Association between perfluoroalkyl substance exposure and asthma and allergic disease in children as modified by MMR vaccination

Association Between Perfluoroalkyl Substance Exposure and Asthma and Allergic Disease in Children as Modified by MMR Vaccination

[a]Clara Amalie Gade Timmermann, [b]Esben Budtz-Jørgensen, [a]Tina Kold Jensen, [c]Christa Elyse Osuna, [d]Maria Skaalum Petersen, [d]Ulrike Steuerwald, [a]Flemming Nielsen, [e]Lars K Poulsen, [df]Pál Weihe, and [ag]Philippe Grandjean

[a]Department of Environmental Medicine, University of Southern Denmark, Odense, Denmark; [b]Section of Biostatistics, University of Copenhagen, Copenhagen, Denmark; [c]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; [d]Department of Occupational Medicine and Public Health, Faroe Islands, Denmark; [e]Allergy Clinic, Copenhagen University Hospital Gentofte, Hellerup, Denmark; and, [f]Faculty of Natural and Health Sciences, University of the Faroe Islands, Faroe Islands, Denmark; [g]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA

Please address all correspondence to*:* Clara Amalie Gade Timmermann, Department of Environmental Medicine, University of Southern Denmark, J.B. Winsløwsvej 17A, 5000 Odense C, Denmark. (T) 4565503742 (E) atimmermann@health.sdu.dk

Running title: PFAS exposure - asthma and allergy - children – MMR

Keywords: Allergy, asthma, children, MMR vaccination, perfluoroalkyl substances

Abstract

Perfluoroalkyl substances (PFAS) are highly persistent chemicals that might be associated with asthma and allergy, but the associations remain unclear. Therefore, this study examined whether pre- and post-natal PFAS exposure was associated with childhood asthma and allergy. Measles, mumps and rubella (MMR) vaccination in early life may have a protective effect against asthma and allergy and is therefore taken into account when evaluating these associations. In a cohort of Faroese children whose mothers were recruited during pregnancy, serum concentrations of five PFAS were measured: Perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) at three timepoints (maternal serum in pregnancy week 34-36 and child serum at ages 5 and 13 years) and determined their association with immunoglobulin E (IgE) (cord blood and at age 7 years) and asthma/allergic diseases (questionnaires at ages 5 and 13 years and skin prick test at age 13 years). A total of 559 children were included in the analyses. Interactions with MMR vaccination were evaluated. Among 22 MMR-unvaccinated children, higher levels of the five PFAS at age 5 years were associated with increased odds of asthma at ages 5 and 13. The associations were reversed among MMR-vaccinated children. Pre-natal PFAS exposure was not associated with childhood asthma or allergic diseases regardless of MMR vaccination status. In conclusion, PFAS exposure at age 5 was associated with increased risk of asthma among a small subgroup of MMR-unvaccinated children but not among MMR-vaccinated children. While PFAS exposure may impact immune system functions, this study suggests that MMR vaccination might be a potential effect-modifier.

Introduction

Perfluoroalkyl substances (PFAS) constitute a group of highly persistent chemicals used in a variety of products including furniture, carpets, clothing, food packaging, and firefighting foams (Fromme et al. 2009). PFAS are found in human serum worldwide (Houde et al. 2006), and children appear to have a greater exposure than teenagers and adults (Trudel et al. 2008; Kato et al. 2009). Recently, higher serum-PFAS concentrations in childhood have been associated with increased odds of concurrent asthma (Dong et al. 2013), perhaps due to a shift towards a T-helper type 2 cell (T_H2) immune response (Dong et al. 2011) as seen in allergic asthma and allergic diseases. However, other studies could not replicate this association (Humblet et al. 2014; Stein et al. 2016). Reported associations between pre-natal PFAS exposure and asthma, wheezing, and other allergic diseases in children also have not been consistent (Wang et al. 2011; Okada et al. 2012, 2014; Granum et al. 2013; Smit et al. 2015).

Furthermore, Measles, mumps and rubella (MMR) vaccination in early life may have a protective effect against asthma or asthma symptoms (Gruber et al. 2003; Roost et al. 2004), an association that was previously substantiated in our analyses (Timmermann et al. 2015). The protective effect is possibly due to the elicitation of a T_H1-biased response (Pabst et al. 1997; Ovsyannikova et al. 2003), and it is possible that a T_H1-biased response induced by MMR vaccination could suppress a PFAS induced T_H2 immune response thus minimizing the effect of PFAS on asthma among MMR-vaccinated children.

The objective of the present study was therefore to investigate whether pre- or postnatal PFAS exposure was associated with asthma and allergic diseases in a birth cohort while considering the potential effectmodification by MMR-vaccination.

Materials and Methods

Sample population

Data were collected as part of the Children's Health and Environment in the Faroe Islands (CHEF) study (Grandjean et al. 2010; Heilmann et al. 2010; Timmermann et al. 2015). The birth cohort was formed in 1997-2000, and a blood sample for measuring environmental exposures was obtained when the women were included in gestational week 34-36. Obstetric information was recorded, and a cord blood sample was obtained at birth. At ages 5, 7, and 13 years, children were invited for physical examinations (including blood sampling) and parents filled out a questionnaire together with a research nurse.

MMR vaccinations were routinely administered at 15 mo-of-age; vaccination dates were obtained from the child's vaccination card at age 5. There were no specific contraindications. Children were considered eligible for this study if they attended the age-5 examination. They were excluded if they had had measles or if no information was provided about this disease (Timmermann et al. 2015).

Written informed consent was obtained from the parents of all children included in the study. The study was carried out in accordance with the Helsinki Declaration and was approved by the ethical review committee serving the Faroe Islands and the U.S. (Harvard) institutional review board (IRB).

Asthma and allergy outcomes

Total Immunoglobulin E (IgE) and A (IgA) concentrations were quantified in cord blood by ImmuniCAP and in-house ELISA. Analyses have been described in more detail elsewhere (Timmermann et al. 2015). IgE measures from samples with IgA concentrations > 50 μg/ml were disregarded due to possible contamination with maternal blood (Bonnelykke et al. 2010).

At age 5, parents were asked whether the child had been diagnosed with or was suspected to suffer from asthma and whether the child had been diagnosed with hypersensitivity or allergy. At age 7, a blood sample was drawn from which total IgE was quantified. At age 13, parents were asked whether the child had ever suffered from asthma. Rhinoconjunctivitis symptoms (in past 12 months) and eczema (ever) were also identified through the parental questionnaire using questions from the International Study of Asthma and Allergies in Childhood (ISAAC) as previously described (Timmermann et al. 2015). The children underwent a skin prick test (SPT) with extracts (Soluprick, ALK, Hørsholm, Denmark) of five common allergens (birch/grass pollen, dog/cat dander, and house dust mite [*Dermatophagoides pteronyssinus*]) at age 13. A wheal size ≥ 3 mm in diameter resulting from any of the five allergens was considered positive.

PFAS analysis

Perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluoro-octanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) were measured in maternal pregnancy serum and child serum at ages 5 and 13 (Grandjean et al. 2012). Analyses were carried out by isotope dilution and online solid-phase extraction followed by high performance liquid chromatography with triple quadropole mass-spectrometric analysis according to the methods of Haug et al. (2009). PFOS was quantified by integration of two adjacent peaks, which represent the branched isomers and the linear isomer. The between batch-imprecision was < 7.7% and the limit of detection (LOD) for the five PFAS was 0.03 ng/ml. All five PFAS were found at concentrations above the LOD in all maternal and child serum samples.

Potential confounders

Information about variables known to be associated with asthma and/or allergic diseases (Bodner et al. 1998; Reichman and Nepomnyaschy 2008; Just et al. 2010; Nagel et al. 2010;

Civelek et al. 2011; Herr et al. 2012; Chang et al. 2013; de Vries et al. 2014; Dogaru et al. 2014; Mu et al. 2014; Netting et al. 2014) was obtained from the recruiting midwife's chart (maternal pre-pregnancy BMI (weight/hight^2), maternal smoking during pregnancy (none/any), premature birth (< 37 weeks / ≥ 37 weeks), birth season (spring: March-May; summer: June-August; fall: September-November; winter: December-February), sex, birth weight and parity (zero/one/more than one previous birth), the parental questionnaires at age 5 (total duration of breastfeeding = exclusive *and* partial breastfeeding in months, number of siblings, parental smoking at home [yes/no], weekly fish dinners, and daycare attendance [yes/no]) and age 13 (fish dinners, domestic pets, and family history of asthma and allergic diseases [no/from one parent's side/from both parents' sides]).

Statistics

Among children included in this study, all missing values were imputed using multiple imputation by chained equations with 40 imputations based on all exposures, outcomes, and potential confounders, as well as three auxiliary variables (Azur et al. 2011), i.e., information about the father's primary education (7^{th}-8^{th} Grade/9^{th}-10^{th} Grade), whether the child had lived abroad between ages 7 and 13 (yes/no), and whether the child is allergic to anything (yes/no or do not know). IgE and PFAS concentrations were right skewed and therefore were $\log_{10}$–transformed to avoid violating model assumptions when performing imputations and conducting association analyses.

Each interaction between MMR vaccination and PFAS concentration measures were tested in relation to all asthma and allergic disease measures (except cord blood IgE, which could not have been affected by subsequent MMR vaccination) in marginal analyses using the unimputed data. Interactions considered to be consistent (interactions with $p < 0.2$ in the same

direction for at least three out of five PFAS measures) were included in the imputation of the asthma and allergic disease measures on which they were found to interact.

All imputations were performed using the mi impute chained command in Stata version 14.0 (StataCorp, College Station, TX). The imputation models are described in further detail in Appendix A. Using the imputed data, associations between serum concentrations of each PFAS and asthma and allergic diseases at ages 5 and 13 were determined in logistic regression models, and associations between each PFAS and total IgE in cord blood and at age 7 were determined in linear regression models. If interactions were identified in the marginal analyses using the unimputed data, an interaction term for PFAS exposure and MMR vaccination was included in the model, and potential confounders were included if associated with the PFAS measures (Appendix B). When investigating interactions, information about birth weight and family history of chronic bronchitis/asthma was also included in the models because these factors are associated with MMR vaccination uptake in the Faroese cohort and so might confound the association between MMR vaccination and asthma/allergic diseases (Timmermann et al. 2015). Since both PFAS concentrations and IgE measures were log-transformed, the estimates of association were converted to express the percent change in IgE associated with a doubled serum-PFAS concentration in the linear regression models and the odds ratio with a doubling of the PFAS exposure in the logistic regression models.

Sensitivity analyses were also performed in which analyses were conducted using the unimputed data and information about maternal education (none/any education above primary school), maternal pregnancy serum dichlorodiphenyldichloroethylene (DDE), and the sum of maternal pregnancy serum polychlorinated biphenyl (PCB) concentrations were included one at a time. A simplified sumPCB concentration was calculated as the sum of congeners CB-138, CB-153, and

CB-180 multiplied by 2. Lastly, subgroup analyses for atopic and non-atopic asthma was performed and separately compared each group to children with no asthma. At age 5, atopic asthma was classified as having both asthma and allergy (41% of asthma cases), and at age 13, atopic asthma was classified as having both asthma and positive SPT (59% of asthma cases). In these analyses only children with complete information about both asthma and allergy/SPT were included. All analyses were performed in Stata version 14.0.

Results

Informed consent was obtained from 648 mothers of whom eight had twins, thus leaving 640 singleton children. Among these, 59 children were not seen at age 5 and 22 were excluded due to having a history of measles infection (n = 7) or not having provided information about measles infection (n = 15) (Timmermann et al. 2015), leaving 559 children (87%) eligible for the study (Figure 1). The 81 (59 + 22) ineligible children had mothers with lower parity. Further more, among the ineligible children, mothers of 76 children had provided a blood sample in which PFAS was measured. These 76 mothers had lower serum concentrations of PFDA and PFNA, and higher levels of PFOA. Finally, 31 of the ineligible children attended the 13-year examination. These 31 children more often had asthma at age 13 compared to the 491 (522-31) children who attended the 13-year examination *and* were eligible for the study. No other significant ($p < 0.05$) differences between the eligible and ineligible children were found with regard to outcomes, exposures, or potential confounders.

The distributions of exposures, outcomes, and potential confounders are shown in Table 1. Among the 559 mothers included in this study, 30 did not provide a blood sample for PFAS analysis during pregnancy. Furthermore, 35 children did not provide a blood sample for PFAS analysis at age 5, and 68 children did not participate in the examination at age 13. An additional

six children did not provide a blood sample for PFAS analysis at age 13. These missing values were imputed along with missing information about the outcomes and covariates. In the unimputed data the median PFHxS and PFOS concentrations were highest (4.5 and 27.4 ng/ml) in maternal pregnancy serum and lowest in child serum at age 13, while the medians for PFOA and PFNA were highest in age-5 child serum (4.0 and 1.0 ng/ml); PFDA concentrations were stable across the three timepoints (0.3 ng/ml). At age 5, 14.2% and 13.5% were reported to have asthma and allergy respectively; at age 13, 16.9% had had asthma, 38.7% had a positive SPT, 12.0% had allergic rhinoconjuctivitis, and 20.8% had had eczema. Only 22 out of 555 children had not received the MMR vaccination scheduled for age 15 months before the 5-year examination (Table 1). PFAS distributions including minimum and maximum values for children with and without asthma at ages 5 and 13 years by MMR vaccination status are shown in Appendix C. None of the small subgroups contain extreme values. As previously shown, children without MMR-vaccination had higher risk of asthma and allergic diseases (Timmermann et al. 2015).

In the marginal analyses on the unimputed data, no MMR vaccination interaction with maternal PFAS concentrations was found in relation to any of the outcome measures, but interactions between MMR vaccination and age-5 or age-13 PFAS in relation to some of the asthma and allergic disease measures were observed. Thus, increased serum-PFAS concentrations at age 5 tended to be associated with higher odds of a history of asthma at ages 5 and 13 and allergy at age 5 among the MMR-*un*vaccinated children, and among MMR-vaccinated children, an increase in serum-PFAS concentrations at age 5 tended to be associated with *reduced* odds of history of asthma at ages 5 and 13 and allergy at age 5. Few of these associations were, however, significant; the interaction between MMR vaccination and PFASs at age 5 was significant for PFOA in relation to asthma at ages 5 and 13 and for PFNA and PFDA in relation to asthma at

age 5. The same pattern was found in the association between PFAS concentrations at age 13 and atopic eczema at age 13 (data not shown).

When using the data with imputations and taking potential confounding factors into account, the pattern described above persisted. As shown in Table 2, a doubling of the serum-PFOA concentration at age 5 was significantly associated with 10-fold increased odds of asthma at ages 5 and 13 among the MMR-unvaccinated children. A doubling of the age 5 serum-PFNA concentration was likewise significantly associated with similarly increased odds of asthma at both ages among the MMR-unvaccinated children, and a doubling of the age 5 serum-PFDA concentration showed a significant, though slightly weaker, association with asthma at age 5 among the MMR-unvaccinated children (Table 2). The interaction between MMR vaccination and age-5 PFAS was significant for PFOA, PFNA and PFDA in relation to asthma at ages 5 and 13 and for PFHxS in relation to asthma at age 5 (Table 2). Results were similar when maternal education, pregnancy serum-DDE, or pregnancy serum-PCB concentrations were included in an analysis using the unimputed data (results not shown).

In the adjusted analyses, in which interactions with MMR vaccination were not included (because no interaction was found in the marginal analyses), no clear patterns of association were found. Specifically, no associations were found between PFAS exposure at age 5 and total IgE at age 7, positive SPT at age 13, allergic rhinoconjunctivitis at age 13 or atopic eczema at age 13 (Table 2). Increased maternal serum-PFHxS concentrations were associated with increased odds of atopic eczema at age 13 (Table 3), but given the large number of analyses conducted, the risk of Type I errors is increased, and this one significant association is likely to be a chance finding. No associations were found between maternal PFAS exposure and the other outcome measures (Table 3). Additionally, increased serum-PFHxS and serum-PFNA

concentrations at age 13 were associated with reduced odds of asthma and allergic rhinoconjunctivitis, respsectively (Table 4). However, since no other significant associations were found with PFAS exposure at age 13, these associations could also be chance findings.

When dividing asthma into atopic and non-atopic asthma, the interactions between MMR vaccination and PFAS at age 5 persisted for atopic asthma, whereas no significant interactions or associations were seen for non-atopic asthma (Table 5). Among MMR-*un*vaccinated children, a doubling in the concentration of the PFAS at age 5 was significantly associated with 9-75-fold higher odds of atopic asthma, and a doubling of the PFNA concentration was likewise significantly associated with 7-fold higher odds of atopic asthma at age 13 (Table 5).

Discussion

Serum-PFAS concentrations at age 5 were associated with increased odds of asthma history at ages 5 and 13 in children who had not been MMR-vaccinated, although the numbers were small. The associations were reversed among MMR-vaccinated children. The same tendencies, though not significant, were seen in the association between serum-PFAS at age 5 and history of allergy at age 5 and between serum-PFAS at age 13 and history of atopic eczema at age 13.

A Taiwanese case-control study reported that increased PFAS exposures were associated with concurrent asthma among 10-15-year-olds (Dong et al. 2013), while a cross-sectional study using the U.S. National Health and Nutrition Examination Survey (NHANES) data (1999-2000, 2003-2004, 2005-2006, 2007-2008) of 12-19 year-olds found that serum-PFOA was associated with increased odds of asthma while PFOS showed a negative association (Humblet et al. 2014), and finally a recent study using NHANES data (2005-2006) of 12-19 year-olds did not find any association between PFAS and asthma but found that higher serum-PFOS concentrations were

associated with reduced odds of sensitization to any allergen, while increased PFOA and PFNA were associated with increased serum IgE (Stein et al. 2016). Neither of these studies examined MMR vaccinations or other factors that might contribute to the different findings.

In regard to pre-natal PFAS exposure, cord blood PFOS and PFOA concentrations have been found to correlate positively with cord blood IgE levels in boys (Wang et al. 2011), while high maternal PFOA concentrations were associated with decreased cord blood IgE levels in girls (Okada et al. 2012) and reduced risk of allergic diseases during the first 24 months (Okada et al. 2014). Recent studies found that maternal PFOA concentrations were inversely associated with current wheezing among Ukrainian but not in Greenlandic children aged 5-9 years (Smit et al. 2015) and other types of maternal PFAS were inversely associated with allergic diseases among 4-year-old children (Goudarzi et al. 2016). Several other analyses, however, showed no associations between pre-natal exposure to PFAS and cord blood IgE (Ashley-Martin et al. 2015) or asthma and allergy in childhood (Wang et al. 2011; Okada et al. 2012; Granum et al. 2013), which is in accordance with the current findings.

The mechanisms underlying immunomodulating effects of various PFAS are far from fully understood, but these chemicals have been shown to affect activation of human immune cells and reduce both pro- and anti-inflammatory cytokine production *in vitro* (Corsini et al. 2014). In mice, PFOS exposure has been shown to lead to an increase in interleukin (IL)-4 and IL-10 and to decrease IL-2 and interferon (IFN)-γ, thereby indicating a shift towards a T_H2 immune response (Dong et al. 2011). If such a shift occurs, it could perhaps explain the positive association between serum-PFAS concentrations and asthma among the MMR-unvaccinated children in the current analyses, while the MMR vaccination may rather shift the immune response towards a more T_H1-prone state (Pabst et al. 1997; Ovsyannikova et al. 2003), thereby

preventing or counteracting a PFAS-induced T_H2-shift. If PFAS exposure affects the T_H1/T_H2 balance, an interaction between MMR vaccination and serum-PFAS concentrations thus seems plausible.

If MMR vaccination modifies the effect of PFAS on asthma as suggested by the current findings, differences in vaccination schedules might explain why the association between PFAS exposure and asthma differs between studies from different parts of the world. However, the range of serum-PFAS concentrations is also important. Both the age 5 and 13-year serum-PFAS concentrations of children in this study were lower than among Taiwanese children, except for PFOA, which was higher (Dong et al. 2013), and the age 5 serum-PFAS concentrations were similar to those of American children whereas the age 13 serum-PFAS concentrations were lower in the present study (Humblet et al. 2014). It is possible MMR vaccination can protect against adverse effects of PFAS on asthma only when PFAS concentrations are relatively low.

It is important to note that a main limitation of this study is that very few cohort children were not MMR-vaccinated, which limited the power to detect interactions between MMR vaccination and PFAS concentrations. Therefore, the present hypothesis about MMR vaccination modifying the association between PFAS and asthma needs to be re-examined in populations with a larger number of MMR-unvaccinated children. In addition, this study conducted a large number of analyses, which increased the risk of chance findings. Therefore, it did not look for single significant associations but rather patterns in the associations.

As common in cohort studies, follow-up was not complete, and this study compensated for missing data by multiple imputations. Assuming that data were missing at random, this approach would be expected to produce more correct estimates of the true associations (Azur et al. 2011). However, those cohort members who did not provide information on history of

measles infection had to be excluded. They differed from those included with regard to maternal serum-PFAS concentrations and asthma at age 13, and one cannot rule out the possibility of some selection bias. However, with 87% of the original cohort included in the present study, it is believed that any potential bias was minimal. These analyses showed that some confounding did occur, but only marginally affected the results. However, in observational studies, the possibility of residual confounding can never be excluded.

Imprecision of the asthma measures must also be considered. At age 13, questionnaire data about asthma have been shown to have a high sensitivity and specificity in relation to clinical asthma (Fuso et al. 2000), but this measure is probably less precise at age 5 due to difficulties in distinguishing asthma from bronchitis. Any misclassification is, however, unlikely to be associated with the PFAS exposure or misclassification of PFAS exposure; therefore any bias likely would have been towards null (Kristensen 1992).

Overall, pre-natal PFAS exposure did not seem to affect the risk of childhood asthma or allergy. Much previous research has focused on pre-natal exposure, but both the pre- and post-natal periods are known to be important in establishing an immune deviation from T_H2 to T_H1 skewed immunity (Romagnani 2014), and in this study, associations were found only with post-natal exposure. In addition, it has been established that children are highly exposed to PFAS in early life through breastfeeding (Mogensen et al. 2015), emphasizing the need to further study the effects of post-natal PFAS exposure.

Among the MMR-unvaccinated children, stronger associations were found between PFAS concentrations and asthma than between PFAS and other allergic diseases, and stronger associations were observed for atopic than for non-atopic asthma. This indicated that PFAS

exposure may affect development of allergy development. However, given the small number of unvaccinated children, these findings should be interpreted with caution.

Conclusions

Serum PFAS concentrations at age 5 years were associated with increased odds of asthma at ages 5 and 13 years among MMR-unvaccinated children. The associations were reversed among MMR-vaccinated children. Similar analyses failed to identify any association with pre-natal PFAS exposure. These findings suggest that MMR vaccination should be considered as a potential effect-modifier in studies of adverse effects of PFAS on immune functions, but larger studies are needed to support the current findings.

Acknowledgments

This work was supported by the National Institute of Environmental Health Sciences, NIH (ES012199); the U.S. Environmental Protection Agency (R830758); the Danish Council for Strategic Research (09-063094); and the Danish Environmental Protection Agency as part of the environmental support program DANCEA (Danish Cooperation for Environment in the Arctic). The authors are solely responsible for all results and conclusions, which do not necessarily reflect the position of any of the funding agencies.

Declaration of interest

The authors report no conflict of interests. The authors alone are responsible for the content of this manuscript.

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Figure legend

Figure 1. Flowchart of the experiment.

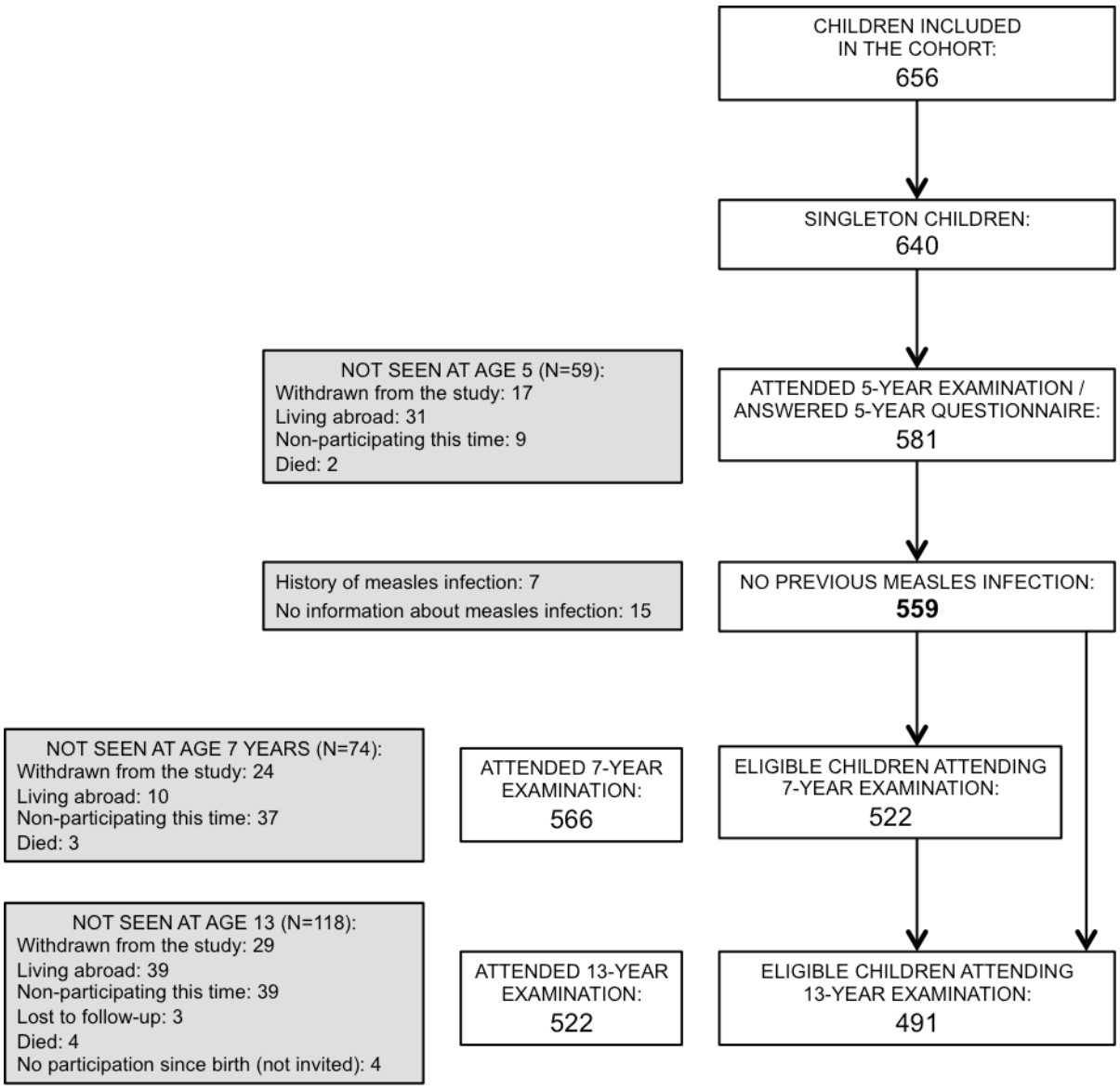

Table 1: Distribution of exposures, outcomes and potential confounders (pre-imputation)

N = 559		Information collected prenatally / at birth		Information collected at age 5 / 7		Information collected at age 13	
Continuous variables		**Median (25^{th}-75^{th} percentile)**	**n missing**	**Median (25^{th}-75^{th} percentile)**	**n missing**	**Median (25^{th}-75^{th} percentile)**	**n missing**
PFHxS (ng/mL)		4.5 (2.2; 8.3)	30	0.6 (0.4; 0.9)	35	0.4 (0.3; 0.5)	74
PFOS (ng/mL)		27.4 (23.3; 33.3)	30	16.8 (13.5; 21.1)	35	6.7 (5.2; 8.5)	74
PFOA (ng/mL)		3.3 (2.5; 4.0)	30	4.0 (3.3; 5.0)	35	2.0 (1.6; 2.5)	74
PFNA (ng/mL)		0.6 (0.5; 0.8)	30	1.0 (0.8; 1.2)	35	0.7 (0.6; 0.9)	74
PFDA (ng/mL)		0.3 (0.2; 0.4)	30	0.3 (0.2; 0.4)	35	0.3 (0.2; 0.4)	74
Total IgE (kU/L)		0.1 (0.0; 0.2)	62	21.8 (9.8; 70)	132	-	
Birth weight (kg)		3.8 (3.4; 4)	0	-		-	
Total months of breastfeeding		-		9 (6; 12)	3	-	
Maternal pre-pregnancy BMI (kg/m^2)		23.1 (21.3; 25.7)	0	-		-	
Categorical variables		**% (n/N)**	**n missing**	**% (n/N)**	**n missing**	**% (n/N)**	**n missing**
Asthma (questionnaire)		-		14.2 (78/550)	9	16.9 (83/491)	68
Allergy (questionnaire)		-		13.5 (74/548)	11	-	
Positive skin prick test		-		-		38.7 (190/491)	68
Allergic Rhinoconjunctivitis (questionnaire)		-		-		12.0 (59/491)	68
Atopic eczema (questionnaire)				-		20.8 (102/491)	68
Sex (girls)		47.2 (265/559)	0	-		-	
Premature birth (<=36 weeks)		1.2 (11/559)	0	-		-	
Birth season	Summer	27.2 (152/559)	0	-		-	
	Fall	24.0 (134/559)					
	Winter	27.6 (154(559)					
Maternal parity	1	33.1 (185/559)	0	-		-	
	>=2	41.3 (231/559)					
Number of siblings	1	-		35.3 (197/558)	1	-	
	2			38.9 (217/558)			
	>=3			19.2 (107/558)			
Received 15-months MMR vaccination		-		96.0 (533/555)	4	-	
Maternal smoking		29.7 (166/559)	0	-		-	
Parental smoking in the home				26.3 (146/556)	3	10 (49/490)	69
Weekly fish dinners	>1 – 2	36.1 (97/269)	290 [a]	40.9 (228/557)	2	43.4 (213/491)	68
	>2	37.2 (100/269)		42.2 (235/557)		15.9 (78/491)	
Use of daycare		-		92.4 (510/552)	7		
Has furry pets		-				54.4 (266/489)	70
Family history of chronic	From one parent					33.2 (163/491)	

bronchitis or asthma	From both parents	-	-	4.7 (23/491)	68
	Do not know			5.3 (26/491) [b]	
Family history of eczema	From one parent			44.0 (216/491)	
in children, allergic	From both parents	-	-	11.2 (55/491)	68
eczema and hay fever	Do not know			6.5 (32/491) [b]	
	From one parent			32.9 (161/490)	
Family history of allergy	From both parents	-	-	7.8 (38/490)	69
	Do not know			11.4 (56/490) [b]	

Only 104 children had complete information i.e. no missing values on outcomes, exposures, or covariates. Compared to children with missing information on one or more of the variables, these 104 children had mothers with lower concentrations of PFHxS (median 2.1 vs. 5.7 ng/mL) and higher concentrations of PFNA (median 0.7 vs. 0.6 ng/mL) tested using Wilcoxon rank-sum test. The 104 children also had lower concentrations of PFOS at age 5 (median 15.2 vs. 17.3 ng/mL) tested using Wilcoxon rank-sum test, and they were more often born during the summer (36 % vs. 25 %) tested using Chi square test. No other significant ($p<0.05$) differences were found.

[a] Information about maternal diet was not collected from the beginning of the study.

[b] "Do not know"-answers were imputed along with the missing values.

- Information was not obtained / not included in this study.

Table 2: Differences in IgE (%) and OR for history of asthma, history of allergic diseases, and SPT associated with a doubling of serum-PFAS concentrations at age 5

N= 559		Age 5				Age 7
Age 5 PFASs	**MMR vaccination before age 5**	**Asthma [a] OR (95% CI)**	**p [i]**	**Allergy [a] OR (95% CI)**	**p [i]**	**IgE % difference (95% CI)**
PFHxS	no	3.57 (0.95; 13.43)	0.03	1.25 (0.41; 3.88)	0.56	-2.88 (-20.47; 18.61)
	yes	0.81 (0.58; 1.14)		0.89 (0.63; 1.25)		
PFOS	no	3.96 (0.55; 28.39)	0.18	6.15 (0.77; 49.23)	0.06	-9.38 (-37.17; 30.71)
	yes	0.98 (0.55; 1.76)		0.80 (0.43; 1.49)		
PFOA	no	10.37 (1.06; 101.93)	0.03	1.73 (0.32; 9.22)	0.21	7.87 (-25.22; 55.61)
	yes	0.76 (0.41; 1.39)		0.55 (0.29; 1.06)		
PFNA	no	12.52 (1.29; 121.67)	0.02	2.40 (0.57; 10.03)	0.11	-5.02 (-27.83; 25.00)
	yes	0.72 (0.44; 1.18)		0.71 (0.43; 1.17)		
PFDA	no	4.04 (1.05; 15.50)	0.02	2.21 (0.69; 7.06)	0.10	-7.59 (-26.58; 16.29)
	yes	0.71 (0.48; 1.06)		0.79 (0.52; 1.19)		

		Age 13				
Age 5 PFASs		**Asthma [a] OR (95% CI)**	**p [i]**	**Positive skin prick test OR (95% CI)**	**Allergic rhinoconjunctivitis** (past 12 months) **OR (95% CI)**	**Atopic eczema OR (95% CI)**
PFHxS	no	2.51 (0.77; 8.16)	0.10	0.95 (0.75; 1.20)	0.80 (0.57; 1.14)	0.92 (0.70; 1.22)
	yes	0.90 (0.63; 1.27)				
PFOS	no	5.41 (0.62; 47.16)	0.12	0.76 (0.49; 1.18)	0.63 (0.33; 1.20)	0.80 (0.46; 1.39)
	yes	0.94 (0.51; 1.74)				
PFOA	no	9.92 (1.06; 93.22)	0.02	1.15 (0.75; 1.77)	1.01 (0.54; 1.89)	0.72 (0.42; 1.25)
	yes	0.65 (0.35; 1.20)				
PFNA	no	6.85 (1.05; 44.69)	0.02	0.79 (0.57; 1.10)	0.63 (0.37; 1.07)	0.79 (0.51; 1.23)
	yes	0.71 (0.44; 1.16)				
PFDA	no	2.87 (0.84; 9.79)	0.03	0.79 (0.59; 1.05)	0.71 (0.47; 1.08)	0.92 (0.64; 1.31)
	yes	0.71 (0.48; 1.06)				

Missing values were imputed using multiple imputation. All analyses were adjusted for family history of eczema in children, allergic eczema and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breastfeeding, fish intake at age 5, number of siblings, and daycare attendance at age 5.

[a] Additionally adjusted for birth weight, and family history of chronic bronchitis/asthma.

[i] p-value for PFAS/MMR vaccination interaction.

Table 3: Differences in IgE (%) and OR for history of asthma, history of allergic diseases, and SPT associated with a doubling of maternal serum-PFAS concentrations

N= 559	**Cord blood**	**Age 5**		**Age 7**
Maternal PFASs	**IgE % difference (95% CI)**	**Asthma OR (95% CI)**	**Allergy OR (95% CI)**	**IgE % difference (95% CI)**
PFHxS	-0.17 (-9.33; 9.93)	0.99 (0.80; 1.22)	1.20 (0.95; 1.53)	6.35 (-6.89; 21.48)
PFOS	-6.28 (-30.29; 25.99)	1.21 (0.64; 2.29)	0.73 (0.38; 1.41)	18.61 (-24.06; 85.24)
PFOA	2.81 (-19.29; 30.93)	1.37 (0.81; 2.32)	0.92 (0.53; 1.57)	-5.15 (-31.92; 32.14)
PFNA	0.94 (-17.67; 23.75)	1.03 (0.67; 1.59)	0.82 (0.52; 1.31)	21.11 (-11.70; 66.12)
PFDA	3.23 (-14.29; 24.35)	1.09 (0.72; 1.65)	0.83 (0.54; 1.27)	24.73 (-5.68; 64.94)

	Age 13			
Maternal PFASs	**Asthma OR (95% CI)**	**Positive skin prick test OR (95% CI)**	**Allergic rhinoconjunctivitis** (past 12 months) **OR (95% CI)**	**Atopic eczema OR (95% CI)**
PFHxS	0.98 (0.79; 1.20)	0.94 (0.79; 1.12)	1.14 (0.88; 1.46)	1.32 (1.08; 1.62)
PFOS	1.61 (0.84; 3.08)	1.25 (0.75; 2.11)	1.05 (0.51; 2.17)	0.75 (0.42; 1.34)
PFOA	1.12 (0.67; 1.88)	1.16 (0.76; 1.77)	1.18 (0.65; 2.15)	1.36 (0.85; 2.19)
PFNA	1.21 (0.77; 1.88)	0.97 (0.69; 1.38)	0.86 (0.52; 1.41)	0.81 (0.55; 1.20)
PFDA	1.26 (0.83; 1.92)	1.02 (0.74; 1.41)	0.88 (0.56; 1.38)	0.92 (0.64; 1.32)

Missing values were imputed using multiple imputation. All analyses were adjusted for maternal parity, family history of eczema in children, allergic eczema and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, maternal fish intake during pregnancy, and duration of breastfeeding.

Table 4: OR for history of asthma, history of allergic diseases, and SPT associated with a doubling of serum-PFAS concentrations at age 13

N= 559		**Age 13**				
Age 13 PFASs	**MMR vaccination before age 5**	**Atopic eczema [a]**		**Asthma**	**Positive skin prick test**	**Allergic rhinoconjunctivitis** (past 12 months)
		OR (95% CI)	**p [i]**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
PFHxS	no	1.27 (0.16; 10.15)	0.66	0.63 (0.41; 0.97)	0.88 (0.64; 1.21)	0.91 (0.58; 1.43)
	yes	0.80 (0.53; 1.20)				
PFOS	no	8.94 (0.27; 299.11)	0.18	0.69 (0.43; 1.09)	0.90 (0.63; 1.29)	0.66 (0.39; 1.11)
	yes	0.82 (0.53; 1.28)				
PFOA	no	4.48 (0.42; 47.69)	0.17	0.93 (0.57; 1.53)	1.03 (0.70; 1.51)	0.69 (0.40; 1.20)
	yes	0.82 (0.49; 1.36)				
PFNA	no	371.98 (0.16; $8.62*10^5$)	0.12	0.81 (0.52; 1.28)	0.82 (0.57; 1.16)	0.55 (0.32; 0.94)
	yes	0.77 (0.49; 1.22)				
PFDA	no	401.88 (0.09; $1.84*10^6$)	0.15	0.84 (0.55; 1.29)	0.81 (0.59; 1.13)	0.68 (0.42; 1.12)
	yes	0.88 (0.58; 1.34)				

Missing values were imputed using multiple imputation. All analyses were adjusted for family history of eczema in children, allergic eczema and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breastfeeding, and fish intake at age 13.

[a] Additionally adjusted for birth weight, and family history of chronic bronchitis/asthma.

[i] p-value for PFAS/MMR vaccination interaction.

Table 5: OR for history of allergic and non-allergic asthma associated with a doubling of serum PFAS concentrations at age 5

		Age 5				Age 13			
Age 5 PFASs	**MMR vaccination before age 5**	**Atopic asthma [a] N=494**		**Non-atopic asthma [b] N=511**		**Atopic asthma [c] N=456**		**Non-atopic asthma [d] N=443**	
		OR (95% CI)	**p [i]**	**OR (95% CI)**	**p [i]**	**OR (95% CI)**	**p [i]**	**OR (95% CI)**	**p [i]**
PFHxS	no	9.30 (1.20; 72.09)	0.02	1.66 (0.32; 8.71)	0.36	1.86 (0.52; 6.69)	0.29	3.31 (0.36; 30.74)	0.26
	yes	0.80 (0.47; 1.38)		0.75 (0.48; 1.17)		0.91 (0.58; 1.42)		0.90 (0.52; 1.55)	
PFOS	no	53.10 (1.50; 1881.81)	0.04	0.08 (0.00; 27.08)	0.47	2.64 (0.24; 29.32)	0.27	8.19 (0.04; 1743.69)	0.55
	yes	1.16 (0.44; 3.03)		0.73 (0.34; 1.58)		0.65 (0.29; 1.49)		1.56 (0.65; 3.77)	
PFOA	no	46.98 (1.26; 1747.34)	0.02	1.20 (0.06; 64.44)	0.62	6.40 (0.80; 50.97)	0.03	20.94 (0.34; 1295.34)	0.12
	yes	0.57 (0.19; 1.66)		0.79 (0.37; 1.72)		0.57 (0.25; 1.31)		0.75 (0.29; 1.95)	
PFNA	no	75.81 (1.24; 4636.88)	0.02	4.24 (0.28; 63.54)	0.17	6.99 (1.06; 45.86)	0.02	14.16 (0.05; 3837.15)	0.34
	yes	0.61 (0.25; 1.52)		0.62 (0.32; 1.18)		0.59 (0.30; 1.15)		0.92 (0.45; 1.88)	
PFDA	no	18.42 (2.02; 168.04)	<0.01	1.72 (0.30; 9.66)	0.37	2.43 (0.69; 8.56)	0.05	1.82 (0.16; 20.17)	0.53
	yes	0.52 (0.25; 1.05)		0.75 (0.45; 1.23)		0.63 (0.36; 1.10)		0.83 (0.48; 1.44)	

Missing values were imputed using multiple imputation. All analyses were adjusted for family history of eczema in children, allergic eczema and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breastfeeding, fish intake at age 5, number of siblings, daycare attendance at age 5, birth weight, and family history of chronic bronchitis/asthma.

[a] Both asthma and allergy at age 5.

[b] Asthma but not allergy at age 5.

[c] Both asthma and positive skin prick test at age 13.

[d] Asthma but no positive skin prick test at age 13.

[i] p-value for PFAS/MMR vaccination interaction.

Appendix A: Imputation models

Model [a]	Use of data from the model	Variables included in the imputation model	Interactions
1	All analyses except associations between PFASs and allergy (questionnaire) at age 13 and atopic eczema at age 13	***Binary*** [b]***:*** MMR vaccination, parental smoking in the home at ages 5 and 13, use of daycare at age 5, has furry pets at age 13, father's primary education, whether the child had lived abroad between ages 7 and 13, asthma at age 5, allergy at age 5, asthma at age 13, positive SPT at age 13, rhinoconjunctivitis at age 13 ***Ordered categorical*** [c]***:*** Number of siblings, weekly maternal fish dinners, weekly fish dinners at ages 5 and 13 ***Non-ordered categorical*** [d]***:*** Family history of chronic bronchitis or asthma, family history of eczema in children, allergic eczema and hay fever, family history of allergy ***Continuous with truncation*** [e]***:*** Total months of breastfeeding ***Continuous*** [f]***:*** Maternal PFHxS, PFOS, PFOA, PFNA and PFDA, child PFHxS, PFOS, PFOA, PFNA and PFDA at age 5, child PFHxS, PFOS, PFOA, PFNA and PFDA at age 13, umbilical cord IgE, IgE at age 7 ***Complete (no missing):*** Sex, premature birth, birth season, birth weight, maternal pre-pregnancy BMI, maternal smoking during pregnancy, maternal parity	Interactions between MMR vaccination and each of the variables PFHxS, PFOS, PFOA, PFNA and PFDA at age 5 were included in the imputation of asthma at age 5, allergy at age 5, asthma at age 13
2	Analyses of association between: - all PFASs and allergy (questionnaire) at age 13 - maternal PFASs + PFASs at age 5 and eczema at age 13 - PFOS, PFOA, PFNA, and PFDA at age 13 and eczema at age 13	All variables from model 1 except asthma at age 13, positive SPT at age 13, and rhinoconjunctivitis at age 13 ***Binary*** [b]***:*** Allergy (questionnaire) at age 13 and atopic eczema at age 13	Interactions between MMR vaccination and each of the variables PFOS, PFOA, PFNA, and PFDA at age 13 were included in the imputation of eczema (age 13)
3	Analyses of association between PFHxS at age 13 and eczema at age 13	All variables from model 1 except asthma at age 13 and positive SPT at age 13 ***Binary*** [b]***:*** Atopic eczema at age 13	An interaction between MMR vaccination and PFHxS at age 13 were included in the imputation of eczema (age 13)

[a] We were unable to create an imputation model including all allergy measures at age 13, and data was therefore imputed in three different models
[b] Binary variables were imputed using logistic regression
[c] Ordered categorical variables were imputed using ordered logistic regression
[d] Non-ordered categorical variables were imputed using multinomial logistic regression
[e] Duration of breastfeeding was imputed using linear regression left truncated at zero
[f] Other continuous variables were imputed using classic linear regression

Appendix B: Associations between parental/child characteristics and PFAS tested in linear regression models, N = 559

		Maternal PFAS % difference [a]					5 years PFAS % difference [a]					13 years PFAS % difference [a]				
		PFHxS	**PFOS**	**PFOA**	**PFNA**	**PFDA**	**PFHxS**	**PFOS**	**PFOA**	**PFNA**	**PFDA**	**PFHxS**	**PFOS**	**PFOA**	**PFNA**	**PFDA**
Sex	Girls	-	-	-	-	-	5.8	1.9	-4.9	-5.4	-3.7	-16.2	-16.4	-10.6	-14.2	-14.6
	p	-	-	-	-	-	*0.29*	*0.51*	*0.08*	*0.13*	*0.38*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*
Premature birth (<=36 weeks)	Yes	-	-	-	-	-	-10.6	-1.9	-7.3	1.0	34.3	3.1	12.9	-3.3	14.4	28.9
	p	-	-	-	-	-	*0.56*	*0.86*	*0.47*	*0.94*	*0.05*	*0.82*	*0.32*	*0.77*	*0.28*	*0.06*
Birth season	Summer	-27.4	-5.5	-8.1	-4.2	4.4	-	-	-	-	-	-	-	-	-	-
	Fall	-22.8	1.6	-5.5	-5.7	0.2	-	-	-	-	-	-	-	-	-	-
	Winter	-27.7	-2.5	-7.4	-7.2	0.7	-	-	-	-	-	-	-	-	-	-
	p	*0.01*	*0.16*	*0.24*	*0.51*	*0.83*	-	-	-	-	-	-	-	-	-	-
Birth weight (kg)		-	-	-	-	-	-5.5	-2.5	-2.6	-1.2	-4.0	-0.2	2.0	-3.1	-0.2	4.2
	p	-	-	-	-	-	*0.28*	*0.38*	*0.36*	*0.73*	*0.32*	*0.95*	*0.57*	*0.31*	*0.95*	*0.28*
Total months of breastfeeding		-0.6	-0.6	-0.9	-0.8	-0.5	2.8	1.7	1.6	1.2	0.8	0.6	0.5	0.6	0.2	-0.1
	p	*0.3* [b]	*<0.01* [b]	*<0.01* [b]	*<0.01* [b]	*0.09* [b]	*<0.01* [b]	*<0.01* [b]	*<0.01* [b]	*<0.01* [b]	*0.01* [b]	*0.03* [b]	*0.05* [b]	*0.02* [b]	*0.47* [b]	*0.65* [b]
Maternal parity (pre-pregnancy)	1	-15.7	-1.9	-22.2	-2.8	5.0	1.0	0.2	2.1	3.7	4.6	6.6	3.8	3.2	2.4	3.8
	>=2	-17.5	0.5	-25.2	9.3	15.7	-2.8	-0.5	0.1	8.3	1.7	5.6	-0.4	1.8	1.3	3.3
	p	*0.08*	*0.70*	*<0.01*	*0.01*	*0.01*	*0.81*	*0.98*	*0.81*	*0.21*	*0.73*	*0.40*	*0.56*	*0.77*	*0.87*	*0.73*
Number of siblings (at age 5)	1	-	-	-	-	-	29.4	17.9	12.6	8.1	-1.2	2.4	0.4	-2.6	-5.1	-1.0
	2	-	-	-	-	-	31.7	17.4	12.8	12.2	-3.9	9.3	1.8	-1.1	-1.2	1.8
	>=3	-	-	-	-	-	8.3	11.7	6.5	10.2	-4.7	-4.3	-4.0	-6.7	-6.2	-0.9
	p	-	-	-	-	-	*0.01*	*0.03*	*0.12*	*0.45*	*0.89*	*0.08*	*0.69*	*0.62*	*0.62*	*0.93*
Received 15-month MMR-vaccination	Yes	-	-	-	-	-	-0.6	8.4	-8.3	4.7	16.5	4.7	7.4	-6.7	11.4	14.7
	p	-	-	-	-	-	*0.96*	*0.28*	*0.25*	*0.63*	*0.16*	*0.65*	*0.44*	*0.41*	*0.24*	*0.18*
Maternal pre-pregnancy BMI (kg/m^2)		-0.7	0.34	0.1	0.5	0.1	-2.8	-1.1	-1.1	-0.3	-0.6	-1.5	-1.0	-0.9	-0.5	-0.5
	p	*0.42*	*0.26*	*0.77*	*0.25*	*0.89*	*<0.01* [b]	*<0.01* [b]	*<0.01* [b]	*0.52* [b]	*0.24* [b]	*<0.01* [b]	*0.02* [b]	*0.02* [b]	*0.26* [b]	*0.26* [b]
Maternal smoking during pregnancy	Yes	-11.0	-3.9	2.9	-7.8	-6.7	-18.2	-11.3	-3.4	-10.7	-8.5	-11.8	-11.3	-1.5	-7.5	-10.5
	p	*0.13* [2]	*0.14* [2]	*0.40* [2]	*0.04* [2]	*0.10* [2]	*<0.01* [b]	*<0.01* [b]	*0.28* [b]	*<0.01* [b]	*0.06* [b]	*<0.01* [b]	*<0.01* [b]	*0.68* [b]	*0.05* [b]	*0.01* [b]
Parental smoking in the home at age 5	Yes	-	-	-	-	-	-13.3	-6.3	-2.4	-6.2	-9.8	-9.1	-10.1	-1.2	-6.7	-4.3
	p	-	-	-	-	-	*0.02* [b]	*0.05* [b]	*0.46* [b]	*0.12* [b]	*0.03* [b]	*0.03* [b]	*0.01* [b]	*0.74* [b]	*0.08* [b]	*0.32* [b]
Parental smoking in the home at age 13	Yes	-	-	-	-	-	-	-	-	-	-	-1.8	-6.9	1.7	-2.7	-4.6
	p	-	-	-	-	-	-	-	-	-	-	*0.77*	*0.22*	*0.75*	*0.65*	*0.48*
Weekly maternal fish dinners during pregnancy	2	-6.8	5.6	-3.5	5.6	4.6	-15.3	-0.4	-0.6	6.4	6.7	-4.9	2.8	-0.4	6.4	9.1
	>2	-2.2	11.3	-4.7	12.3	12.3	-23.2	2.5	-5.9	11.5	14.9	-4.7	8.8	-4.0	12.2	20.8
	p	*0.89* [b]	*0.03* [b]	*0.61* [b]	*0.10* [b]	*0.14* [b]	*<0.01* [b]	*0.82* [b]	*0.29* [b]	*0.14* [b]	*0.16* [b]	*0.60* [b]	*0.25* [b]	*0.69* [b]	*0.09* [b]	*0.01* [b]
Weekly fish dinners at age 5	>1 – 2	-	-	-	-	-	-12.7	3.7	-1.2	7.0	23.7	-6.0	9.7	-1.0	12.1	14.0
	>2	-	-	-	-	-	-22.6	5.2	-5.9	17.7	31.1	-7.2	13.9	-4.0	17.8	26.6
	p	-	-	-	-	-	*<0.01* [b]	*0.48* [b]	*0.20* [b]	*<0.01*	*<0.01*	*0.39* [b]	*0.03* [b]	*0.57* [b]	*0.01* [b]	*<0.01* [b]

										[b]	[b]					
Weekly fish dinners at age 13	>1 – 2	-	-	-	-	-	-	-	-	-	-	-0.9	6.7	0.6	12.7	12.2
	>2	-	-	-	-	-	-	-	-	-	-	10.3	20.2	-1.2	26.7	33.0
	p	-	-	-	-	-	-	-	-	-	-	*0.17* [b]	*<0.01* [b]	*0.93* [b]	*<0.01* [b]	*<0.01* [b]
Use of daycare at age 5	Yes	-	-	-	-	-	10.8	-3.4	7.9	11.1	16.2	5.3	-3.2	10.4	-3.8	-13.4
	p	-	-	-	-	-	*0.31*	*0.53*	*0.17*	*0.13*	*0.06*	*0.48*	*0.63*	*0.11*	*0.56*	*0.05*
Has furry pets at age 13	Yes	-	-	-	-	-	-	-	-	-	-	4.6	-0.0	0.2	-1.8	-1.4
	p	-	-	-	-	-	-	-	-	-	-	*0.26*	*0.99*	*0.95*	*0.62*	*0.72*
Family history of chronic bronchitis or asthma	From one parent	-0.4	0.9	1.9	-1.7	4.1	-8.6	-5.2	-1.5	-1.0	-2.2	-5.9	-5.6	1.8	-1.7	-3.1
	From both parents	17.8	8.1	20.2	0.6	5.2	-12.6	1.4	-0.3	-5.0	12.7	-15.5	-0.7	3.5	1.2	1.6
	p	*0.59*	*0.42*	*0.03*	*0.91*	*0.65*	*0.25*	*0.27*	*0.91*	*0.82*	*0.35*	*0.10*	*0.36*	*0.82*	*0.89*	*0.76*
Family history of eczema in children, allergic eczema and hay fever	From one parent	-14.1	-7.3	-7.8	-8.1	-7.7	-4.2	-6.7	0.5	0.8	-0.1	-4.6	-5.9	-1.0	-1.7	-8.1
	From both parents	-14.1	-0.3	-4.5	4.1	12.0	-7.2	-6.2	3.2	1.7	1.6	-10.5	-9.1	-5.1	-7.8	-6.3
	p	*0.18* [b]	*0.02* [b]	*0.08* [b]	*0.05* [b]	*0.01* [b]	*0.65*	*0.10*	*0.82*	*0.96*	*0.97*	*0.19* [b]	*0.16* [b]	*0.64* [b]	*0.40* [b]	*0.18* [b]
Family history of allergy	From one parent	-23.8	-1.3	-2.1	-5.9	-4.3	-3.1	-3.5	0.1	-2.5	-7.6	-9.6	-7.8	-0.0	-4.7	-9.4
	From both parents	-24.1	2.0	-0.3	0.4	-1.0	-8.9	-6.4	0.8	0.9	2.7	-3.9	2.1	1.0	0.8	4.0
	p	*<0.01*	*0.79*	*0.86*	*0.35*	*0.67*	*0.67*	*0.46*	*0.99*	*0.83*	*0.25*	*0.10* [b]	*0.10* [b]	*0.99* [b]	*0.47* [b]	*0.05* [b]

Factors were considered associated with PFAS if they were associated ($p<0.20$) with at least two PFAS measures in the same direction at a particular time point.

[a] The estimates of association were converted to express percent differences in PFAS.

[b] In adjusted models including both breast feeding, pre-pregnancy BMI, maternal smoking during pregnancy, parental smoking at age 5, fish intake, family disposition for eczema/hay fever, and family disposition for allergy, parental smoking at age 5 was no longer associated with PFAS concentrations, maternal fish intake during pregnancy was no longer associated with PFAS at ages 5 and 13, and fish intake at age 5 was no longer associated with PFAS concentrations at age 13. Also, family disposition for eczema/hay fever and family disposition for allergy were no longer associated with PFAS concentrations at age 13, but when family disposition for allergy was removed from the adjusted model, family disposition for eczema/hay fever was still associated with PFAS at age 13.

Appendix C: Distributions of PFASs by MMR vaccination status at age 5 and asthma status at ages 5 and 13 years (pre-imputation)

	MMR vaccinated		**MMR unvaccinated**	
	Age 5 asthma Median (min; max)	**No asthma at age 5** Median (min; max)	**Age 5 asthma** Median (min; max)	**No asthma at age 5** Median (min; max)
Maternal PFASs (ng/mL)	**N=69**	**N=426**	**N=8**	**N=13**
PFHxS	4.2 (1.0; 26.5)	4.6 (0.6; 25.7)	3.0 (1.3; 9.0)	6.2 (1.3; 11.7)
PFOS	28.4 (10.6; 66.7)	27.4 (9.4; 68.8)	28.3 (15.9; 34.0)	24.0 (15.4; 36.4)
PFOA	3.6 (1.3; 6.2)	3.2 (0.8; 8.4)	2.8 (1.3; 4.0)	3.5 (2.1; 4.6)
PFNA	0.6 (0.2; 1.9)	0.6 (0.2; 2.5)	0.7 (0.3; 1.1)	0.6 (0.3; 0.8)
PFDA	0.3 (0.1; 0.8)	0.3 (0.0; 1.2)	0.3 (0.1; 0.7)	0.3 (0.2; 0.5)
Age 5 PFASs (ng/mL)	**N=66**	**N=427**	**N=6**	**N=14**
PFHxS	0.6 (0.0; 3.0)	0.6 (0.1; 19.5)	1.0 (0.3; 1.3)	0.5 (0.2; 1.6)
PFOS	16.2 (3.3; 33.3)	17.1 (6.2; 48.2)	18.7 (9.2; 27.2)	14.1 (8.8; 27.8)
PFOA	4.0 (0.8; 7.9)	4.0 (1.5; 15.4)	5.0 (3.6; 11.4)	3.5 (2.7; 7.3)
PFNA	0.9 (0.5; 3.2)	1.0 (0.4; 6.2)	1.3 (0.6; 3.9)	0.8 (0.5; 1.6)
PFDA	0.3 (0.1; 0.8)	0.3 (0.1; 1.2)	0.4 (0.1; 0.7)	0.2 (0.1; 0.8)
	Age 13 asthma Median (min; max)	**No asthma at age 13** Median (min; max)	**Age 13 asthma** Median (min; max)	**No asthma at age 13** Median (min; max)
Maternal PFASs (ng/mL)	**N=73**	**N=372**	**N=9**	**N=9**
PFHxS	4.3 (0.7; 26.5)	4.6 (0.6; 25.7)	3.0 (1.3; 9.0)	8.3 (1.3; 11.7)
PFOS	28.0 (15.8; 66.7)	27.5 (9.4; 57.0)	30.6 (15.9; 36.4)	21.4 (15.4; 33.0)
PFOA	3.5 (1.1; 6.5)	3.2 (0.8; 8.4)	3.8 (1.3; 4.1)	2.8 (2.1; 4.0)
PFNA	0.6 (0.3; 1.9)	0.6 (0.2; 1.8)	0.8 (0.3; 1.1)	0.6 (0.3; 0.6)
PFDA	0.3 (0.1; 0.8)	0.3 (0.0; 0.7)	0.3 (0.1; 0.7)	0.3 (0.2; 0.3)
Age 5 PFASs (ng/mL)	**N=70**	**N=376**	**N=7**	**N=10**
PFHxS	0.6 (0.0; 1.6)	0.6 (0.1; 19.5)	0.6 (0.3; 1.3)	0.5 (0.2; 1.6)
PFOS	16.5 (3.3; 31.3)	17.1 (6.2; 40.8)	18.6 (9.2; 27.8)	13.1 (10.4; 23.3)
PFOA	4.0 (0.8; 7.9)	4.0 (1.3; 15.4)	5.0 (3.6; 11.4)	3.1 (2.7; 7.3)
PFNA	0.9 (0.5; 3.1)	1.0 (0.4; 5.3)	1.2 (0.5; 3.9)	0.8 (0.5; 1.6)
PFDA	0.3 (0.1; 1.0)	0.3 (0.1; 1.2)	0.3 (0.1; 0.7)	0.2 (0.1; 0.8)
Age 13 PFASs (ng/mL)	**N=73**	**N=392**	**N=8**	**N=10**
PFHxS	0.4 (0.1; 1.2)	0.4 (0.1; 4.1)	0.4 (0.2; 0.7)	0.3 (0.2; 0.6)
PFOS	6.8 (1.5; 14.0)	6.7 (1.0; 16.6)	6.7 (3.9; 9.1)	6.4 (3.5; 8.4)
PFOA	2.0 (0.7; 4.0)	2.0 (0.6; 6.1)	2.1 (1.2; 3.5)	2.0 (1.1; 3.0)
PFNA	0.7 (0.2; 1.7)	0.7 (0.2; 2.1)	0.7 (0.3; 1.2)	0.7 (0.4; 0.9)
PFDA	0.3 (0.1; 0.8)	0.3 (0.1; 1.2)	0.2 (0.2; 0.7)	0.3 (0.1; 0.5)