on immune effects of pre-natal exposure to PFAS have been absent. In 2012, however, Grandjean et al. (2012) reported that elevated PFAS concentrations in maternal blood were associated with reduced humoral immune responses to routine childhood vaccinations in Faroese children. Thus, the objective of the present study was to investigate whether pre-natal exposure to PFAS is associated with altered vaccination responses and clinical health outcomes such as common infections and asthma- and allergy-related diseases in early childhood.

Address for Correspondence: Dr Berit Granum, Division of Environmental Medicine, Norwegian Institute of Public Health, PO Box 4404 Nydalen, N-0403 Oslo, Norway. Tel: 4721076696. Fax: 4721076686. E-mail: berit.granum@fhi.no

Materials and methods

Study population

The BraMat cohort was established between April 2007 and March 2008, as previously described (Stølevik et al., 2011).

Abbreviations

BMI, Body mass index;
ELISA, Enzyme-linked immunoassay;
Hib, *Heamophilus influenzae* type b;
IQR, Interquartile range;
LC-MS/MS, Liquid chromatography-triple quadrupole mass spectrometry;
LOQ, Limit of quantification;
PFAS, Perfluoroalkyl substances;
PFHxS, Perfluorohexane sulfonate;
PFNA, Perfluorononanoate;
PFOA, Perfluorooctanoate;
PFOS, Perfluorooctane sulfonate.

In short, all pregnant women already enrolled in the Norwegian Mother and Child Cohort Study (MoBa) and who were scheduled to give birth in Oslo or Akershus were invited to participate (recruitment rate ~25%). MoBa is a nation-wide prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health, including 108,000 pregnancies between 1999–2008 (Magnus et al., 2006). Exclusion criteria for the BraMat cohort were autoimmune diseases of the mother and use of steroids, anti-inflammatory, or epileptic drugs during pregnancy. There were no plurality births. The Norwegian Regional Committee for Medical and Health Research Ethics and the Data Inspectorate approved this study. All mothers gave their written informed consent.

The present study was based on 99 participants for whom we collected maternal blood at time of delivery. When investigating serological outcomes, the participants were limited to 56, for whom we had both maternal blood samples at delivery and blood samples from the children at 3 years-of-age.

Blood sampling and handling

Venous blood from the mothers was collected into lithium-heparinized vacutainers (Becton Dickinson and Company (BD), Plymouth, UK) 0–3 days after delivery. Plasma was prepared by centrifugation (1300 x g for 5 min at room temperature) and aliquots were stored at −80°C until further use. Venous blood was collected from the 3-year-old children either at their doctor's office, at home by a technician, or at a commercial laboratory (Fürst Medical Laboratory, Oslo, Norway). Blood was collected into BD Vacutainer®SST™II serum gel separation tubes with butterfly blood collection sets (BD, Franklin Lakes, NJ). The blood was allowed to clot for at least 30 min before centrifugation (1000–1300 g for 10 min at room temperature) and aliquots were stored at −20°C until further use. Blood samples from the children were collected at a mean age of 35 months (range: 31–38 months).

PFAS measurements in maternal plasma

The concentrations of PFAS in maternal blood samples collected at the time of delivery were used as a measure of pre-natal exposure to PFAS. Nineteen PFAS were determined using liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS) according to a previously-described method (Haug et al., 2009a). Due to low PFAS concentrations and/or detection frequency, statistical analyses were performed only for PFOA, perfluorononanoate (PFNA), perfluorohexane sulfonate (PFHxS), and PFOS. The limit of quantification (LOQ) was 0.050 ng/ml for all PFAS. Concentrations below LOQ were set to 0.035 ng/ml (LOQ divided by the square root of two, i.e. 1.414). For quantification of PFOS, the total area of the linear and branched isomers was integrated.

Serological health outcomes

Vaccination responses

On the basis of good vaccination coverage (93–94%) and low background exposures in the population, we chose to examine antibody levels specific for four vaccines in the Norwegian Childhood Vaccination Program: measles, rubella, tetanus, and *Haemophilus influenza* type b (Hib). Vaccines against tetanus and Hib are given at ages 3, 5, and 12 months, and measles and rubella at age 15 months. The antibody titers were measured as previously described (Stølevik et al., 2013). Children not following the Norwegian Childhood Vaccination Program ($n = 4$) were excluded from the statistical analyses regarding vaccination responses.

Allergen-specific IgE

The serum samples were analyzed for allergen-specific IgE antibodies using ImmunoCAP Phadiatop® Infant (Thermo Fisher Scientific, Uppsala, Sweden), which is designed to differentiate between atopic and non-atopic persons. The concentration of antibodies was expressed as Phadia Arbitrary Units/l (PAU/l), with a cut-off for positive response set to 0.35 PAU/l, as recommended by the manufacturer.

Clinical health outcomes

At the age of 1, 2, and 3 years, a questionnaire was sent to the participants. The questionnaire covered topics on the child's infectious diseases, allergy, and asthma (Table 1). Concerning infectious diseases, the mothers were asked how many episodes of the following diseases/complaints the child had experienced in the last 12 months: common colds and other upper respiratory tract infections (hereafter called common cold), otitis media, pneumonia, gastroenteritis with vomit or diarrhea, and urinary tract infection. Concerning allergy and asthma, the mothers were asked: Has the child been diagnosed with asthma or asthma bronchitis by a doctor? Has the child had periods of more than 10 days of dry cough, chest tightness, or wheeze (hereafter called wheeze)? Has the child had eczema or itchiness (in the face or at joints such as the groin, popliteal fossa, ankle, elbow, and wrist)? Has the child been diagnosed with atopic eczema by a doctor? Has the child been diagnosed with allergy by a doctor?

Data for all 3 years merged (0–3 years-of-age) and data for the third year were investigated. Regarding the binary health outcomes data (yes/no answers) for all 3 years merged, a positive answer in one or more of the annual questionnaires was noted as a 'yes'. For health outcomes reporting number of episodes, the merged data contain the sum of the number of reported incidences for all 3 years.

Due to a low number of positive cases, statistical analyses were not performed for the health outcomes pneumonia ($n = 6$), urinary tract infections ($n = 5$), and doctor-diagnosed allergy ($n = 6$). Statistical analyses for doctor-diagnosed asthma and atopic eczema were, for the same reasons, performed only for all 3 years merged (0–3 years). Only one child was reported not to have a common cold during the study period. Binary statistical analyses (common cold yes/no) were therefore performed for the third year only.

Statistical analyses

Poisson regression analyses were used for health outcomes consisting of count data (number of episodes of common cold

Table 2. Concentrations of the four most abundant PFAS (ng/ml) in maternal plasma from the 99 participants.

Compound		Mean	Median	Min–Max	IQR
PFOA	Perfluorooctanoate	1.1	1.1	0.2–2.7	0.8–1.4
PFNA[a]	Perfluorononanoate	0.3	0.3	<0.05–0.9	0.2–0.4
PFHxS[b]	Perfluorohexane sulfonate	0.3	0.3	<0.05–2.8	0.4–0.3
PFOS	Perfluorooctane sulfonate	5.6	5.5	1.4–11.0	3.8–7.1

IQR, interquartile range; PFAS, perfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.
[a]One sample (1.0%) was below LOQ.
[b]Six samples (6.1%) were below LOQ.

Table 3. Levels of vaccine-induced antibodies at the age of 3 years.

Vaccine	*n*	Mean	Median	Min–Max	IQR
Rubella (OD)	50	1.7	1.9	0.8–2.4	1.5–2.1
Measles (OD)	50	0.8	0.8	0.1–1.8	0.6–1.0
Tetanus (IU/ml)	49	0.2	0.1	0.1–0.7	0.1–0.3
Hib (μg/ml)	51	2.5	0.8	0.1–36.0	0.5–1.7

AU, arbitrary units; Hib, *Haemophiluz influenzae* type b; IU, international units; IQR, inter-quartile range; OD, optical density.

Serological health outcomes

Vaccination responses

Table 3 shows the levels of vaccine-induced antibodies in serum samples from the children at 3 years of age. In multivariate models, increased concentrations of all four PFAS in maternal blood were significantly associated with reduced levels of anti-rubella antibodies in the children at 3 years-of-age (Table 4). Based on the β-values, the strength of the association between rubella antibody-levels and PFAS concentrations were PFNA > PFOA > PFHxS > PFOS. No significant associations were found between the concentrations of PFAS and vaccine antibody levels to the other vaccines.

Allergen-specific IgE antibodies

Eighteen per cent (10/56) of the children's serum samples were found to have a positive Phadiatop® Infant (≥0.35 PAU/l). In bivariate logistic regression analyses, no significant associations were found between a positive Phadiatop® Infant and the concentration of the four PFAS (data not shown).

Clinical health outcomes

With regard to infectious diseases, the maternal concentrations of PFOA and PFNA were positively-associated with the number of episodes of common cold for both the children's third year of life and all 3 years merged. PFHxS was positively-associated with the children's number of episodes of common cold for all 3 years merged in the bivariate analysis only (Table 5). When analyzing common cold for the third year of life as a binary variable (yes/no), no statistically significant associations were found (Table 5).

The concentrations of PFOA and PFHxS were positively associated with the number of episodes of gastroenteritis for all 3 years merged (Table 5). No statistically significant associations were found when analyzing gastroenteritis as a binary variable (yes/no). No significant associations were found in bivariate logistic regression analyses between the concentrations of the four PFAS and the reported eczema and itchiness, wheeze, otitis media, and doctor-diagnosed atopic eczema or asthma (Table S1).

Discussion

In the present study, increased concentrations of PFOA, PFNA, PFHxS, and PFOS in maternal blood were found to be associated with decreased antibody levels to the rubella vaccine in the children at 3 years-of-age. Increased concentrations of PFOA and PFNA were associated with increased number of episodes of common cold. Furthermore, increased levels of PFOA and PFHxS were associated with increased number of episodes of gastro-enteritis. These results are indicative of immunosuppressive effects of pre-natal exposure to PFAS on both response to pediatric vaccines and immune-related clinical health outcomes in humans.

Vaccine responses in serum are relevant indicators of immunotoxicity (van Loveren et al., 2001; Luster et al., 2005; WHO, 2012), thus antibody levels to four vaccines included in the Norwegian Childhood Vaccination Program were examined. A significant inverse association was found between the concentration of PFAS and anti-rubella antibody levels. Grandjean et al. (2012) reported a similar result in that they found an inverse association between maternal concentrations of PFAS and the level of anti-diphtheria antibodies in the children at 5 years of age. As in the study by Grandjean et al., no associations were found between maternal PFAS and antibody levels against the tetanus vaccine. The different vaccines may stimulate different components of the immune system, which can explain the vaccine-dependent differences in the effect of PFAS exposure. However, the results from the Grandjean et al. study and our study indicate that pre-natal exposure to PFAS may suppress responses to some pediatric vaccines. The time between the last vaccination and the time of blood collection may be an important covariate. For the vaccines included in the present study, this time period is between 20–23 months, but this information was not available on an individual level. However, the child's age at the time of the 3-year follow-up was included in the statistical analyses as a potential confounding variable, but did not remain in the final multivariate analyses.

To our knowledge, there are three birth cohort studies examining clinical immunotoxicological outcomes in the child and pre-natal exposure to PFAS (at concentrations similar or higher than in BraMat) (Fei et al., 2010; Wang et al., 2011; Okada et al., 2012). In addition, in the C8 Project Panel Study, women ($n = 878$) providing blood samples during or close to the time of pregnancy were interviewed concerning infectious diseases among their children (C8 Project Panel Study, 2012). None of these studies found any associations between pre-natal PFAS exposure and the health outcomes investigated. Our observations are in agreement with the studies by Okada et al. (2012) and Wang et al. (2011) in that there were no significant associations between pre-natal exposure to PFAS and eczema, wheeze, otitis

Table 4. Results of bivariate and multivariate regression analyses of pre-natal exposure to PFAS and anti-vaccine antibody levels.

PFAS	Vaccine	Bivariate n	Bivariate β (95% CI)	Bivariate p-value	Multivariate[a] n	Multivariate[a] β (95% CI)	Multivariate[a] p-value
PFOA	Rubella	50	*−0.40 (−0.64, −0.17)*	*0.001*	50	*−0.40 (−0.64, −0.17)*	*0.001*
PFNA		50	*−1.26 (−2.32, −0.20)*	*0.021*	47	*−1.38 (−2.35, −0.40)*	*0.007*[b]
PFHxS		50	*−0.38 (−0.66, −0.11)*	*0.008*	50	*−0.38 (−0.66, −0.11)*	*0.008*
PFOS		50	*−0.08 (−0.14, −0.02)*	*0.007*	50	*−0.08 (−0.14, −0.02)*	*0.007*
PFOA	Measles	50	−0.13 (−0.35, 0.09)	0.236			
PFNA		50	−0.55 (−1.51, 0.41)	0.256			
PFHxS		50	−0.04 (−0.30, 0.22)	0.770			
PFOS		50	−0.05 (−0.10, 0.01)	0.093			
PFOA	Hib	51	−0.05 (−3.85, 3.74)	0.978			
PFNA		51	4.90 (−10.7, 20.5)	0.531			
PFHxS		51	−0.48 (−4.64, 3.67)	0.817			
PFOS		51	−0.16 (−1.02, 0.70)	0.705			
PFOA	Tetanus	49	0.01 (−0.09, 0.10)	0.921			
PFNA		49	−0.01 (−0.41, 0.39)	0.966			
PFHxS		49	0.07 (−0.03, 0.18)	0.156			
PFOS		49	−0.002 (−0.03, 0.02)	0.873			

Italics, $p \leq 0.05$.

Hib, *Haemophiluz influenzae* type b; PFAS, perfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

[a]Potential confounding variables initially included in multivariate analyses were maternal allergy, paternal allergy, maternal education, child's gender, and/or age at 3-year follow-up.

[b]Confounding variable remaining in final model was paternal allergy.

Table 5. Results of bi- and multivariate regression analyses of pre-natal exposure to PFAS and the health outcomes common cold and gastroenteritis.

PFAS	Health outcomes	Bivariate n	Bivariate p-value	Multivariate[a] n	Multivariate[a] β (95% CI)	Multivariate[a] p-value
No. of episodes of common cold			β (95% CI)			
PFOA	3rd year	78	*0.42 (0.16, 0.72)*	*0.002*	*0.42 (0.16, 0.72)*	*0.002*
	All 3 years	65	*0.17 (0.01, 0.33)*	*0.044*	*0.42 (0.21, 0.62)*	*<0.001*[b]
PFNA	3rd year	77	*1.24 (0.08, 2.40)*	*0.036*	*1.24 (0.08, 2.40)*	*0.036*
	All 3 years	64	0.57 (−0.10, 1.23)	0.094	*0.74 (0.05, 1.43)*	*0.035*[c]
PFHxS	3rd year	78	0.24 (−0.03, 0.51)	0.084	0.24 (−0.03, 0.51)	0.084
	All 3 years	65	*0.17 (0.01, 0.33)*	*0.036*	0.15 (−0.02, 0.32)	0.078[d]
PFOS	3rd year	78	0.03 (−0.03, 0.10)	0.274		
	All 3 years	65	0.01 (−0.02, 0.05)	0.501		
Common cold (Y/N)			OR (95% CI)			
PFOA	3rd year	83	1.24 (0.32, 4.83)	0.762		
PFNA	3rd year	82	0.11 (0.001, 22.5)	0.412		
PFHxS	3rd year	83	1.71 (0.20, 14.8)	0.628		
PFOS	3rd year	83	1.13 (0.85, 1.51)	0.394		
No. of episodes of gastroenteritis			β (95% CI)			
PFOA	3rd year	82	0.21 (−0.21, 0.64)	0.327		
	All 3 years	66	*0.31 (0.002, 0.61)*	*0.048*	*0.31 (0.00, 0.61)*	*0.048*
PFNA	3rd year	81	−0.46 (−2.27, 1.35)	0.617		
	All 3 years	65	−0.10 (−1.36, 1.17)	0.883		
PFHxS	3rd year	82	0.33 (−0.05, 0.71)	0.087	0.33 (−0.05, 0.71)	0.087
	All 3 years	66	*0.35 (0.10, 0.61)*	*0.007*	*0.35 (0.10, 0.61)*	*0.007*
PFOS	3rd year	82	0.06 (−0.03, 0.14)	0.225		
	All 3 years	66	0.03 (−0.04, 0.10)	0.367		
Gastroenteritis (Y/N)			OR (95% CI)			
PFOA	3rd year	84	1.16 (0.37, 3.65)	0.803		
	All 3 years	93	3.13 (0.37, 26.2)	0.294		
PFNA	3rd year	83	0.16 (0.001, 17.5)	0.440		
	All 3 years	92	0.06 (0.00, 171)	0.482		
PFHxS	3rd year	84	8.10 (0.28, 237)	0.225		
	All 3 years	93	11.41 (0.24, 5496)	0.440		
PFOS	3rd year	84	1.11 (0.87, 1.42)	0.390		
	All 3 years	93	1.07 (0.72, 1.58)	0.733		

Italics, $p \leq 0.05$.

PFAS, perfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

[a]Potential confounding variables initially included in the multivariate analyses regarding common cold were previous breastfeeding, older siblings, maternal allergy, maternal education, child's gender, child's birth season and/or gross income of the household. Variables initially included in multivariate analyses regarding gastroenteritis were previous breastfeeding, older siblings, maternal allergy, maternal education, and/or gross income of household.

[b]Confounding variables remaining in final model were previous breastfeeding and birth season.

[c]Confounding variable remaining in final model was previous breastfeeding.

[d]Confounding variables remaining in final model were previous breastfeeding and maternal education.

media, and atopic dermatitis. Fei et al. (2010) investigated the associations between pre-natal exposure to PFOS and PFOA and the sum of any hospitalization of the child due to infectious diseases, whereas the C8 Project Panel Study investigated the association between PFOA exposure during pregnancy and categories of infection reported for the children. In the present study, the clinical health outcomes that were associated with pre-natal exposure to PFAS were common cold and gastroenteritis that in most cases do not require hospitalization. Additionally, when investigating possible associations between maternal PFAS concentrations and infectious diseases as binary outcomes (yes/no answers), no significant associations were observed. In contrast, when examining the number of episodes of infectious diseases, positive associations were found between maternal concentrations of PFOA and PFNA and common cold, as well as for PFOA and PFHxS concentrations and gastroenteritis. This may suggest that the number of episodes of infectious diseases may be a more sensitive indicator for immunosuppressive effects of pre-natal exposure to PFAS than binary reported outcomes.

Since the concentration of PFAS in cord blood is not analyzed, there are no direct measures of fetal exposures to PFAS in our study. However, strong and highly significant correlations between the concentrations of PFAS in maternal and cord blood have been reported, suggesting that maternal PFAS concentrations can be used as a marker of pre-natal exposure (Glynn et al., 2012; Gutzkow et al., 2012). In the present study, PFAS concentrations in breast milk were not available, thus we cannot differentiate between pre-natal and post-natal exposure. Blood and breast milk concentrations of PFAS have been found to be highly correlated (Haug et al., 2011). We can, therefore, not exclude that the observed effects in the present study may be partially explained by post-natal PFAS exposure.

Our findings are in line with evidence of immunosuppression reported in several animal experiments on exposure to PFOS and PFOA. Suppressed IgM responses to T-cell-dependent and -independent antigens and natural killer (NK)-cell function has been demonstrated in adult mice and 8-week-old offspring of dams exposed to PFOS during gestation (Keil et al., 2008; Peden-Adams et al., 2008; Dong et al., 2009; Zheng et al., 2009; DeWitt et al., 2012). In agreement, host resistance to influenza A infection was suppressed in female mice exposed to PFOS (Guruge et al., 2009). Also in human donors, PFOS and PFOA plasma concentrations have been reported to be associated with reduced NK-cell activity (Brieger et al., 2011). The mechanisms behind the immunomodulatory effects of PFAS are unclear. However, dependent on the type of PFAS, both innate and adaptive immune cells may be affected. For some substances, inhibition of NF-κB activation and/or activation of peroxisome proliferator-activated receptor (PPAR)-α may contribute to altered proliferation and cytokine secretion (Corsini et al., 2012; DeWitt et al., 2012).

Based on their persistence and bio-accumulating properties, the use of PFOS and its salts was restricted in 2000–2009 (European Union, 2006; Stockholm Convention on Persistent Organic Pollutants, 2009). However, the industry started a phase-out of both PFOS and PFOA in 2000–2009 (3M Company, 2000; United States Environmental Protection Agency [US EPA], 2006), consistent with the observed decrease in blood concentrations of these substances in humans in the last decade (Haug et al., 2009b; Kato et al., 2011; Glynn et al., 2012). The concentration of other PFAS in humans has, however, increased in the same time period (Kato et al., 2011; Glynn et al., 2012). Until recently, both human and animal studies have focused mostly on the effects of PFOS and PFOA. Our findings on immunosuppressive effects of all the four PFAS studied (although they are not highly correlated ($r = 0.26$–0.60)) illustrate the importance of studying and possibly also regulating other PFAS than PFOA and PFOS.

The strength of the study is the extensive information about the children and their mothers from MoBa and the Medical Birth Registry of Norway, as well as from the annual BraMat questionnaires. The potential selection bias in MoBa (38.5% participation rate) has been evaluated (Nilsen et al., 2009), and, although there were differences between participants and non-participants on a number of exposure and outcome variables, no statistical differences when comparing eight exposure-outcome associations were found. The authors suggested that self-selection in MoBa may not be a validity problem in studies on exposure–outcome associations. A selection bias due to the low recruitment rate in the BraMat cohort (25%), however, cannot be excluded. Blood samples were collected for about half of the participants when the children were 3 years old. Since there was only a significant statistical difference for paternal allergy with regard to participants with or without blood samples, there are most likely no selection biases at the 3-year follow-up. A weakness of the present study is the small study population where minor differences in the incidence of the different health outcomes may strongly impact the statistical analyses. In addition, some of the statistically significant associations may be due to chance because of the multiple exposure–health outcome comparisons made. However, our significant serological and clinical findings are all consistently suggesting immunosuppressive effects of pre-natal exposure to PFAS and are supported by both human and animal studies (Keil et al., 2008; Grandjean et al., 2012).

Conclusion

In the present study, PFAS concentrations were associated with reduced antibody levels to the rubella vaccine and increased number of episodes of common cold and gastroenteritis, suggesting that pre-natal exposure to various PFAS may lead to immunosuppression in early childhood. Our findings illustrate the necessity for further investigations of the immunotoxic potential of different PFAS in current and/or increasing use.

Acknowledgments

We are grateful to all the participating families in Norway who take part in the BraMat sub-cohort study within the Norwegian Mother and Child Cohort Study (MoBa) and the NewGeneris study. We thank the technicians Bodil Hasseltvedt, Else-Carin Groeng, Astri Grestad, Berit Arvesen Stensby, Åse Eikeset, Azemira Sabaredzovic, Anne-Cathrine Kristoffersen, and Dr Kirsti Vainio at the Norwegian Institute of Public Health for their assistance in blood processing and serological analyses. We are also grateful to the staff at the maternity wards and laboratories at Oslo and Akershus University Hospitals for their contribution to the recruitment of participants and assistance in blood sampling and processing. Finally we are grateful to Dr Anne Lise Brantsæter at the Norwegian Institute of Public Health and MS Petter Mowinckel at Oslo University Hospital for assistance with the statistical analyses. This work was supported by the EU Integrated Project NewGeneris, 6th Framework Programme, Priority 5: Food Quality and Safety (FOOD-CT-2005-016320) and the Norwegian Institute of Public Health.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

3M Company. 2000. *Phase-out plan for PFOS-based products*. St. Paul, MN: U.S. Environmental Protection Agency Public Docket AR226-0588 U.S. EPA.

Apelberg, B. J., Witter, F. R., Herbstman, J. B., 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.

Brieger, A., Bienefeld, N., Hasan, R., et al. 2011. Impact of perfluorooctanesulfonate and perfluorooctanoic acid on human peripheral leukocytes. *Toxicol. In Vitro* 25:960–968.

C8 Project Panel Study. 2012. Probable link evaluation of infectious diseases. Available from: http://www.c8sciencepanel.org/prob_link.html [Last accessed 1 Oct 2012].

Corsini, E., Sangiovanni, E., Avogadro, A., et al. 2012. *In vitro* characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). *Toxicol. Appl. Pharmacol.* 258:248–255.

DeWitt, J. C., Peden-Adams, M. M., Keller, J. M., and Germolec, D. R. 2012. Immunotoxicity of perfluorinated compounds: Recent developments. *Toxicol. Pathol.* 40:300–311.

Dong, G. H., Zhang, Y. H., Zheng, L., et al. 2009. Chronic effects of perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/6 mice. *Arch. Toxicol.* 83:805–815.

European Union. 2006. Directive 2006/122/ECOF the European parliament and of the council of 12 December 2006 amending for the 30th time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (perfluorooctane sulfonates). Available form: http://eur-lex.europa.eu/LexUriServ/ LexUriServ.do?uri=CELEX:32006L0122:en:NOT [Last accessed 22 Oct 2012].

Fei, C., McLaughlin, J. K., Lipworth, L., and Olsen, J. 2010. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. *Environ. Res.* 110:773–777.

Fromme, H., Tittlemier, S. A., Volkel, W., et al. 2009. Perfluorinated compounds: Exposure assessment for the general population in Western countries. *Int. J. Hyg. Environ. Health* 212:239–270.

Glynn, A., Berger, U., Bignert, A., et al. 2012. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: Serial sampling during pregnancy and nursing, and temporal trends 1996–2010. *Environ. Sci. Technol.* 46:9071–9079.

Grandjean, P., Andersen, E. W., Budtz-Jorgensen, E., et al. 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA* 307:391–397.

Guruge, K. S., Hikono, H., Shimada, N., et al. 2009. Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female $B_6C_3F_1$ mice. *J. Toxicol. Sci.* 34:687–691.

Gutzkow, K. B., Haug, L. S., Thomsen, C., et al. 2012. Placental transfer of perfluorinated compounds is selective: A Norwegian Mother and Child sub-cohort study. *Int. J. Hyg. Environ. Health* 215:216–219.

Haug, L. S., Huber, S., Becher, G., and Thomsen, C. 2011. Characterisation of human exposure pathways to perfluorinated compounds: Comparing exposure estimates with biomarkers of exposure. *Environ. Int.* 37:687–693.

Haug, L. S., Salihovic, S., Jogsten, I. E., et al. 2010. Levels in food and beverages and daily intake of perfluorinated compounds in Norway. *Chemosphere* 80:1137–1143.

Haug, L. S., Thomsen, C., and Becher, G. 2009a. A sensitive method for determination of a broad range of perfluorinated compounds in serum suitable for large-scale human biomonitoring. *J. Chromatogr. A* 1216:385–393.

Haug, L. S., Thomsen, C., and Becher, G. 2009b. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. *Environ. Sci. Technol.* 43:2131–2136.

Holsapple, M. P., Paustenbach, D. J., Charnley, G., et al. 2004. Symposium summary: Children's health risk–what's so special about the developing immune system? *Toxicol. Appl. Pharmacol.* 199:61–70.

Houde, M., Martin, J. W., Letcher, R. J., et al. 2006. Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* 40:3463–3473.

Kato, K., Wong, L. Y., Jia, L. T., et al. 2011. Trends in exposure to polyfluoroalkyl chemicals in the U.S. Population: 1999-2008. *Environ. Sci. Technol.* 45:8037–8045.

Keil, D. E., Mehlmann, T., Butterworth, L., and Peden-Adams, M. M. 2008. Gestational exposure to perfluorooctane sulfonate suppresses immune function in $B_6C_3F_1$ mice. *Toxicol. Sci.* 103:77–85.

Lau, C., Anitole, K., Hodes, C., et al. 2007. Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicol. Sci.* 99:366–394.

Luster, M. I., Johnson, V. J., Yucesoy, B., and Simeonova, P. P. 2005. Biomarkers to assess potential developmental immunotoxicity in children. *Toxicol. Appl. Pharmacol.* 206:229–236.

Magnus, P., Irgens, L. M., Haug, K., et al. 2006. Cohort profile: The Norwegian Mother and Child Cohort Study (MoBa). *Int. J. Epidemiol.* 35:1146–1150.

Nilsen, R. M., Vollset, S. E., Gjessing, H. K., et al. 2009. Self-selection and bias in a large prospective pregnancy cohort in Norway. *Pediatr. Perinat. Epidemiol.* 23:597–608.

Okada, E., Sasaki, S., Saijo, Y., et al. 2012. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. *Environ. Res.* 112:118–125.

Peden-Adams, M. M., Keller, J. M., Eudaly, J. G., et al. 2008. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicol. Sci.* 104:144–154.

Stockholm Convention on Persistent Organic Pollutants (POPs). 2009. Available form: http://chm.pops.int/Convention/Media/Publications/tabid/506/language/en-US/Default.aspx [Last accessed 22 Oct 2012].

Stølevik, S. B., Nygaard, U. C., Namork, E., et al. 2011. Prenatal exposure to polychlorinated biphenyls and dioxins is associated with increased risk of wheeze and infections in infants. *Food Chem. Toxicol.* 49:1843–1848.

Stølevik, S. B., Nygaard, U. C., Namork, E., et al. 2013. Prenatal exposure to polychlorinated biphenyls and dioxins from the maternal diet may be associated with immunosuppressive effects that persist into early childhood. *Food Chem. Toxicol.* 51:165–172.

Thomsen, C., Haug, L. S., Stigum, H., et al. 2010. Changes in concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated biphenyls in Norwegian breast-milk during twelve months of lactation. *Environ. Sci. Technol.* 44:9550–9556.

United States Environmental Protection Agency (US EPA). 2006. *2010/1015 PFOA Stewardship Program.* Available form: http://www.epa.gov/oppt/pfoa/pubs/stewardship [Last accessed 22 Oct 2012].

van Loveren, H., and Piersma, A. 2004. Immunotoxicological consequences of perinatal chemical exposures. *Toxicol. Lett.* 149:141–145.

van Loveren, H., van Amsterdam, J. G., Vandebriel, R. J., et al. 2001. Vaccine-induced antibody responses as parameters of influence of endogenous and environmental factors. *Environ. Health Perspect.* 109:757–764.

Vestergren, R., and Cousins, I. T. 2009. Tracking the pathways of human exposure to perfluorocarboxylates. *Environ. Sci. Technol.* 43:5565–5575.

Wang, I. J., Hsieh, W. S., Chen, C. Y., et al. 2011. The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. *Environ. Res.* 111:785–791.

WHO. 2012. *Guidance for Immunotoxicity Risk Assessment for Chemicals.* Geneva: World Health Organization. IPCS harmonization project document; No. 10. ISBN 978 92 4150 330 3.

Zheng, L., Dong, G. H., Jin, Y. H., and He, Q. C. 2009. Immunotoxic changes associated with a 7-day oral exposure to perfluorooctanesulfonate (PFOS) in adult male C57BL/6 mice. *Arch. Toxicol.* 83:679–689.

DOI: 10.3109/1547691X.2012.755580

Table 1. Demographics of the subjects included in the study.

	0–3 years merged	3rd year	3rd year with blood samples
Health outcomes			
Common cold (yes), *n* (%)	94 (98.9)	70 (82.4)	45 (83.3)
No. of episodes of common cold, mean ± SD	10.9 ± 6.4	3.0 ± 3.1	3.2 ± 3.5
Gastroenteritis (yes), *n* (%)	87 (93.5)	64 (76.2)	47 (83.9)
No. of episodes of gastroenteritis, mean ± SD	3.1 ± 2.3	1.3 ± 1.1	1.37 ± 1.1
Otitis media (yes), *n* (%)	27 (37.0)	16 (18.8)	10 (17.9)
Doctor diagnosed asthma (yes), *n* (%)[a]	11 (14.5)		
Doctor diagnosed atopic eczema (yes), *n* (%)[a]	14 (18.4)		
Eczema/Itchiness (yes), *n* (%)	32 (45.7)	21 (25.9)	16 (30.2)
Wheeze (yes), *n* (%)	29 (38.2)	18 (21.2)	11 (19.6)
Child			
Gender (boy), *n* (%)	42 (55.3)	48 (56.5)	24 (42.9)
Birth weight (g), mean ± SD	3584 ± 441	3576 ± 440	3565 ± 476
Breast-feeding (months), mean ± SD	11.9 ± 5.1	12.8 ± 5.1	12.6 ± 5.0
Start of day-care center (months), mean ± SD	13.1 ± 3.8	13.2 ± 3.8	12.9 ± 4.0
Family			
Parity (≥ 1), *n* (%)	38 (50.0)	41 (48.2)	25 (44.6)
Older siblings (yes), *n* (%)	32 (50.8)	35 (49.3)	22 (39.3)
Previous breast-feeding (months), mean ± SD	6.6 ± 9.0	6.4 ± 8.8	5.7 ± 8.1
Maternal asthma (yes), *n* (%)	10 (13.2)	11 (12.9)	7 (12.5)
Maternal allergy (yes), *n* (%)	36 (49.3)	39 (47.6)	27 (50.0)
Paternal asthma (yes), *n* (%)	5 (6.3)	8 (9.4)	4 (7.1)
Paternal allergy (yes), *n* (%)	19 (26.4)	22 (27.2)	11 (20.8)[b]
Maternal education (years), *n* (%)			
<13	18 (24.7)	21 (25.6)	7 (13.2)
13–16	32 (43.8)	34 (41.5)	27 (50.9)
>16	23 (31.5)	27 (32.9)	19 (35.8)
Gross income of household (>700.000 NOK), *n* (%)	49 (67.1)	55 (67.1)	38 (70.4)

NOK. Norwegian kroner.
[a]Due to the low number of cases, only data from all 3 years merged are included in the statistical analyses.
[b]Statistically significant differences ($p \leq 0.05$) between participants with or without blood samples collected at 3 years of age.

and gastroenteritis), otherwise logistic or linear regression analyses were applied on binary and continuous health outcomes, respectively. Multivariate regression analyses were performed when the *p*-value was ≤0.1 in bivariate analyses between the different health outcomes and the concentration of PFAS.

Potential confounding variables were extracted from BraMat and MoBa questionnaires (~15th and 30th week of gestation and ~6 months after delivery; Version 5 of the quality-controlled data files) and the Medical Birth Registry of Norway. The criterion for inclusion of potential confounding factors in the multivariate regression analysis was $p \leq 0.25$ in the bivariate analysis between the confounding variable in question and both PFAS concentrations and health outcomes. Variables found to be potential confounders were older siblings, previous breastfeeding (number of months with breastfeeding of older siblings), maternal and paternal allergy, paternal asthma, maternal education, gross income of the household, birth season, child's gender, and/or child's age at the 3-year follow-up (for vaccination responses and allergen-specific IgE only). Older siblings and previous breast-feeding were highly correlated ($r = 0.82$). Therefore, in analyses where >1 of these variables were shown to be potential confounders, only previous breast-feeding was included in the multivariate model. Additional variables initially evaluated for confounding effects were: APGAR score after 1 min, passive smoking for the child, child's age when starting at a day-care center, breastfeeding of the index child, maternal asthma, maternal body mass index (BMI; before pregnancy), maternal age at the time of delivery, maternal passive smoking during pregnancy, and maternal smoking during the last 3 months before pregnancy.

For all regression models, a manual backward deletion method was used, starting with all included variables in the model. At each deletion step, the least significant confounding variable was manually removed until only statistically significant ($p \leq 0.05$) variables remained in the model. For logistic regression analyses, Hosmer-Lemeshow test, Cook's D, and residuals were used to investigate the robustness of the models, whereas for Poisson and linear regression analyses, Cook's D, and residuals were used.

Statistical analyses were performed using the statistical software PASW Statistics 17 (SPSS Inc., Chicago, IL).

Results

Questionnaires and demography

Of the 99 BraMat participants, questionnaires for all 3 years were received from 76 (76.8%) participants, whereas 93 (93.9%), 89 (89.9%), and 85 (85.9%) questionnaires were received at the 1-, 2-, and 3-year follow-up, respectively. Demographic data for health outcomes and potential confounding variables are shown in Table 1. With regard to participants with or without blood samples at 3 years of age, there was a statistically significant difference only for the confounding variable paternal allergy.

PFAS concentrations in maternal plasma

Table 2 shows the concentrations of PFNA, PFOA, PFHxS, and PFOS in maternal plasma collected at time of delivery. For PFNA and PFHxS, one and six samples were below LOQ, respectively. The correlation coefficients for the four PFAS ranged from 0.26–0.60 ($p \leq 0.011$).