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Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1–4 years among 359 children in the Odense Child Cohort

Louise Dalsager [a,b,*], Nikolas Christensen [c,d], Steffen Husby [c], Henriette Kyhl [c,e], Flemming Nielsen [a], Arne Høst [c], Philippe Grandjean [a,f], Tina Kold Jensen [a,b]

[a] Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Odense, J.B. Winsløwsvej 17A, 5000 Odense, Denmark
[b] Rigshospitalet, Copenhagen University Hospital, Department of Growth and Reproduction, Blegdamsvej 9, 2100 Copenhagen, Denmark
[c] Hans Christian Andersen Children's Hospital, Odense University Hospital, Sdr. Boulevard 29, 5000 Odense C, Denmark
[d] Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
[e] Odense Patient data Exploratory Network (OPEN), Odense, Denmark
[f] Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA

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ABSTRACT

Introduction: Perfluorinated alkylated substances (PFAS) are persistent industrial chemicals that have resulted in global environmental exposures. Previous epidemiological studies have reported possible effects on the immune system after developmental PFAS exposure, but the possible impact on childhood infectious disease is unclear.
Objectives: To investigate the association between prenatal exposure to PFAS and symptoms of infections at age 1–4 years.
Methods: The Odense Child Cohort is an on-going prospective study on children's health, where serum concentrations of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorodecanoic acid (PFDA) and perfluorononanoic acid (PFNA) were measured in 649 pregnant women before gestational week 16. Of these women, 359 reported on symptoms of infection in their child every two weeks for a one-year period. The association between prenatal exposure to PFAS and the symptoms was estimated using a logistic regression model and a negative binomial regression model. For the latter, the outcome was reported as an incidence rate-ratio (IRR), and all models were adjusted for maternal age, educational level, parity and child age.
Results: On average, the children experienced symptoms of infection 23% of the time during one year. PFOS exposure in the high tertile compared to the low tertile was associated with a statistically significant increased proportion of days with fever (IRR: 1.65 (95% CI: 1.24, 2.18), P-trend < 0.001) and an increased odds of experiencing days with fever above the median (OR: 2.35 (95% CI: 1.31, 4.11). The latter tendency was also apparent for PFOA (OR: 1.97 (95% CI: 1.07, 3.62). Further, higher concentrations of PFOS and PFOA tended to increase the number of episodes of co-occurrence of fever and coughing and fever and nasal discharge during the one-year study period.
Conclusion: We found a positive association between prenatal exposure to PFOS and PFOA and the prevalence of fever, which may be a sensitive marker of infection. This finding is in agreement with an immunotoxic effect of prenatal exposure to PFAS. The wider implications for childhood infectious disease deserve attention.

1. Introduction

Perfluorinated compounds (PFASs) are highly persistent industrial chemicals that have resulted in global environmental dissemination (Lau et al., 2007). The most common types are PFOS (perfluorooctane sulfonic acid), and PFOA (perfluorooctanoic acid), which are ubiquitously present in human serum, where they show an estimated elimination half-life of four years for PFOA and five years for PFOS (Calafat et al., 2007; Kato et al., 2011; Olsen et al., 2007). They cross the placenta (Needham et al., 2011) and are released through human milk (Mogensen et al., 2015), thereby causing early-life exposure that may be worthy of concern. Due to changes in production, serum concentrations of some PFASs have decreased, although others have increased, including perfluorohexane sulfonic acid (PFHxS,) perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) (Glynn et al., 2012).

* Corresponding author at: Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Winsløwsparken 17, 5000 Odense, Denmark.
E-mail address: ldalsager@health.sdu.dk (L. Dalsager).

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Among potential toxic effects, developmental PFAS exposures have been linked to lower efficiency of routine childhood immunizations (Grandjean et al., 2012; Granum et al., 2013). Increased prevalence of self-reported common cold and gastroenteritis was found during the first three years of life in children with elevated prenatal exposure to PFOA, PFNA and PFHxS *in utero* (Granum et al., 2013). However, among 1400 members of the Danish Birth Cohort, hospitalization for infectious diseases such as appendicitis or pneumonia was not associated with maternal PFOA and PFOS concentrations in serum during the first eleven years of life (Fei et al., 2010). On the other hand, a recent report showed that the PFAS analyses in this study might have been imprecise (Bach et al., 2015), thus limiting the validity of this finding. A key concern in these studies is how infection is defined and reported. In children, fever is an almost universal sign of infection, and other causes of elevated body temperature are rare in temperate countries (Sullivan and Farrar, 2011).

The aim of this study was therefore to investigate the association between pregnancy serum concentrations of PFOS, PFOA, PFDA, PFNA and PFHxS and fever and other symptoms of infections during a one-year period among children aged 1–4 years.

2. Methods

2.1. Study participants and data collection

The participants were derived from the Odense Child Cohort. Briefly, all women living in the municipality of Odense who were pregnant between January 1st 2010 and December 31st 2012 were invited to participate (a total of 6707). They were recruited either at a voluntary meeting about ultrasound examinations, at their first antenatal visit, or at the ultrasound examination at Odense University Hospital between gestational age (GA) 10–16 weeks (Kyhl et al., 2015). Of the eligible women, 4017 were informed and 2874 (43%) agreed to participate. Today 2446 singleton children are still being followed. At the time of inclusion, a blood sample was drawn and the participants filled out a questionnaire on general health, lifestyle and social factors. Questionnaires focusing on the child's health and well-being were completed at age three months, 18 months and 3 years, and together with the birth records, these were the sources of data on co-variables. Information on maternal age and educational level was obtained from the questionnaire completed at the time of inclusion, while information on parity, smoking and child sex was derived from the birth record. Educational level was missing in the questionnaires for 119 women and the information was then retrieved from the obstetric records. Duration of breastfeeding and day-care attendance were reported in the questionnaire at age 18 months.

2.2. PFAS measurements

From the serum samples collected, a subset of 649 samples was analyzed for PFAS. Of these, 200 were selected randomly while the remaining 449 were selected based on the availability of information from questionnaires, birth records, and a clinical examination of the child at three months of age. The blood sample preparation and storage were uniform, as appropriate for PFAS analysis (Kato et al., 2013). After centrifugation, serum was pipetted into polypropylene cryo tubes and immediately frozen to −80 °C and stored up to three years until analysis. As is standard in this field, we specifically avoided using sampling or storage tubes containing fluoropolymers (such as Teflon-coated materials) that could be a source of PFAS contamination (Berger et al., 2011). Although sample contamination or adsorption is always a possibility, our repeated analyses of serum samples over time as well as analyses of duplicate samples have shown excellent precision and have never revealed any indication of variance attributable to sampling and storage (Jensen et al., 2015). The serum-PFAS concentrations were analyzed using on-line solid phase extraction followed by liquid chromatography and triple quadropole mass spectrometry (LC–MS/MS) at Environmental Medicine, University of Southern Denmark. The quantified PFASs include perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA). For all compounds, the Limit of Quantification (LOQ) was 0.03 ng/mL. Results from the first 200 samples as well as more detailed information of PFAS analyses have been previously published (Vorkamp et al., 2014).

2.3. Symptoms of infection in children

In June 2014, 1647 of the 2547 families, who were enrolled and active in the Odense Child Cohort at that time, were invited to participate in a study of childhood infections in which symptoms of infection had to be reported by text messages every second week (26 times) during one year. At the time of invitation the children were between 1.0 and 3.3 years old. It was not possible to invite all 2547 due to a clerical error in the informed consent materials. Of the families invited, a total of 1540 (93%) accepted to participate.

Symptoms of infection were assessed using mobile-phone questionnaires (SMS-Track Aps, Esbjerg, Denmark). The participants received a text message every second Sunday and were asked to evaluate the occurrence of 11 symptoms during the previous two weeks. The parents reported every symptom with a value between zero and 14 reflecting the number of days the symptom had been present within the two-week period. Data on the following symptoms were collected: days without symptoms, fever, stuffed or runny nose, cough, wheezy or whistling breathing, eye inflammation, ear pain, discharge from ear, feeling unwell, diarrhea, blood in stool, and vomiting. All parents were provided with written information on when and how to report the symptoms. In the present study, we focused on fever, cough, nasal discharge, diarrhea and vomiting. Fever was considered the most relevant outcome for assessing infection, but the other symptoms were included as likely symptoms of common cold and gastroenteritis, which have previously been found to be associated with three of the five PFASs investigated here (Granum et al., 2013). Participants were instructed that a rectal temperature above 38.5 °C was required to be certain about the presence of fever.

The study was performed in accordance with the second Helsinki Declaration and approved by the regional Ethical Review Committee (Project ID S-20,090,130). All participants received written and oral information and gave their written consent.

2.4. Statistical analysis

Due to a non-normal distribution of the serum-PFAS concentrations, these were log-transformed and reported as medians. The PFAS concentrations were examined in regard to relevant characteristics of the participants, and *t*-tests and F-tests were conducted. Characteristics of participants included in the analysis were compared to those who dropped out using a *t*-test for continuous variables and a Chi^2 test for categorical variables.

The reported number of days with each symptom was summarized for all 26 periods to create a mean for the whole year. Since not all participants answered the 26 text messages and therefore did not report the presence of symptoms for all days during the whole year, the time at risk differed. This was taken into account by calculating the proportion of days with a given symptom out of the total number of days for which information on symptoms were provided. Further, to quantify the extent of the missing replies to the text messages, the mean reply rate for the 26 periods was calculated.

The associations between the PFAS exposures and the symptoms of infection were assessed using regression models, and the outcomes were analyzed both as dichotomous and ordinal data. First, the number of days with a symptom was transformed into a binary variable reflecting whether or not a child's proportion of days with a symptom