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Perfluoroalkyl substances, airways infections, allergy and asthma related health outcomes – implications of gender, exposure period and study design

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ABSTRACT

Introduction: Exposure to perfluoroalkyl substances (PFASs) has been inconsistently associated with asthma, allergic diseases and airways infections in early childhood. The aim of the study was, therefore, to investigate the effect of childhood exposure to PFASs on asthma and allergy related outcomes and on airways infections before and during puberty using the prospective birth cohort Environment and Childhood Asthma (ECA) Study. Aspects of gender, exposure period and study design (cross-sectional and longitudinal) were also taken into consideration.

Material and methods: Included in the study was 378 participants with PFAS measurements at age 10 years and follow-up data at ages 10 years (cross sectional data) and 16 years (longitudinal data). Eight PFASs with at least 70% of measurements above the limit of quantification (LOQ) in the child's serum were included in the present study: perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS) and perfluorooctane sulfonate (PFOS). The PFAS levels were converted into interquartile range (IQR). In addition, perfluorooctane sulfonamide (PFOSA) detected in 60% of the samples, was recoded into "not detected /detected". Binomial, multinomial and linear regression were used, followed by Bonferroni adjustment to correct for multiple comparisons. Sensitivity analyses evaluating the effect of extreme PFAS values and gender were performed.

Results: In the cross sectional data at 10 years a positive statistically significant association was seen between PFHpA and asthma in girls. In the longitudinal data, PFNA, PFDA and PFUnDA were inversely associated with atopic dermatitis (AD) in girls and with PFHxS in all participants and in boys. Further, PFNA and PFHpS were positively associated with rhinitis in girls and with PFOA in all participants. There seems to be a suggestive pattern of increased risk of allergic sensitisation in all participants and a decreased risk in boys, but due to different results in main and sensitivity analyses these findings should be interpreted with caution. No associations were found between PFASs and lung function. For airways infections and longitudinal data, PFDA was inversely associated with common cold, while positive association was found for PFHpA, PFOA, PFHpS and PFOS and lower respiratory tract infections (LRTI).

Discussion and conclusion: Our results lend further support for an immunosuppressive effect of PFASs on AD and LRTI. Gender seems to be important for some exposure-health associations. No clear pattern in exposure-health associations was observed with regard to exposure period or study design, with the exception of asthma where significant findings have mostly been reported in cross-sectional studies.

1. Introduction

Perfluoroalkyl substances (PFASs) are synthetic fluorinated compounds widely used due to their water-, oil-, soil-, and stain-resistant properties. Over the last 60 years, the industry has found a variety of usages for these fluorinated compounds, like in coating on frying pans, waterproof outdoor clothes, non-stick food packaging and a wide range of commercial household products like polishes, (ski) waxes, paints, cleaning products and fire-fighting foams. However, PFASs are considered environmental pollutants and are ubiquitously distributed in

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humans and wildlife (EFSA-CONTAM-Panel, 2018). The major exposure route for humans is trough food and drinks. Among the most prevalent PFASs, seafood is the food group with both the highest concentrations and the food contributing most to the total exposure (EFSA-CONTAM-Panel, 2018; Haug et al., 2010). Inhalation and ingestion of house dust may also contribute to the total exposure (Haug et al., 2011).

A normal maturation of the immune system depends upon specific processes that differ in both time and location within the body. Thus, the child's immune system is a moving toxicological target for interactions with environmental chemicals. The immune system matures mainly during gestation, although critical adjustments both in the level and spectrum of immune response capacities continue to change after birth and into adolescence. Therefore, different stages during childhood can exhibit differential vulnerabilities. Gender-related effects are also common with developmental immunotoxicity (IPCS, 2012). Immunotoxic effects can be divided into autoimmunity, hypersensitivity and immunosuppression, in which the two latter are examined the present study.

Experimental *in vitro* and animal studies suggest that PFASs have immunotoxic effects. In addition, some human studies on prenatal exposure to PFASs and vaccine responses or infectious diseases, suggest immunosuppression (Dalsager et al., 2016; DeWitt et al., 2012; Goudarzi et al., 2017; Grandjean et al., 2012; Granum et al., 2013; Impinen et al., 2019; NTP, 2016). When it comes to possible effects of PFASs on asthma and allergy related outcomes (hypersensitivity), the results are conflicting.

Longitudinal studies may be better at studying disease development due to the temporality between exposure and diseases, while cross-sectional studies may be better suited to investigate exacerbation of disease symptoms. The different study designs may therefore reflect different mechanism of PFAS exposure on immune diseases. PFAS exposure later in childhood has been studied in only seven studies, one with a longitudinal design and six with a cross-sectional design, where ages varied between 8 and 19 years (Agier et al., 2019; Averina et al., 2018; Dong et al., 2013; Humblet et al., 2014; Stein et al., 2016; Zhu et al., 2016).

The Environment and Childhood Asthma (ECA)-study is a prospective birth cohort study with follow-up examinations at the ages two (nested case-control study), 10 and 16 years. Thus, by using the ECA cohort, we have the possibility to study both cross-sectional and longitudinal data with PFASs measured in cord blood and at 10 years, and health outcomes measured at birth, 2 years, 10 years and 16 years. The effect of prenatal exposure and health outcomes up to 10 years of age has previously been published (Impinen et al., 2018). Therefore, in the present study exposure to PFASs at age 10 years and health outcomes collected at the 10 and 16 years follow-up examinations were explored.

The aim of the study was to investigate the effect of childhood exposure to PFASs on asthma and allergy related outcomes and on airways infections before and during puberty, taking gender, exposure period and study design (cross-sectional and longitudinal) into consideration.

2. Methods

2.1. Study design and health outcomes

The present study includes data from the 10 and 16years follow-up investigations for the prospective, birth cohort Environment and Child Asthma (ECA) Study in Oslo described in detail elsewhere (Hovland et al., 2013; Lodrup Carlsen, 2002). In short, two main hospitals in Oslo, Norway, recruited 3754 healthy new-borns weighing at least 2000 g, between January 1992 and March 1993. Lung volume was measured by tidal flow loops at birth in 802 of the 3754 children included in the cohort. Follow-up investigations were performed at 2 years (nested case-control study), 10 years and 16 years. At the 10 and 16 years follow-up, only participants that had lung function measurements at birth and/or attended the 2 years follow-up were invited (n = 1215). Of these, 1019 (84%) and 540 (44%) participants attended the 10 and 16 years follow-up, respectively (Supplementary Fig. 1). Of the 540 children attending both the 10 and 16 years investigations, PFAS measurements (at age 10 years) were available for 378 children (31%).

The clinical follow-up investigations at 10 and 16 years included:

- 10 years: Anthropometry, skin prick test (SPT) and blood sampling for allergic sensitisation, spirometry including treadmill test and methacholine challenge, and a parental interview (Lodrup Carlsen et al., 2006).
- 16 years: Anthropometry, SPT and blood sampling for allergic sensitisation, spirometry including methacholine challenge, and separate interviews with the parents and the participants. The follow-up included 550 adolescents, where 540 participated at both 10 and 16 years (Hovland et al., 2014; Lodrup Carlsen et al., 2014).

Lung function at 10 and 16 years were measured by maximally forced expiratory lung volume loops according to European Guidelines using Sensormedics V-max (Sensormedics Diagnostics, Yorba Linda, CA, USA) spirometer. SPT was performed according to the European standards with the following standardized allergen extracts (Soluprick, ALK-Abello, Denmark): house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farina*), pets (dog, cat, rabbit), grass, tree and mugwort pollens and moulds, as well as cow's milk, wheat, peanut, and cod.

All investigations required at least 4 weeks without symptoms of respiratory tract infection, no use of antihistamines for 120 h, leukotriene antagonists for 12 or 48 h or inhaled corticosteroids for 12 h.

The health outcomes included in the present study are listed in Table 1.

Written informed consent forms were obtained from all parents at all follow-up examinations, as well from the children at 16 years of age. The study was approved by the Regional Ethics Committee (Oslo, Norway) and the Norwegian Data Inspectorate and reported to the Norwegian Biobank Registry (Oslo, Norway).

2.2. PFAS measurements in serum at 10 years of age

In serum at age 10 years, 19 PFASs were determined using liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS) according to a previously described method (Haug et al., 2009). The limit of quantification (LOQ) was 0.050 ng/mL for all PFASs. For quantification of perfluorooctane sulfonate (PFOS), the total area of the linear and branched isomers was integrated. For 10 PFASs, all samples were below LOQ and thus omitted from the statistical analyses: perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluorododecanoate (PFDoDa), perfluorotridecanoate (PFTrDA), perfluorotetradecanoate (PFTeDa), perfluorobutane sulfonate (PFBS), perfluorodecane sulfonate (PFDS), N-methylperfluorooctane sulfonamide (MeFOSA) and N-ethylperfluorooctane sulfonamide (EtFOSA). The 9 PFASs included in the statistical analyses were perfluorooctane sulfonamide (PFOSA; recoded into not detected/detected [328/60]); perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorohexadecanoate (PFHxS), perfluoroheptane sulfonate (PFHpS) and perfluorooctane sulfonate (PFOS) (≥70% of the samples above LOQ). The values below LOQ were imputed by dividing LOQ with the square root of 2.

2.3. Confounding

Prior to the statistical analysis, we constructed separate directed acyclic graphs (DAGs) for the asthma related outcomes (asthma and

Table 1
Health outcomes used in the present study at 10 and 16 years of age.

Outcome	Age (years)	Definition
Lung function	10	Percent predicted values of forced expiratory volume in 1 s (FEV1) according to reference algorithm by Stanojevic
	16	(Stanojevic et al., 2008)
Asthma	10	Asthma ever: a positive response to at least two of the following: dyspnoea, chest tightness and/or wheezing 0–3 and/or 4–10 years, doctor's diagnosis of asthma, use of asthma medication (ß-2 agonsist, sodium chromoglycate, corticosteroids, leukotriene antagonists and/or aminophylline) 0–3 and/or 4–10 years
	10–16	A positive response to at least two of the following: doctor's diagnosis of asthma, asthma symptoms, use of anti-asthmatic medication between 10 and 16 years of age
	16	A positive response to at least two of the following: doctor's diagnosis of asthma, asthma symptoms, use of anti-asthmatic medication last 12 months
Atopic dermatitis (AD)	10	Parent-reported doctor diagnosis of AD (ever or ongoing)
	10–16	Parent-reported AD between 10 and 16 years of age and/or dermatitis at 16 years
	16	Parent-reported AD last 12 months
Rhinitis	10	At least one of the following parent-reported symptoms (without a cold) last 12 months: runny nose, blocked nose or sneezing
	16	
Allergic sensitisation/skin prick test (SPT)	10	At least one positive SPT: ≥ 3 mm when compared to the negative control
	16	
Common cold	10–16	Parent-reported number of episodes between 10 and 16 years of age
	16	Parent-reported number of episodes last 12 months
Lower respiratory tract infections (LRTI)	10–16	Parent-reported number of episodes of bronchitis and pneumonia between 10 and 16 years of age
	16	Parent-reported number of episodes of bronchitis and pneumonia last 12 months

lung function), allergy related outcomes (AD, rhinitis and skin prick test) and airways infections (common cold and LRTI) (version 2.3; www.dagitty.net). Supplementary Fig. 2 shows the DAGs for the outcomes at 16 years. Depending on the covariates available, similar models were adapted for the 10 years outcomes. Covariates examined were sex, maternal smoking during pregnancy, passive smoking at home (parental smoking) and active smoking at 16 years (the participant), parental atopy, mother's education, number of older siblings, the child's physical activity level at 10 and 16 years, body mass index (BMI) at 10 and 16 years and the degree of pubertal development. A confounder is defined as a variable that influences both exposure and health outcome. Therefore, covariates that may affect the exposure at 10 years and the outcome at 10 and 16 years respectively were included in the DAGs. The correlation between PFASs measured in cord blood and the samples at 10 years were low (Supplementary Table 1), thus cord blood PFASs levels were not included in the DAGs.

Covariates included in models for lung function, asthma, AD, rhinitis and SPT at 10–16 years/16 years were BMI at 16 years, puberty status at 16 years (growth spurt: not started-ongoing, growth spurt done), mothers' education (less than 12 years, college, university) and physical activity level at 16 years (frequency of activities leading to breaking sweat and shortness of breath). Whereas airways infections models included puberty status at 16 years, mothers' education and physical activity level at 16 years. For the 10 years data the models for asthma, AD and rhinitis were adjusted for age at follow-up, physical activity at 10 years and mothers' education, while SPT and predicted FEV1 were adjusted for physical activity at 10 years, mothers' education and BMI at 10 years.

2.4. Statistical methods

Continuous variables are reported as mean and standard deviation (SD) and tested with student's *t*-test for possible differences between gender and included/not included participants. Categorical data are reported as counts and percentages and tested with Chi square test or Kendall's exact test (if $n < 5$ per subgroup). Correlations between the PFASs were tested with Pearson.

In the regression analyses, the PFASs concentrations (PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFHpS and PFOS concentrations in serum at 10 years) were converted into interquartile range (IQR). Thus, the risk estimates are interpreted as changed risk with an increase of one IQR difference.

Binomial logistic regression models were used for the binary health outcomes (asthma, AD, rhinitis and SPT). LRTI was recoded into yes/no and treated as a binary outcome. Multinomial regression models were used for common cold that had three categories. Linear regression models were used for the lung function measurement (FEV1 % predicted). The effect estimates are reported in Risk Ratios, Odds Ratios, and Coefficients with 95% confidence intervals, for the binary, multinomial and linear regressions respectively. All models were adjusted for potential confounding as described above. For some exposure-health associations, there was not possible to fit a model including the selected covariates, thus results from the unadjusted analysis are shown.

A $p < 0.05$ is generally considered statistical significant. However, to avoid likelihood of false positives (Type 1 error) due to multiple testing, we used the Bonferroni method and divided the p-value 0.05 by the number of hypotheses being tested (9 PFASs) giving a threshold of significance of $p < 0.006$.

Previous studies give some indication that gender may play a role in the exposure-health associations. Therefore, all regression analyses were also stratified by gender.

2.4.1. Additional analyses

Sensitivity analyses were performed to check the influence of extreme PFAS values. These data points were identified by visual inspection of the distribution of the data (histograms), and were clearly above the normal distribution curve: 1 excluded for PFOA, PFDA and PFHpS, 2 excluded for PFNA and PFUNDA, 6 excluded for PFHxS and 8 excluded for PFOS. Since no data points were excluded for PFHpA, and PFOSA was treated as a binary outcome, no sensitivity analyses were performed for these two PFASs.

All statistically significant exposure-health associations were tested for linearity by using generalized additive models in which is a method of fitting a smooth relationship between two or more variables (function gam() from the R package mgcv). The fit between the models with or without the smooth function were assessed by using the Akaike Information Criteria (AIC). The models were defined as being different from each other if AIC > 3. For the exposures-health associations that were found to be non-linear, we present the results from linear models for ease of interpretability and comparison with the other exposures whose associations were linear. However, they only reflect a linear averaging over the true non-linear associations and these estimates should be interpreted with caution.

For statistical analyses, STATA version 15 (StataCorp LLC, TX, USA) and R sofware version 3.5 (www.r-project.org) were used.

Table 2
PFAS concentrations (ng/mL) in serum at 10 years for all participants, girls and boys. Bold: p value < 0.05 comparing levels between girls and boys.

	Total n = 378					Girls n = 185					Boys n = 193					
	N*	mean	SD	median	IQR	N	mean	SD	median	IQR	N	mean	SD	median	IQR	p
PFHpA	378	0.14	0.12	0.11	0.13	185	0.14	0.12	0.11	0.13	193	0.15	0.13	0.12	0.14	0.63
PFOA	378	4.62	1.86	4.36	1.77	185	**4.32**	1.36	4.13	1.63	193	**4.90**	2.21	4.53	1.86	**0.002**
PFNA	378	0.63	0.30	0.60	0.29	185	**0.57**	0.22	0.54	0.28	193	**0.69**	0.34	0.62	0.31	< **0.001**
PFDA	378	0.19	0.11	0.18	0.13	185	**0.17**	0.10	0.17	0.14	193	**0.21**	0.12	0.19	0.12	< **0.001**
PFUnDA	378	0.18	0.13	0.16	0.13	185	**0.17**	0.09	0.15	0.12	193	**0.20**	0.15	0.18	0.14	**0.01**
PFHxS	378	3.33	9.62	1.32	0.86	185	2.68	7.08	1.20	0.81	193	3.95	11.53	1.43	1.26	0.20
PFHpS	378	0.37	0.26	0.32	0.20	185	**0.32**	0.18	0.28	0.16	193	**0.43**	0.32	0.38	0.21	< **0.001**
PFOS	378	20.9	8.75	19.4	9.23	185	**19.0**	7.80	17.52	8.02	193	**22.8**	9.22	21.7	8.86	< **0.001**
PFOSA	60	0.42	0.23	0.38	0.25	29	**0.35**	0.16	0.35	0.17	31	**0.48**	0.26	0.41	0.32	**0.02**

*Percentage of imputed values (< LOQ): PFHpA 24%, PFDA 9%, PFUnDa 5% and PFHpS 0.3%.
IQR: interquartile range; SD: standard deviation.

3. Results

3.1. PFAS concentrations

Table 2 shows the PFASs concentrations in serum at 10 years as mean with standard deviations (SD), median and interquartile range (IQR). Most PFAS concentrations were quite normally distributed (PFOA, PFNA, PFDA, PFUnDA, PFHpS and PFOS) while some were skewed towards the left (PFHpA and PFHxS). Among the measured PFASs, PFOS had the highest concentrations with a mean of 20.9 ng/mL, followed by PFOA and PFHxS, with means 4.62 and 3.33 ng/mL, respectively. Serum PFAS concentrations were generally slightly higher in boys than in girls; being statistically significant for PFOA, PFNA, PFDA, PFUnDA, PFHpS and PFOS ($p < 0.05$). The inter-correlations for PFASs ranged from no correlation to strong correlation (correlation coefficients: 0–0.73 (Supplementary Table 2).

3.2. Population description

At the 16 years follow-up, 378 of the 540 participants had serum samples available for PFAS-analysis (49% girls) (Supplementary Table 3). The mean age was 10.6 years (SD 0.8) and 16.7 years (0.4) at the 10 and 16 years follow-up, respectively. Of the mothers, 55% had university/college education. At both ages 10 and 16 years, boys had a higher level of physical activity compared to girls. At age 16 years, more girls than boys had finished their growth spurt.

Prevalence of the different health outcomes at ages 10 and 16 years are shown in Table 3. The most frequent health outcomes were common cold with 87% reporting > 5 times from 10 to 16 years, a positive SPT at 16 years (46.5%) and rhinitis at 16 years (34%). Asthma at age 10 years and a positive SPT at both 10 and 16 years were significantly more frequent among boys than girls (asthma: 34.7% vs 23.8%; SPT at 10 years: 18.8% vs 8.2%; SPT at 16 years: 28.5% vs 18.1%, respectively). Whereas, more girls than boys had more than three episodes of common cold at age 16 years (40.0% vs 25.4%, respectively).

The adolescents attending the 16 years follow-up (n = 540) were at the 10 years follow-up slightly younger, weighted less and were shorter compared to the adolescents not attending the 16 years follow-up (n = 479) (age 10.7 and 10.9 years, weight 37.8 and 39.6 kg, and height 145 vs 147 cm, respectively) (Hovland et al., 2013). There were no differences in disease prevalence, except for any positive SPT where there was a lower percentage among the included versus the not included children (26.0 and 32.7%, respectively). In the present study, the 378 participants with PFAS measurements and clinical follow-up at both 10 and 16 years, were slightly younger, had a lower BMI, and were less physically active at 10 years compared to the participants not included in the study (Supplementary Table 3) (age 10.6 and 10.8 years, BMI 17.7 and 18.2, > 3 times/week with physical activity 57 and 68%, respectively). With regard to smoking, household smoking reported at age 10 years was less prevalent for the included compared to the participants not included, whereas household smoking was more prevalent at 16 years. Furthermore, a higher percentage of the mothers of the included children had a university degree compared to the mothers of the children not included. Except for FEV1 % predicted, there were no differences in disease prevalence for the included and not included children (Table 3).

3.3. Lung function and asthma

No significant associations were found between PFAS exposure and FEV1 % predicted at 10 and 16 years of age after the Bonferroni adjustment. Before the adjustment, however, there was a positive association between PFOA and PFOS and FEV1 % predicted for all participants at age 10 years (coefficient [95% CI]: 1.20 [0.33;2.07] per IQR of 1.77 ng/mL and 1.08 [0.11; 2.05] per IQR of 9.23 ng/mL, respectively), and for PFOA in boys (1.31 [0.26;2.36] per IQR of 8.86 mg/mL) (Supplementary Table 4). These findings were no longer statistically significant in the sensitivity analysis, indicating that the extreme values may influence the findings. The association between PFOS and FEV1 % predicted was non-linear in the main analysis but linear in the sensitivity analysis (Supplementary Fig. 3).

In the cross-sectional design at age 10 years, PFHpA was positively associated with asthma in girls (1.31 [1.08; 1.60] for an IQR of 0.13 ng/mL) (Supplementary Table 5). No statistically significant associations were found for the other PFASs. For the longitudinal designs (10–16 years and 16 years), no statistically significant associations were observed.

Unadjusted statistical analyses for FEV1 % predicted and asthma are shown in Supplementary Tables 6 and 7, respectively.

3.4. Atopic dermatitis (AD), rhinitis and allergic sensitisation

No statistically significant associations (at p-value < 0.006) were detected between PFASs and AD based on the cross-sectional design data at age 10 years (Table 4). For the longitudinal designs, inverse associations were found between PFNA and PFUnDA and AD in girls between the ages 10 and 16 years with risk estimates of similar magnitude (RR[95% CI]: 0.51[0.35;0.73] per IQR of 0.28 ng/mL and 0.45[0.29;0.69] per IQR of 0.12 ng/mL, respectively) (Table 4, Supplementary Fig. 4A). Before the Bonferroni adjustment, there were also inverse associations between PFDA and AD in girls (0.64[0.42;0.98] per IQR of 0.14 ng/mL), and between PFHxS and AD in all participants and in boys [0.79[0.34;0.99] per IQR of 0.86 ng/mL and 0.71[0.52;0.99] per IQR of 1.26 ng/mL, respectively). Before the Bonferroni adjustment, an inverse relation was also seen for PFHxS and AD in boys at age 16 years (0.59[0.37;0.95] per IQR of 1.26 ng/mL), whereas PFHpA was positively associated with AD in girls (1.38[1.04;1.83] per IQR of 0.13 ng/mL). In the additional analyses, all

Table 3
Clinical characteristics of the participants at the different ages for all (n = 378), girls (n = 185) and boys (n = 193). Not included: Participants without PFAS measurements at age 10 years. Bold: p value < 0.05 comparing levels between girls and boys or between all included and not included participants.

		All		Girls		Boys			Not included		
Outcomes	Age	Mean	SD	Mean	SD	Mean	SD	p-value	Mean	SD	p-value
FEV1 % predicted*	10	98.3	9.1	98.2	8.9	98.4	9.2	0.80	**96.7**	**9.8**	**0.01**
	16	100	0.6	102	0.9	99.9	0.6	0.11	**96.8**	**10.8**	**< 0.001**
		N, all	**%**	**N, girls**	**%**	**N, boys**	**%**	**p-value**	**N, all**	**%**	**p-value**
Asthma	10	111	29.4	**44**	**23.8**	**67**	**34.7**	**0.02**	191	30.5	0.71
	10–16	71	18.8	33	17.8	38	19.7	0.65	35	21.6	0.45
	16	68	18.0	31	16.8	37	19.2	0.54	27	16.7	0.71
AD	10	84	22.2	40	21.6	44	22.8	0.78	131	20.4	0.49
	10–16	73	19.3	37	20.0	36	18.7	0.74	27	16.7	0.47
	16	54	14.3	27	14.6	27	14.0	0.87	19	11.7	0.43
Rhinitis	10	58	15.3	24	13.0	34	17.6	0.21	121	19.0	0.14
	16	129	34.1	56	30.3	73	37.8	0.12	49	30.3	0.38
SPT	10	102	27.1	**31**	**8.2**	**71**	**18.8**	**< 0.001**	191	30.2	0.29
	16	175	46.5	**68**	**18.1**	**107**	**28.5**	**< 0.001**	67	41.4	0.27
Common cold	10–16										
1–2 times		6	1.6	3	1.6	3	1.6		3	1.9	
3–5 times		42	11.1	22	11.9	20	10.4		15	9.3	
> 5 times		330	87.3	160	86.5	170	88.1	0.92	143	88.8	0.81
	16										
none		43	11.4	**19**	**10.3**	**24**	**12.4**		11	6.8	
1–2 times		212	56.1	**92**	**49.7**	**120**	**62.2**		89	54.9	
> 3 times		123	32.5	**74**	**40.0**	**49**	**25.4**	**0.01**	62	38.3	0.17
LRTI	10–16	67	17.7	27	14.6	40	20.7	0.12	27	16.7	0.75
	16	11	3.3	4	2.5	7	4.1	0.54	2	1.5	0.27

*FEV1 % predicted at 10y: n = 377 for all, n = 184 for girls, n = 193 for boys, n = 639 for not included; FEV1 % predicted at 16y: n = 372 for all, n = 179 for girls, n = 193 for boys, n = 162 for not included.

Table 4
Associations between PFAS exposure and AD between ages 10 and 16 years and at 16 years for all, girls and boys. Results are shown for the main analyses and for sensitivity analyses where the results differed from the main analysis.

	All				Girls				Boys			
	RR[§]	CIL	CIU	P	RR	CIL	CIU	P	RR	CIL	CIU	P
10 years	N = 376				N = 185				N = 191			
PFHpA	0.93	0.80	1.08	0.35	0.99	0.81	1.22	0.93	0.81	0.65	1.01	0.06
PFOA	1.01	0.90	1.13	0.87	1.17	0.95	1.44	0.15	0.98	0.84	1.15	0.82
PFNA	0.96	0.83	1.10	0.54	0.88	0.68	1.15	0.35	0.96	0.81	1.14	0.65
PFDA	0.93	0.79	1.09	0.38	0.85	0.65	1.10	0.22	0.95	0.76	1.17	0.61
PFUnDA	0.90	0.77	1.07	0.23	0.79	0.60	1.05	0.11	0.95	0.79	1.14	0.59
PFHxS	1.00	0.98	1.01	0.62	1.00	0.98	1.02	0.98	0.99	0.97	1.01	0.52
PFHpS	1.03	0.96	1.11	0.43	1.00	0.82	1.23	0.98	1.03	0.95	1.11	0.54
PFOS	0.98	0.85	1.13	0.77	0.98	0.79	1.23	0.89	0.95	0.78	1.16	0.61
PFOSA	0.81	0.55	1.21	0.31	0.69	0.37	1.28	0.24	0.85	0.50	1.44	0.54
10–16 years	N = 375				N = 184				N = 191			
PFHpA	0.95	0.76	1.20	0.69	1.15	0.87	1.51	0.32	0.69	0.45	1.07	0.10
PFOA	0.97	0.78	1.21	0.80	1.24	0.86	1.78	0.24	0.89	0.64	1.23	0.48
PFNA	0.83	0.65	1.08	0.16	**0.51***	**0.35**	**0.73**	**< 0.001**	0.98	0.75	1.28	0.89
PFDA	0.86	0.66	1.12	0.28	**0.64[a]***	**0.42**	**0.98**	**0.04**	1.13	0.85	1.50	0.40
PFUnDA	0.95	0.75	1.21	0.69	**0.45***	**0.29**	**0.69**	**< 0.001**	1.16	0.97	1.38	0.10
PFHxS	**0.79***	**0.34**	**0.99**	**0.04**	0.91	0.74	1.10	0.33	**0.71***	**0.52**	**0.99**	**0.04**
PFHpS	0.98	0.83	1.17	0.84	1.01	0.73	1.39	0.96	0.97	0.80	1.18	0.77
PFOS	0.93	0.73	1.20	0.59	1.04	0.73	1.47	0.84	0.88	0.62	1.24	0.45
PFOSA	1.14	0.68	1.93	0.62	0.83	0.35	1.96	0.67	1.39	0.69	2.77	0.35
16 years	N = 375				N = 184				N = 191			
PFHpA	1.06	0.82	1.36	0.67	**1.38**	**1.04**	**1.83**	**0.02**	0.72	0.43	1.19	0.20
PFOA	1.04	0.87	1.24	0.68	1.21	0.81	1.82	0.35	0.99	0.76	1.27	0.92
PFNA	0.86	0.94	1.16	0.37	0.72	0.43	1.21	0.21	0.95	0.68	1.34	0.78
PFDA	0.92	0.68	1.25	0.60	0.77	0.46	1.30	0.33	1.04	0.72	1.52	0.82
PFUnDA	0.99	0.76	1.30	0.97	0.75	0.43	1.31	0.31	1.10	0.85	1.43	0.47
PFHxS	0.78	0.60	1.02	0.07	0.93	0.79	1.10	0.43	**0.59***	**0.37**	**0.95**	**0.03**
PFHpS	0.97	0.80	1.12	0.79	1.11	0.78	1.58	0.55	0.91	0.68	1.21	0.52
PFOS	0.85	0.62	1.17	0.33	1.14	0.77	1.68	0.51	0.62	0.37	1.05	0.08
PFOSA	0.80	0.38	1.68	0.55	0.67	0.21	2.12	0.50	0.94	0.36	2.50	0.90

*Remained significant in sensitivity analysis.
[a] Unadjusted analysis.
[§] Change in risk ratio per IQR increase.

Table 5
Associations between PFAS exposure and rhinitis at ages 10 and 16 years for all, girls and boys. Results are shown for the main analyses and for sensitivity analyses where the results differed from the main analysis.

	All				Girls				Boys			
Rhinitis	RR[§]	CIL	CIU	P	RR	CIL	CIU	p	RR	CIL	CIU	P
10 years	N = 377				N = 185				N = 192			
PFHpA	0.91	0.68	1.21	0.51	0.89	0.55	1.45	0.64	0.86	0.62	1.18	0.35
PFOA	0.84	0.61	1.15	0.28	0.84	0.48	1.49	0.56	0.77	0.53	1.11	0.16
PFNA	1.06	0.84	1.32	0.63	1.48	0.92	2.37	0.10	0.87	0.66	1.13	0.30
PFDA	1.11	0.85	1.44	0.44	1.25	0.78	2.02	0.36	0.96	0.76	1.21	0.74
PFUnDA	1.04	0.83	1.32	0.73	0.86	0.49	1.50	0.59	1.05	0.86	1.29	0.62
PFHxS	0.98	0.93	1.02	0.39	0.94	0.81	1.09	0.42	0.99	0.95	1.03	0.64
PFHpS	0.88	0.67	1.14	0.34	0.99	0.64	1.52	0.97	0.73	0.52	1.03	0.08
PFOS	0.98	0.74	1.30	0.92	0.97	0.58	1.62	0.92	0.90	0.66	1.23	0.52
PFOSA	0.49	0.20	1.18	0.11	0.24	0.03	1.69	0.15	0.53	0.20	1.41	0.20
16 years	N = 375				N = 184				N = 193			
PFHpA	0.94	0.80	1.10	0.44	0.99	0.77	1.26	0.92	0.83	0.68	1.03	0.09
PFOA	**1.08**	**1.01**	**1.14**	**0.02**	1.16	0.90	1.50	0.25	1.06	0.84	1.32	0.63
PFNA	1.02	0.89	1.17	0.81	**1.56***	**1.18**	**2.06**	**0.002**	0.84[a]	0.67	1.04	0.10
PFDA	1.06	0.91	1.24	0.45	1.24	0.93	1.66	0.15	0.97	0.80	1.19	0.79
PFUnDA	0.99	0.85	1.15	0.90	0.98	0.72	1.34	0.91	0.97	0.82	1.15	0.74
PFHxS	1.00	0.98	1.01	0.84	0.99	0.96	1.03	0.77	1.00	0.98	1.01	0.89
PFHpS	1.01[a]	0.91	1.10	0.99	**1.36***	**1.28**	**1.45**	**< 0.001**	0.81[a]	0.71	1.06	0.16
PFOS	1.03	0.90	1.19	0.69	1.15	0.91	1.45	0.24	0.92**	0.72	1.19	0.55
$PFOS_{sens}$									**0.64**[a]	**0.46**	**0.88**	**0.006**
PFOSA	0.78	0.51	1.20	0.26	1.17	0.66	2.05	0.59	**0.49**	**0.26**	**0.94**	**0.03**

*Remained significant in sensitivity analysis.
**Non-linear association.
[a] Unadjusted analysis.
[§] Change in risk ratio per IQR increase.

associations except for PFHpA, remained statistically significant in the sensitivity analyses with similar p-values as in the main analyses. All the statistically significant associations between PFASs and AD were linear.

In the cross-sectional design at 10 years, no associations were found between PFASs and rhinitis (Table 5). In the longitudinal design, PFNA and PFHpS were positively associated with rhinitis at age 16 years in girls (RR[95% CI]: 1.56[1.18;2.06] per IQR of 0.28 ng/mL and 1.36[1.28;1.45] per IQR of 0.16 ng/mL, respectively) (Table 5, Supplementary Fig. 4B). Before the Bonferroni adjustment, PFOA was positively associated with rhinitis in all participants (1.08[1.01;1.14] per IQR of 1.77 ng/mL), whereas there was an inverse association for PFOSA in boys (0.49[0.26;0.94]). With regard to the additional analyses, only the associations for rhinitis in girls remained statistically significant in the sensitivity analyses. Regarding rhinitis in boys and PFOS there was an inverse relation not observed in the main analysis (0.64[0.46–0.88] per IQR of 8.86 ng/mL). In the main analyses, all statistically significant associations were linear, except for PFOS and rhinitis in boys (Supplementary Fig. 5A). It was not possible to fit a gam model for PFOS and rhinitis in boys for the sensitivity analysis. However, by visually inspection of the graph, there seems to be a linear association when omitting the extreme values.

In the Boneferroni adjusted analyses at age 10 years, there was a positive association between PFOA and PFHxS and SPT in all participants (RR[95% CI]: 1.11[1.07;1.15] per IQR of 1.77 ng/mL and 1.01[1.00;1.02] per IQR of 0.86 ng/mL, respectively) and between PFHxS and SPT in boys (1.00[1.00;1.01] per IQR of 1.26 ng/mL) (Table 6). However, inverse associations were seen for PFNA and PFHpS and SPT in boys (0.94[0.92;0.95] per IQR of 0.31 ng/mL and 0.97[0.96;0.99] per IQR of 0.21 ng/mL, respectively). Before the Bonferroni adjustment, there was also an inverse association between PFHpA and SPT in boys (0.91[0.84;0.99] per IQR of 0.14 ng/mL). Regarding the additional analyses, only the association between PFNA and SPT in boys remained statistically significant in the sensitivity analysis. Additionally, a positive association was observed for PFHpS in all participants (1.23[1.19;1.28] per IQR of 0.20 ng/mL), as well as an inverse relation for PFOS in boys (0.87[0.85;0.90] per IQR of 8.86 mg/mL). All the statistically significant associations between PFASs and SPT at 10 years were linear, except for PFHpS in all participants and PFHxS in boys (Supplementary Fig. 5B–E). In the sensitivity analysis, the association between PFHpS and SPT in all participants became linear. Although there seems to be a pattern that PFASs may give increased risk of allergic sensitisation in all participants but a decreased risk in boys in the cross-sectional design, the findings should be interpreted with caution due to difference between the main and sensitivity analyses and the non-linear nature of some of the associations.

At age 16 years, SPT was positively associated with PFOA, PFHpS and PFOS in all participants (1.07[1.05;1.08] per IQR of 1.77 ng/mL, 1.06[1.04;1.07] per IQR of 0.20 ng/mL and 1.09[1.03;1.15] per IQR of 9.23 ng/mL, respectively), and with PFOA and PFHpS in boys (1.05[1.03;1.06] per IQR of 1.86 ng/mL and 1.04[1.03;1.05] per IQR of 0.21 ng/mL, respectively) (Table 6). In the additional analysis, the results for PFOA and PFHpS in all participants and for PFHpS in boys remained statistically significant in the sensitivity analyses. The associations between PFOA and SPT were non-linear for both all participants and boys (Supplementary Fig. 5F–I). However, for all participants, the association between PFOA and SPT became linear in the sensitivity analysis.

Unadjusted statistical analyses for AD, rhinitis and SPT are shown in Supplementary Tables 8–10, respectively.

3.5. Airways infections

In the main analyses, there were no statistically significant associations between PFASs and common cold between the ages 10 and 16 years. A pattern of a decreased risk of common cold at 16 years with increasing PFAS levels was observed before the Bonferroni adjustment (Table 7). After the Bonferroni adjustment, the only statistically significant association was between PFDA and having common cold at least 3 times the last 12 months (all participants; OR[95% CI]: 1–2 times last 12 months 0.78[0.55;1.09] and ≥ 3 times last 12 months 0.56[0.37;0.84] per IQR of 0.13 ng/mL (ref. 0 times)). However, this

Table 6
Associations between PFAS exposure and skin prick test (SPT) at ages 10 and 16 years for all, girls and boys. Results are shown for the main analyses and for sensitivity analyses where the results differed from the main analysis.

	All				Girls				Boys			
	N = 376				N = 184				N = 192			
10 years	**RR**[§]	**CIL**	**CIU**	**P**	**RR**	**CIL**	**CIU**	**P**	**RR**	**CIL**	**CIU**	**P**
PFHpA	0.91	0.74	1.11	0.33	0.86	0.58	1.29	0.46	**0.91**	**0.84**	**0.99**	**0.02**
PFOA	**1.11**	**1.07**	**1.15**	**< 0.001**	1.19	0.79	1.80	0.39	1.02[a]	0.82	1.27	0.84
PFNA	1.06	0.91	1.22	0.46	1.35	0.91	2.02	0.14	**0.94***	**0.92**	**0.95**	**< 0.001**
PFDA	1.15	0.99	1.35	0.07	1.37	0.91	2.04	0.13	1.00	0.98	1.02	0.96
$PFDA_{sens}$	**1.26**	**1.06**	**1.51**	**0.01**								
PFUnDA	1.05	0.91	1.21	0.53	0.92	0.59	1.42	0.69	0.98[a]	0.83	1.16	0.84
PFHxS	**1.01**	**1.00**	**1.02**	**0.002**	0.94	0.84	1.07	0.39	**1.00[a]****	**1.00**	**1.01**	**0.003**
PFHpS	1.04**	0.96	1.13	0.35	1.23	0.95	1.60	0.12	**0.97**	**0.96**	**0.99**	**< 0.001**
$PFHpS_{sens}$	**1.23**	**1.19**	**1.28**	**< 0.001**								
PFOS	1.10	0.95	1.26	0.21	0.97	0.65	1.44	0.86	0.98	0.96	1.01	0.17
$PFOS_{sens}$									**0.87**	**0.85**	**0.90**	**< 0.001**
PFOSA	0.86	0.53	1.40	0.55	0.95	0.39	2.27	0.90	0.73	0.43	1.22	0.23
16 years	N = 375				N = 185				N = 191			
PFHpA	1.02	0.91	1.14	0.75	1.02	0.82	1.27	0.85	0.99[a]	0.87	1.13	0.93
PFOA	**1.07*/****	**1.05**	**1.08**	**< 0.001**	1.13	0.86	1.47	0.38	**1.05[a]****	**1.03**	**1.06**	**< 0.001**
PFNA	1.03	0.94	1.14	0.54	1.11	0.86	1.43	0.43	0.97[a]	0.86	1.10	0.66
$PFNA_{sens}$	**1.16**	**1.10**	**1.22**	**< 0.001**								
PFDA	1.12	0.87	1.45	0.37	1.13	0.86	1.48	0.37	1.02[a]	0.90	1.16	0.76
$PFDA_{sens}$	**1.18**	**1.08**	**1.28**	**< 0.001**								
PFUnDA	1.02	0.93	1.13	0.63	1.00	0.78	1.28	0.99	0.99[a]	0.89	1.11	0.90
PFHxS	1.00	1.00	1.01	0.30	0.99	0.95	1.02	0.46	1.00[a]	1.00	1.01	0.19
PFHpS	**1.06***	**1.04**	**1.07**	**< 0.001**	1.09	0.91	1.30	0.35	**1.04[a]****	**1.03**	**1.05**	**< 0.001**
PFOS	**1.09**	**1.03**	**1.15**	**0.001**	0.99	0.80	1.23	0.93	1.07[a]	0.97	1.17	0.18
PFOSA	1.04	0.78	1.39	0.77	1.06	0.66	1.71	0.81	0.94[a]	0.66	1.35	0.75

Categories: negative SPT and any positive SPT.
*Remained significant in sensitivity analysis.
**Non-linear association.
[a] Unadjusted analysis.
[§] Change in risk ratio per IQR increase.

association did not remain statistically significant in the sensitivity analysis. In the sensitivity analyses after Bonferroni adjustment, inverse associations between PFHpS and the highest category of common cold (0.56[0.39;0.78] per IQR of 0.20 ng/mL), as well as between PFOS and both categories of common cold (1–2 times last 12 months 0.47[0.29;0.75] and ≥ 3 times last 12 months 0.37[0.22;0.63] per IQR of 9.23 ng/mL) became statistically significant for all participants. Furthermore, in boys an inverse associations were seen for PFOS and the lowest category of common cold ((1–2 times last 12 months 0.38[0.19;0.74] per IQR of 8.86 ng/mL). Although these associations were not statistically significant in the main analysis, together with the findings before the Bonferonni adjustment, they give some support of a decreased risk of common cold with increasing PFAS levels. The statistically significant associations between PFASs and common cold at 16 years were linear.

In all participants, there was an increased risk of LRTI between 10 and 16 years of age with increasing levels of PFHpA, PFHpS and PFOS (RR[95% CI: 1.28[1.08;1.51] per IQR of 0.13 ng/mL, 1.12[1.09;1.16] per IQR of 0.20 ng/mL and 1.34[1.17;1.55] per IQR of 9.23 ng/mL, respectively) (Table 8). Furthermore, there was a positive association for PFOA in girls (1.49[1.15;1.92] per IQR of 1.63 ng/mL) and for PFHpS and PFOS in boys (1.01[1.06;1.14] per IQR of 0.21 ng/mL and 1.33[1.26;1.39] per IQR of 8.86 ng/mL, respectively). Before the Bonferroni adjustment, there was also a positive association between LRTI in all participants and PFOA (1.10[1.02;1.19] per IQR of 1.77 ng/mL). Regarding the additional analyses, the associations between LRTI in all participants and PFHpA, PFHpS and PFOS remained statistically significant in the sensitivity analyses. In addition, in the sensitivity analysis, PFNA and LRTI in boys became positively associated after the Bonferroni adjustment (1.39[1.14;1.68] per IQR of 0.31 ng/mL). With regard to linearity, the associations between LRTI in all participants and PFOA and PFOS were non-linear in the main analyses (Supplementary Fig. 6A–D). The association between LRTI in all participants and PFOA became linear in the sensitivity analyses.

At 16 years of age, PFHpA was positively associated with LRTI in all participants and in girls (1.69[1.28;2.24] and 2.20[1.27;3,82] per IQR of 0.13 ng/mL, respectively), but these findings did not remain statistically significant in the sensitivity analyses. In addition, the association for PFHpA in girls was non-linear (Supplementary Fig. 6E).

Unadjusted statistical analyses for common cold and LRTI are shown in Supplementary Tables 11 and 12, respectively.

4. Discussion

The present study examined the association between PFAS concentrations in serum samples from 10 year old children and asthma and allergy related health outcomes and airways infections at age 10 years (cross-sectional design), the period between 10 and 16 years and/or at age 16 years (longitudinal designs). To compare our findings to previous studies, and further investigate the importance of gender, exposure period and study design, a summary of comparable studies on asthma and allergy related outcomes and airways infections are presented in Supplementary Tables 13 and 14, respectively.

4.1. Asthma related outcomes

In the cross-sectional design at age 10 years, there were positive associations between PFOA and PFOS and FEV1 % predicted in all participants, and for PFOA in boys. However, these findings are considered to be weak since they were no longer statistically significant after the Bonferroni adjustment. In addition, none of the associations remained statistically significant in the sensitivity analysis, and the association between PFOS and FEV1 % predicted were non-linear in the main analysis. In the longitudinal design at age 16, no associations were observed between the PFASs and FEV1 % predicted.

To our knowledge, only one study on lung function measurements

Table 7
Associations between PFAS exposure and common colds between ages 10 and 16 years and at 16 years for all, girls and boys. Results are shown for the main analyses and for sensitivity analyses where the results differed from the main analysis.

		All				Girls				Boys			
		OR[§]	CIL	CIU	P	OR	CIL	CIU	P	OR	CIL	CIU	P
10–16 years	**Times Ref. 1–2**	N = 375				N = 184				N = 191			
PFHpA	3–5	0.58	0.27	1.29	0.18	0.50	0.10	2.45	0.39	0.75[a]	0.32	1.75	0.50
	> 5	0.66	0.34	1.35	0.26	0.92	0.22	3.86	0.91	0.64	0.29	1.28	0.25
PFOA	3–5	1.23	0.33	4.58	0.76	1.32	0.19	9.21	0.78	1.41[a]	0.29	6.89	0.67
	> 5	1.29	0.36	4.64	0.70	1.67	0.26	1.09	0.59	1.38	0.29	6.54	0.69
PFNA	3–5	1.15	0.39	3.40	0.80	0.70	0.14	3.58	0.67	1.46[a]	0.38	5.63	0.58
	> 5	1.01	0.35	2.89	0.99	0.69	0.14	3.27	0.64	1.19	0.31	4.47	0.80
PFDA	3–5	1.69	0.46	6.18	0.43	0.79	0.12	5.07	0.80	2.26[a]	0.44	11.7	0.33
	> 5	1.36	0.39	4.80	0.63	0.74	0.12	4.41	0.74	1.59	0.32	7.89	0.57
PFUnDA	3–5	2.38	0.48	11.86	0.29	1.22	0.14	1.06	0.86	1.58[a]	0.36	6.89	0.54
	> 5	2.04	0.42	10.0	0.38	1.46	0.18	11.7	0.72	1.22	0.29	5.22	0.79
PFHxS	3–5	0.99	0.93	1.04	0.60	0.98	0.92	1.05	0.61	1.04[a]	0.73	1.49	0.81
	> 5	0.97	0.93	1.03	0.33	0.95	0.88	1.02	0.14	1.05	0.73	1.49	0.80
$PFHxS_{sens}$	3–5					0.93	0.82	1.05	0.25				
	> 5					**0.89**	**0.79**	**1.0**	**0.05**				
PFHpS	3–5	1.08	0.54	2.14	0.83	0.99	0.33	2.97	0.98	1.77[a]	0.37	8.41	0.47
	> 5	0.92	0.47	1.82	0.82	0.75	0.26	2.16	0.59	1.59	0.34	7.48	0.56
PFOS	3–5	1.26	0.34	4.55	0.73	0.86	0.16	4.75	0.86	2.54[a]	0.38	17.3	0.34
	> 5	1.16	0.33	4.07	0.82	1.07	0.21	5.45	0.93	1.99	0.30	13.2	0.48
PFOSA		/				/				/			
16 years	**Times last 12 months Ref. 0**	N = 375				N = 184				N = 191			
PFHpA	1–2	0.91	0.66	1.26	0.57	1.14	0.63	2.05	0.67	0.81	0.55	1.19	0.28
	≥ 3	0.74	0.51	1.08	0.12	0.97	0.53	1.80	0.93	0.62	0.36	1.05	0.07
PFOA	1–2	0.98	0.76	1.26	0.86	1.29	0.66	2.51	0.45	0.93	0.71	1.23	0.61
	≥ 3	0.73	0.51	1.06	0.10	0.86	0.42	1.76	0.68	0.81	0.52	1.25	0.33
$PFOA_{sens}$	1–2	0.83	0.57	1.22	0.35								
	≥ 3	**0.64**	**0.42**	**1.0**	**0.05**								
PFNA	1–2	0.86	0.66	1.12	0.25	0.84	0.44	1.59	0.58	0.84	0.62	1.14	0.27
	≥ 3	**0.63**	**0.44**	**0.91**	**0.01**	0.81	0.41	1.57	0.53	**0.55**	**0.33**	**0.91**	**0.02**
PFDA	1–2	0.78	0.55	1.09	0.15	0.81	0.41	1.58	0.53	0.76	0.50	1.12	0.18
	≥ 3	**0.56**	**0.37**	**0.84**	**0.006**	0.73	0.36	1.45	0.37	**0.47**	**0.26**	**0.84**	**0.01**
PFUnDA	1–2	0.81	0.62	1.07	0.13	1.33	0.64	2.75	0.44	0.71	0.50	1.00	0.06
	≥ 3	**0.64**	**0.44**	**0.92**	**0.02**	1.08	0.51	2.29	0.84	**0.54***	**0.32**	**0.92**	**0.02**
PFHxS	1–2	0.98	0.96	1.00	0.10	0.98	0.93	1.00	0.11	0.99	0.96	1.01	0.36
	≥ 3	0.97	0.94	1.00	0.10	0.87	0.74	1.01	0.08	0.99	0.96	1.03	0.70
$PFHxS_{sens}$	1–2	**0.94**	**0.89**	**1.00**	**0.04**								
	≥ 3	**0.91**	**0.89**	**1.00**	**0.02**								
PFHpS	1–2	**0.81**	**0.67**	**0.99**	**0.04**	**0.65***	**0.42**	**1.00**	**0.05**	0.83	0.65	1.06	0.13
	≥ 3	0.80	0.63	1.01	0.06	**0.62**	**0.39**	**1.00**	**0.05**	0.89	0.71	1.23	0.33
$PFHpS_{sens}$	1–2	**0.70**	**0.54**	**0.92**	**0.01**					0.71	0.49	1.02	0.06
	≥ 3	**0.56**	**0.39**	**0.78**	**0.001**					**0.56**	0.34	0.92	**0.02**
PFOS	1–2	0.82	0.61	1.09	0.18	0.88	0.54	1.43	0.61	0.76	0.53	1.11	0.15
	≥ 3	**0.67**	**0.47**	**0.96**	**0.03**	0.72	0.41	1.26	0.25	0.73	0.46	1.19	0.21
$PFOS_{sens}$	1–2	**0.47**	**0.29**	**0.75**	**0.002**					**0.38**	**0.19**	**0.74**	**0.005**
	≥ 3	**0.37**	**0.22**	**0.63**	**< 0.001**					**0.39**	**0.18**	**0.84**	**0.02**
PFOSA	1–2	0.61	0.27	1.37	0.23	0.66	0.18	2.36	0.52	0.60	0.21	1.75	0.35
	≥ 3	0.48	0.19	1.18	0.11	0.77	0.21	2.84	0.70	0.24	0.06	1.01	0.05

/Not possible to fit a valid model.
*Remained significant in sensitivity analysis.
[a] Unadjusted analysis.
[§] Change in odds ratio per IQR increase.

has reported an inverse association between prenatal exposure to PFOA and forced vital capacity (FVC) at age four years (Manzano-Salgado et al., 2019) (Supplementary Table 13). In the present study and two other studies, no association were observed between PFASs and lung function measurements regardless of gender, exposure period or study design (Agier et al., 2019; Impinen et al., 2018).

In the cross-sectional design at age 10 years, PFHpA, but none of the other PFASs, was positively associated with asthma ever in girls (RR [95%CI]: 1.31[1.08; 1.60] per IQR increase). Our observation on asthma is in line with the findings in four of the five other studies with a cross-sectional design all reporting on an increased risk of asthma with increasing PFAS levels (OR range: 1.8–4.1 per IQR increase or OR = 2 per doubling of the PFAS level) (Averina et al., 2018; Dong et al., 2013; Humblet et al., 2014; Zhu et al., 2016). In 9–16 years olds, PFOA, PFNA, PFDA, PFHxS and PFOS were positively associated with asthma in all participants (Dong et al., 2013). Whereas, only PFHxS and PFOS were positively associated with asthma in 16 years old children (Averina et al., 2018), and PFOA in 12–19 years old adolescents (Humblet et al., 2014). In the latter study, PFOS was inversely related to asthma. Stratified analyses based on gender were performed in the Taiwan Genetic and Biomarker Study (Dong et al., 2013; Zhu et al., 2016). Both genders showed a positive association between PFASs and asthma ever in 9–16 years old, where boys and girls reached statistical significance for five and three of the PFASs examined, respectively (OR range: 2.6–4.4 and 1.4–5.0 per IQR increase, respectively).

In the present study, no significant associations were observed for the longitudinal designs for asthma up to age 16 years. In the three longitudinal studies on asthma reporting on significant findings,

Table 8
Associations between PFAS exposure and LRTI between ages 10 and 16 years and the last 12 months at 16 years for all, girls and boys. Results are shown for the main analyses and for sensitivity analyses where the results differed from the main analysis.

	All				Girls				Boys			
	RR[§]	CIL	CIU	P	RR	CIL	CIU	p	RR	CIL	CIU	p
10–16 years	N = 375				N = 184				N = 191			
PFHpA	**1.28***	**1.08**	**1.51**	**0.004**	1.29	0.94	1.78	0.11	1.20	0.97	1.50	0.10
PFOA	**1.10**[a]**	**1.02**	**1.19**	**0.01**	**1.49**	**1.15**	**1.92**	**0.002**	1.11	0.97	1.26	0.12
PFNA	1.12	0.94	1.32	0.20	1.17	0.75	1.83	0.50	1.09	0.90	1.31	0.38
$PFNA_{sens}$	**1.32**	1.07	1.63	**0.01**					**1.39**	**1.14**	**1.68**	**0.001**
PFDA	1.09	0.86	1.39	0.46	1.08	0.67	1.73	0.75	1.09	0.83	1.44	0.53
PFUnDA	1.00	0.80	1.26	0.99	0.94	0.57	1.55	0.82	1.02	0.81	1.28	0.89
PFHxS	0.98	0.95	1.02	0.38	0.99	0.93	1.05	0.68	0.98	0.94	1.03	0.48
PFHpS	**1.12***	**1.09**	**1.16**	**< 0.001**	1.20	0.91	1.60	0.20	**1.01**	**1.06**	**1.14**	**< 0.001**
PFOS	**1.34**[a]**/****	**1.17**	**1.55**	**< 0.001**	1.23**	0.91	1.66	0.17	**1.33**[a]	**1.26**	**1.39**	**< 0.001**
$PFOS_{sens}$					**1.75**	**1.12**	**2.74**	**0.01**				
PFOSA	0.84	0.44	1.59	0.59	1.63	0.71	3.74	0.25	0.42	0.14	1.17	0.10
16 years	N = 330				N = 160				N = 170			
PFHpA	**1.69**	**1.28**	**2.24**	**< 0.001**	**2.20**[a]****	**1.27**	**3.82**	**0.005**	1.45	0.88	2.39	0.15
PFOA	1.14	0.81	1.59	0.45	1.61[a]	0.72	3.58	0.25	1.00	0.64	1.59	0.99
PFNA	0.94	0.52	1.70	0.84	2.17[a]	0.72	6.54	0.17	0.56	0.19	1.67	0.30
PFDA	1.34	0.84	2.14	0.26	1.95[a]	0.64	5.87	0.24	1.11	0.65	1.90	0.69
PFUnDA	0.90	0.46	1.75	0.75	1.84[a]	0.68	4.97	0.23	0.49	0.14	1.70	0.27
PFHxS	0.93	0.74	1.18	0.57	0.83[a]	0.28	2.43	0.73	0.95	0.76	1.19	0.64
PFHpS	0.80	0.40	1.58	0.52	0.84[a]	0.24	3.02	0.79	0.74	0.31	1.76	0.49
PFOS	0.82	0.40	1.69	0.60	1.11[a]	0.41	3.00	0.84	0.62	0.22	1.78	0.38
PFOSA	0.57	0.07	4.32	0.58	1.80[a]	0.19	16.6	0.60	/			

/Not possible to fit a valid model.
*Remained significant in sensitivity analysis.
[a] Unadjusted analysis.
[§] Change in risk ratio per IQR increase.

prenatal exposure to PFUnDA was inversely related to asthma at age 7 years (OR[95%CI]: 0.68[0.48;0.97] per IQR increase) (Impinen et al., 2019), and PFNA to asthma at age four years (RR[95%CI]: 0.74[0.57;0.96] per doubling in PFNA level) (Manzano-Salgado et al., 2019), whereas PFOS at age 16 years were positively associated with asthma at age 18 years (doubling of OR per IQR) (Averina et al., 2018) (Supplementary Table 13). It should be noted that none of these results were adjusted for multiple comparisons, thus there is a chance for false positive findings. In the other three studies with a longitudinal design, no associations between PFASs and asthma were found (Granum et al., 2013; Impinen et al., 2018; Smit et al., 2015). Regardless of study design, gender differences were assessed in five studies on asthma and the asthma related outcomes, in which a gender difference were observed in three of the studies, but without a clear pattern.

Interpreting the findings with regard to asthma is challenging because asthma is a heterogeneous disease in terms of severity, natural history and treatment responsiveness, and this heterogeneity reflects different underlying mechanisms of the disease (endotype) (Kuruvilla et al., 2019). Thus, even though the participants may display similar clinical symptoms of asthma, the different endotypes may play an important role in how PFASs can affect disease development and/or exacerbations of disease symptoms. None of the published studies have classified asthma patients according to possible endotypes. Interestingly, there is some support for an increased risk of asthma with increased PFAS levels in studies with a cross-sectional but not in the longitudinal designed studies. Based on the studies summarised in Supplementary Table 13, we hypothesise that the increased risk of asthma seen in the cross-sectional studies may reflect an exacerbation of already existing disease, while the lack of consistent findings in longitudinal studies can suggest that PFASs play a minor role in diseases development.

In previous studies, there is little evidence for an effect on PFASs on wheeze. Two out of nine studies found a negative association between prenatal PFAS exposure and wheeze at 5–9 or 7 years of age, but without an adjustment for multiple comparisons (Impinen et al., 2019; Smit et al., 2015) (Supplementary Table 13).

Overall, no firm conclusions can be drawn regarding the effect of PFASs on asthma and asthma related health outcomes.

4.2. Allergy related outcomes

With regard to cross-sectional designed studies, no significant associations between PFASs and AD were reported in the present study and the study by Averina et al. (Averina et al., 2018) (Supplementary Table 13). However, in the longitudinal designs in the present study, PFNA and PFUnDA were inversely related to AD in girls between the age of 10 and 16 years (RR[95%CI]: 0.51[0.35;0.73] and 0.45[0.29;0.69] per IQR increase, respectively). Before adjustment for multiple comparisons, similar associations were also observed for PFDA in girls (0.64[0.42;0.98]) and for PFHxS in all participants and in boys (0.79[0.34;0.99] and 0.71[0.52;0.99] per IQR increase, respectively). As well as between PFHxS and AD in boys at age 16 years (boys: 0.59[0.37;0.95] per IQR increase). These findings are in line with previous studies (Impinen et al., 2019; Manzano-Salgado et al., 2019; Okada et al., 2014) (Lowe et al., 2019) (Supplementary Table 13). Impinen and co-workers found a reduced risk for AD ever with increasing prenatal exposure to PFUnDA (OR[95%CI]: 0.69[0.55;0.86] per IQR increase) in the MoBa-study (Impinen et al., 2019). In the large Hokkaido Study (n = 2062), they found a reduced risk for eczema with increased PFUnDA and PFTrDA in girls (OR[95%CI]: 0.5[0.3;0.81] and 0.39[0.23;0.64] per IQR increase, respectively) and PFTrDA in all participants (0.62[0.45;0.86] per IQR increase) (Okada et al., 2014). In Lowe et al., PFUnDA was inversely associated with both atopic and non-atopic AD (multinomial OR[95%CI]: 0.06[0.01;0.76] and 0.05[< 0.01;0.18], respectively) (Lowe et al., 2019). In the INMA cohort, eczema was inversely related to PFOS (RR[95%CI]: 0.86[0.75;0.98] per doubling of PFOS) (Manzano-Salgado et al., 2019). No adjustment for multiple comparisons was performed in the three latter studies. On the contrary, a study reported an increased risk of AD with increasing levels of PFOA (Kaplan-Meier estimate, p = 0.014)

(Wen et al., 2019). Before adjustment for multiple comparisons, a positive association were also observed in the present study between PFHpA and AD at 16 years in girls (1.38[1.04;1.83] per IQR increase). However, this association did not remain statistically significant in the sensitivity analysis and should therefore be interpreted with caution. Interestingly, no associations between PFASs and AD were observed in the ECA study when examining the effect of prenatal PFAS exposure on AD ever at age 2 and 10 years (Impinen et al., 2018). There are also other studies reporting on no associations between PFASs and AD (Averina et al., 2018; Granum et al., 2013; Okada et al., 2012; Smit et al., 2015; Wang et al., 2011). Overall, there is increasing evidence for a reduced risk of AD with increasing PFAS levels, especially in girls. Furthermore, most of the associations with AD are seen in relation to exposure to perfluorinated carboxylic acids.

Even though no effects of PFAS exposure on AD were observed in the two studies with a cross-sectional design, as opposed to the reduced risk seen in many of longitudinal designed studies, more studies are needed before a conclusion can be drawn with regard to possible effects of the study design. Ten out of the 12 studies on AD examined the effect of prenatal exposure to PFASs (Supplementary Table 13). Most atopic children has the highest incidence of AD during the first months of life, whereas the highest period prevalence is during the first three years of life (WAO, 2019). It could, therefore be expected that the effect of prenatal exposure to PFAS would be most evident with respect to AD development in early childhood. No clear pattern could, however, be identified in that there were overlap in the participants ages in studies finding inverse, positive and no associations (0–7 years, 5–16 years and 0–18 years, respectively).

To our knowledge, rhinitis has only been included in four studies with either a cross-sectional or a longitudinal design (Supplementary Table 13). In cross-sectional designs, the present study and the study by Averian et al. (Averina et al., 2018) showed no associations, whereas Stein *et al.* reported a positive association between PFOA and rhinitis in 12–19 years old adolescents (Stein et al., 2016). With regard to longitudinal design, we found an increased risk of rhinitis in girls at age 16 years with increasing levels of PFNA and PFHpS. In the main analysis, there was no association between PFOS and rhinitis in boys at age 16 years, but an inverse association were observed in the sensitivity analysis. This may be due to the non-linear association in the main analysis (Supplementary Fig. 5A). Furthermore, no significant associations were reported by Impinen *et al.* investigating the effect of prenatal exposure to PFASs using the same cohort as in the present study (Impinen et al., 2018). In the study by Averina et al, no associations were reported between PFAS exposure at age 16 years and rhinitis at age 18 years (Averina et al., 2018). Based on these studies, no conclusion can be drawn with regard to the effect of PFAS exposure on rhinitis due to few studies and inconsistent findings.

In the present study, there was a pattern of a positive association between PFASs and allergic sensitisation in all participants, regardless of study design. In boys, both inverse and positive associations were observed in the cross-sectional design, whereas only positive associations were seen in the longitudinal design. These findings should, however, be interpreted with caution since: (1) At age 10 years, of the five statistically significant associations (after Bonferroni adjustment) only one remained statistically significant in the sensitivity analysis (PFNA in boys). Furthermore, several of the relative risks were close to one, thus questioning the biological relevance of these findings (PFHpS boys: 0.97; PFHxS all participants and boys: 1.01); (2) At age 16 years, only two of the five statistically significant associations both remained statistically significant in the sensitivity analyses and had a linear association (PFHpS, all participants and boys). Three previously published studies examining allergic sensitisation (SPT or serum IgE) were identified (Impinen et al., 2018; Stein et al., 2016; Wang et al., 2011) (Supplementary Table 13). In cross-sectional studies, cord blood levels of PFOA and PFOS were positively associated with serum IgE (Wang et al., 2011), whereas in adolescents PFOS and IgE were inversely related (Stein et al., 2016). It should be noted that no adjustment for multiple comparisons were performed in the two latter studies, thus increasing the chance of false positive findings. In the ECA-study, prenatal exposure to PFASs were not associated with SPT and/or serum IgE levels at 2 or 10 years of age (Impinen et al., 2018). Overall, there is little evidence for an association between PFAS exposure and allergic sensitisation.

When it comes to rhinitis and allergic sensitisation, there are inconsistent findings or too few studies to be able to draw any conclusion on possible effects of gender, exposure period and study design.

4.3. Airways infections

None of the PFASs were associated with common cold between the ages 10 and 16 years, and only the highest frequency category (≥3 times/last year) of common cold at age 16 years remained significantly associated with PFDA after correcting for multiple comparison. However, at age 16 years, the overall pattern was a decreased risk of common cold with increasing PFASs levels. A similar pattern was reported in the MoBa study, but the RR's were close to one and the authors questioned the biological relevance of their findings (Impinen et al., 2019). In two other studies, however, prenatal exposure to PFOA or PFUnDA was associated with an increased risk of common cold (Granum et al., 2013; Impinen et al., 2018) (Supplementary Table 14). There are no clear patterns with regard to gender differences, exposure period or study design that may explain these conflicting findings.

Regarding LRTI between the ages 10 and 16 years in all participants, PFHpA, PFOA, PFHpS and PFOS were positively associated with LRTI (RR range: 1.1–1.3). In the sensitivity analysis, there was also a positive association for PFNA. Even though the association between LRTI and PFOA and PFOS were non-linear in the main analysis, and that PFOA and PFNA did not reach statistically significance after the Bonferroni adjustment, the overall trend was an increased risk of LRTI with increasing PFAS levels. The same pattern were also observed in the stratified analysis, where positive associations were observed between LRTI and PFOA in girls and for PFHpS and PFOS in boys (RR range: 1.0–1.5). In the sensitivity analysis, PFOS was associated with increased risk of LRTI in girls and with PFNA for boys. At age 16 years, LRTI was positively associated with PFHpA in all participants and in girls. The latter association was non-linear. In the same cohort, prenatal exposure to PFOA, PFUnDA, PFOS and PFOSA were positively associated with LRTI at age 10 years (β range: 0.1–0.5 per doubling of PFAS level) (Impinen et al., 2018). Positive associations between prenatal PFOA, PFHxS, PFOS and PFHpS exposure and LRTI at age 3 years were also reported in the MoBa cohort (RR range: 1.2–1.3 per IQR increase) (Impinen et al., 2019). Whereas, one study found no significant associations between prenatal exposure to PFASs and LRTI (Manzano-Salgado et al., 2019). These results lend support for an immunosuppressive effect of PFASs with regard to LRTI. Since no studies on LRTI had a cross-sectional design, no conclusions can be drawn with regard to study design. The increased risk of LRTI observed in longitudinal studies at different exposure periods, however, suggests that PFAS may induce immunosuppression both after prenatal and childhood exposure.

Studies on other airways infections than common cold and LRTI lends further support for immunosuppressive effects of PFASs. There are studies reporting a positive association between prenatal exposure to PFOA and PFOS and the number of days with fever (incidence rate-ratio[95% CI]: 1.65[1.23;2.18]) (Dalsager et al., 2016), and between PFOS and PFHxS and total infectious disease (defined as having at least one of the following infections: otitis media, pneumonia, respiratory syncytial virus infection, varicella) (Q4 vs. Q1 OR[95% CI]: 1.61[1.18;2.21] p for trend = 0.008 and 1.55[0.98;2.45] p for trend = 0.045, respectively) (Goudarzi et al., 2017). Furthermore, prenatal exposure to PFASs has been related to increased risk of throat infections (RR range: 1.10–1.47), whereas pseudocroup and ear

infections showed inconsistent results (Impinen et al., 2019). The majority of the findings in Impinen *et al.* were found in girls only indicating a gender difference in the effect of PFASs on these diseases.

4.4. Gender

Gender may play a role in that several exposure-health associations were observed in one of the genders only (Supplementary Tables 13 and 14). The cause for this observed gender difference in exposure-health effects is, however, unknown. It can be hypothesised that they may be due to different PFAS exposure levels between genders. In the present study however, differences in PFAS levels are likely of less importance in that boys had only slightly higher PFAS levels than girls.

There are indications that PFASs can affect sex hormones. In a paper from the NHANES-study, Zhou and co-workers investigates sex hormones and interactions with PFASs and asthma. They found that increases in PFAS concentrations were associated with asthma among adolescents, and that these associations seemed stronger in those with higher estradiol levels (Zhou et al., 2017). Whether this can explain some of the gender differences in the associations between serum PFAS concentrations and the health outcomes in our study is not known.

4.5. Health outcomes

Both decreased risk of AD and increased risk of LRTI and other respiratory tract infections related to PFAS exposure can be due to immunosuppressive effects on the immune system. With regard to asthma, the triggers of disease are not reported for the different study populations. Both airways infections and exposure to allergens are known triggers of asthma. It could be hypothesised that immunosuppressive effects of PFASs resulting in increased risk of airways infections could be an underlying cause for the increased risk of asthma observed in the cross-sectional studies. On the other hand, immunostimulatory effects of PFASs resulting in increased risk of allergic sensitisation could also be an underlying cause for asthma. However, based on the studies on allergic sensitisation, there is insufficient evidence regarding PFAS exposure and increased risk of allergic sensitisation.

Comorbidities between asthma and allergic diseases are prevalent. In the ECA-cohort, at least one allergic comorbidity was present in 59% of the subjects with asthma from 10 to 16 years (Hovland et al., 2014). Thus, exploring asthma and allergy comorbidity phenotypes as the health outcome with regard to PFAS exposure should be considered in future studies.

4.6. PFASs

The functional groups, carbon chain length and isomer forms of PFASs may be of importance for their effect on human health. Included in the present study are perfluoroalkyl carboxylates (PFHpA, PFOA, PFNA, PFDA, PFUnDA), sulfonates (PFHxS, PFHpS, PFOS) and sulfonamides (PFOSA). The carbon chain lengths are between 7 and 11 for the carboxylates and 6 and 8 for the sulfonates, whereas PFOSA has an 8-carbon chain. Several PFASs exist in both branched or linear isomers. However, we have measures of the total PFAS levels including both linear and branched, without the possibility to differ between the different isomers. In general, in humans the sulfonates are eliminated more slowly than the corresponding carboxylates of the same chain length, the elimination rate decreases with increasing chain length, and increases with increased branching. Elimination of PFASs also show sex differences and age-dependencies within certain species. However, the evidence for sex differences in elimination of PFASs in humans is not as strong as in rats (ATSDR, 2018).

In the present and previous published studies, no clear response pattern with regard to either functional groups or carbon chain length could be identified for any of the outcomes, except for AD where the majority of the significant associations were seen for the carboxylates, and mainly carboxylates with a carbon chain consisting of ≥ 9 carbons. These findings indicate a role of both the functional group and chain length for this health outcome.

Surprisingly the less abundant PFASs (PFHpA, PFNA, PFDA, PFUnDA, PFHpS) were more often significantly associated with the health outcomes in the present study. This pattern cannot be explained by either carbon chain length or functional groups.

In the present study, separate statistical analyses were performed for each of the nine PFASs. Alternatively, the sum of the PFASs or sum of the carboxylates/sulfonates could be used. However, the sum of the PFASs would be dominated by the most abundant PFASs, PFOA and PFOS. Alternatively, the separate carboxylates or sulfonates could be included in the same statistical analyses. Since several of the carboxylates were correlated, the statistical analyses could be affected if including all carboxylates in the same statistical analyses (correlation coefficients: PFDA vs PFNA = 070, PFDA vs PFUnDA = 0.73, PFNA vs PFUnDA = 0.64). Other modelling approaches examining the PFASs as a mixture, and thus take consideration of possible confounding across the PFASs, are an option and will be explored in future analyses.

4.7. Strengths and limitations

The present study is a cohort based on participants living in the capitol of Norway and thus, is not designed to be representative of the national population. The longitudinal design of the ECA study with a broad inclusion of participants at the maternity ward and later follow-up examinations including thorough clinical characterisations at ages 10 and 16 years (performed by personnel blinded to the results of previous assessments) reduce the risk of selection bias and risk of misclassification of the health outcomes. The definition of asthma was based on a set of combination of symptoms, medication and clinical diagnosis, further reducing the risk of misclassifications. In addition, core questions from the standardized ISAAC questionnaires were included, making it possible to compare the findings with other studies. Finally, the risk of exposure misclassification is regarded as minimal in that the PFAS levels are based on measured concentrations in blood samples using a well-established and reliable method, as well as the personnel being blinded for any information on the participants when performing the analyses. The high sensitivity and accuracy of the PFAS analytical method and the well-characterised clinical outcomes increases the overall study sensitivity with regard to detecting effects of PFAS exposures on immune health.

Loss to follow-up between 10 and 16 years can be a common feature of long-term cohort studies. However, the effect of this potential bias is considered being low due to the fact that the children attending the 16 years follow-up were similar in terms of health outcomes compared to the ones not attending (Hovland et al., 2013). Furthermore, socio-economic factors, such as the parent's educational level, may affect both the exposure and health outcomes, thus a selection bias or loss to follow-up with regard to socio-economic status could affect the findings. At the 16 years follow-up, mothers of participants with PFAS measurements (n = 378) had higher education compared to the ones without PFAS measurements (n = 162). This is most likely due to chance and not loss to follow-up for mothers with low education in that no similar differences were seen between the ones attending (n = 540) versus not attending (n = 479) the 16 years follow-up irrespective of available PFAS measurements. In addition, the selection of participants to be included in the present study was based on whether the participants had sufficient volume of both cord blood and serum samples at age 10 years available for PFAS measurements.

Multiple health effects and PFASs were examined, thus increasing the risk of false positive findings. However, to reduce the risk of false positives, adjustments for multiple comparisons were applied. Furthermore, additional analyses such as sensitivity analyses excluding extreme PFAS values and check for linearity in the exposure-health associations were performed. These sensitivity analyses indicated that

some extreme PFASs values may influence the results.

5. Conclusion

Our results lend further support for an immunosuppressive effect of PFASs on AD and LRTI, observed in longitudinal study designs. The study also strengthen the evidence of gender differences in that several exposure-health associations were observed in one of the genders only, underlining the importance of performing stratified analyses separating boys and girls. Either due to few studies or inconsistent findings in previously published literature, no clear pattern were seen for the different PFAS exposure periods on the health outcomes. Similarly, no clear pattern were seen regarding study design, with the exception of asthma where effects were mainly observed in the cross-sectional studies.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105259.

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