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Per- and polyfluoroalkyl substances (PFAS) and immune system-related diseases: results from the Flemish Environment and Health Study (FLEHS) 2008–2014

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Abstract

Background The successive FLEHS campaigns assess internal exposure to pollutants and associated early biological and health effects in participants of different age groups.

Materials and methods Mother–newborn pairs ($N = 220$ in 2008–2009, age 18–42 years; $N = 269$ in 2013–2014, age 18–44 years), 197 adolescents 14–15 years (2010–2011), 201 adults 20–40 years (2008–2009) and 205 adults 50–65 years (2014) were recruited. For the various groups of subjects different sets of PFAS were assessed. Perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorobutane sulfonate (PFBS) were determined in cord plasma and peripheral serum as these were the PFAS compounds for which we had access to high quality measurements and which were expected to be present in the highest concentrations. Participants filled out a questionnaire based on the European Community Respiratory Health Survey questionnaire on asthma and allergy. In these cross-sectional studies associations were assessed using stepwise multiple logistic regression, with confounders (including smoking and familial occurrence of the disease) and potential covariates selected on the basis of experience in our previous studies and a literature search. Forest plots of odds ratios summarize the associations between the various PFAS on the one hand and the different immune outcomes on the other hand.

Results For several self-reported immune system-related diseases inverse associations with PFAS serum concentrations were observed. These inverse associations were more pronounced in mothers and adults than in adolescents. A significant inverse association was observed in adults and mothers (for mothers based on measurements on cord plasma) between PFNA, PFOS, and PFHxS and asthma (for mothers also for PFOA), in mothers between PFHxS, PFNA and PFOS and allergic rhinitis, in mothers and adults between PFHxS and PFOS and some forms of allergy (for mothers also for PFOA), in adults between PFOA and eczema, and in adolescents between PFOS and systemic allergy.

Conclusion Internal exposure to PFAS was associated with changes in immunological processes consistent with what has been reported in the literature. Whereas these changes were observed in many publications to be

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associated with adverse health effects, our findings suggest that they can also lead to inverse associations with certain immune system-related diseases.

Keywords PFAS, Perfluorooctane sulfonate, Perfluorohexane sulfonate, Perfluorobutane sulfonate, Perfluorononanoic acid, Asthma, Eczema, Allergies

Background

PFAS are since decades on the market as surfactants repelling water, oil and stains and are used in textile coatings, non-stick cookware, electronics, mist suppressants, and firefighting foams [1]. Their production, use and disposal resulted in the widespread contamination of the environment. Until recently, toxicological research was essentially limited to perfluorooctane sulfonate (PFOS), and perfluorooctanoic acid (PFOA). Nowadays also perfluorohexane sulfonate (PFHxS) and perfluorononanoic acid (PFNA) get a lot of attention in scientific publications.

PFAS are persistent, but are very different from well-known persistent organic pollutants such as polychlorinated biphenyls (PCBs). They do not contain an aromatic ring, do not accumulate in fatty tissues and are much more soluble in water than polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs) [2]. PFAS are thermally, chemically, and biologically inert and so are very useful in many products, but they are also non-biodegradable and bioaccumulative in the environment and food chains.

PFAS can persist for a long time both in the environment and in the human body, where it may pose a threat to human health [3]. Average half-life in humans has been estimated to be 5.4 years for PFOS, 3.8 years for PFOA and 8.5 years for PFHxS by Zhang et al. [4] and to be 1.77 years for PFOA, 2.87 years for PFHxS and 2.93 years for linear PFOS by Xu et al. [5]. According to EFSA [6], estimated half-lives for PFOA, PFOS, PFHxS and PFNA vary, respectively, from 2.3 to 8.5 years, 3.1 to 7.4 years, 4.7 to 8.5 years and 1.7 to 3.2 years. PFAS can be transferred from the mother to the child through the placenta and through breastfeeding [7]. Internal exposure to PFAS has been observed in populations of different age groups of many countries including Belgium (Flanders) as described by Colles et al. [8], Salihovic et al. [9], Richterova et al. [10] and Kirk et al. [11].

Evidence is accumulating indicating that PFAS could potentially cause harmful renal, hepatic, immunotoxic, reproductive, and endocrine disrupting effects [6, 12–15]. PFAS are probably also carcinogenic [3, 5, 16–21] and possibly induce neurobehavioral and developmental effects [15]. There is some evidence indicating that in early life phases (in utero, childhood) humans might be more sensitive to adverse health effects of PFAS [15, 22].

The FLEHS (Flemish Environment and Health Study) campaigns in Flanders, the northern part of Belgium, are intended to provide data on internal exposure to pollutants (such as polycyclic aromatic hydrocarbons, organochlorines, flame retardants, pesticides, metals) and associated early biological and health effects in persons of different ages randomly sampled from the general population. Data on the concentrations measured in umbilical cord plasma and in adult serum samples from FLESHS-2 (2007–2011) and FLEHS-3 (2012–2015) and in serum samples from adolescents living in an industrially contaminated site (2010–2011) with no specific records of PFAS production were reported by Colles et al. [8]. Colles et al. [8] also present a detailed description of the determinants of differences in internal exposure to PFAS in these populations with regard to personal characteristics, reproductive life, dietary factors, life style factors, socio-economic factors and season. There is ample evidence indicating that PFAS disrupt immunological mechanisms [12, 13]. In the literature both positive and inverse associations between serum PFAS concentrations and immune system-related diseases are reported [23–26] The current study was conducted based on the data of the Colles et al. [8] study. More specifically we present here, for the cohorts participating in the Colles et al. study [8], data concerning the association of internal exposure to PFAS with immune system-related diseases.

Methods

Participants

Flemish reference studies

The selection and recruitment of the mothers and adults participating in this study that are representative for the Flemish population in terms of location of residence, social class, age and gender (for the adults) have been described in detail by Colles et al. [8] and Schoeters et al. [27]. Table 1 summarizes some data concerning the campaigns on which this study is based. Briefly, a stratified clustered multi-stage design was used to select participants in primary sampling units (PSUs) as a random sample of the Flemish population. For mothers these PSUs consisted of maternity units, whereas for adults PSUs were provincial institutes in FLEHS-2, and general practitioner offices in FLEHS-3. Within each PSU, individuals were randomly selected. Inclusion criteria were: (1) residing in Flanders for at least 10 years, (2) giving written

Table 1 Summary data on participants with mention of compounds measured

Campaign and population	Abbreviation in forest plots	*n*	Age	Period	Compounds measured
FLEHS 2 Mothers	FL2-MOTH	220	18–42	August 2008–July 2009	PFOS, PFOA
FLEHS 3 Mothers	FL3-MOTH	269	18–44	November 2013–November 2014	PFOS, PFOA, PFHxS, PFNA
FLEHS 2 adults	FL2-ADU	201 (94 male, 107 female)	20–40	June 2008–April 2009	PFOS, PFOA
FLEHS 3 adults	FL3-ADU	205 (97 male, 108 female)	50–65	May 2014–November 2014	PFOS, PFOA, PFHxS, PFNA
FLEHS 2 adolescents	FL2-ADO	197 (114 male, 83 female)	14–15	May 2010–February 2011	PFOS, PFOA

informed consent, and (3) being able to complete an extensive Dutch questionnaire. For the adults, exclusion criteria were: severe kidney disease (glomerular filtration rate < 60 ml/min) and active anti-cancer therapy (chemotherapy or radiotherapy). A map of Flanders (Fig. 1) shows in which area's adult participants resided. More details about the study design and recruitment strategy of FLEHS-2 and the FLEHS-3 studies on mothers have been previously reported [27–29].

Adolescent study population near industrial contaminated site

As mentioned in Table 1, during the FLEHS-2 study, PFOS and PFOA were also measured in 197 adolescents aged 14–15 years, 114 boys and 83 girls, sampled from May 2010 to February 2011, recruited in the municipalities Menen and Wevelgem near the "Grensland" industrial area (FL2-ADO) (Fig. 1). Their selection and recruitment were described in more detail by van Larebeke et al. [30]. The industrial contaminated site had no specific records of PFAS production. It was home to a large-scale recycling plant of ferrous and non-ferrous metals, timber industry, pigment industry, several incinerators and, until 2005, a large-scale incinerator for municipal waste. Inclusion criteria were: (1) residing in Flanders for at least 10 years; (2) giving written informed consent; and (3) being able to complete an extensive Dutch questionnaire. More details about study design and recruitment can be found elsewhere [27, 28, 31–33].

Field work and sampling

Adult and adolescent participants were examined by trained research nurses and this included collection of blood samples, measurement of the height and weight and filling out of a questionnaire. The tubes used for blood collection were on beforehand tested by the laboratory to make sure that they were not contaminated. Also special procedures were followed by the research nurses who performed the sampling of blood in order to avoid contamination. Forty mL peripheral blood was collected from each adult or adolescent participant. Blood samples were centrifuged and/or fractionated at the local sampling centre and afterwards transported to the central laboratory where they were stored at − 80 °C in a biobank within 12 h after sampling. Assessment of internal exposure to PFAS of mothers was based on

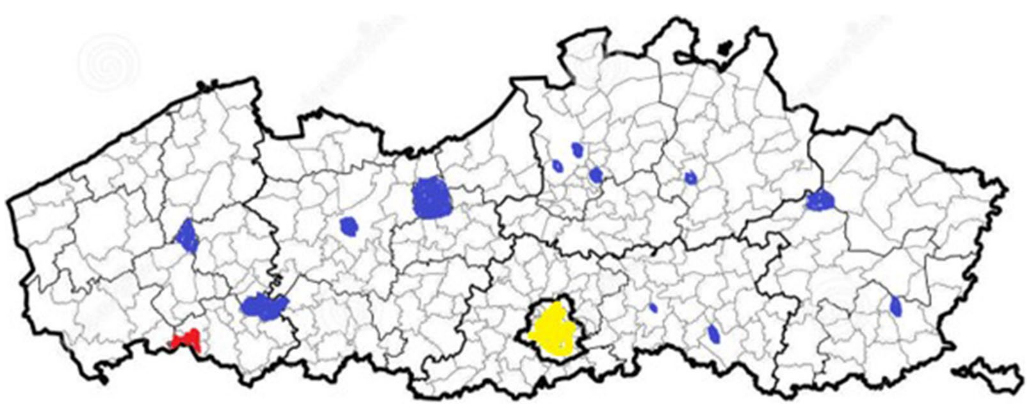

Fig. 1 Map of Flanders showing the administrative borders of municipalities. Between the most western and most eastern point: about 230 km. In red the industrial "Grensland" area (without known PFAS contamination) where the 197 adolescents participating in FLEHS-2 resided. In blue the area's where the 205 adults participating in the FLEHS 3 campaign resided, as a random sample of the Flemish population. In yellow the agglomeration of Brussels which is not part of Flanders and is not involved in this study

measurements of cord plasma. Cord blood was obtained by the obstetric team after the delivery and within 24 h handled and frozen at the laboratory of the participating hospital, between − 20 °C and − 80 °C. Processed samples were transferred to the central laboratory within 4 weeks. Plasma and serum were conserved at − 80 °C. All laboratories worked with pseudonymized samples. Every sample is coded by a unique Sample ID; each Sample ID is linked to one participant by a Subject ID.

Data derived from questionnaires

As described in detail by van Larebeke et al. [30] participants, and in the case of adolescents also their parents, completed a self-reporting questionnaire on socioeconomic status and personal and lifestyle factors, including: age, country of birth of their parents, weight, height, housing, residence history, occurrence of in-house structural modifications or painting, family composition, density of nearby traffic, in-house use of pesticides and in-house exposures to pollutants and chemicals (in terms of use or exposure to solvents, insecticides, herbicides, passive smoking, air refreshers, sprays for impermeabilizing clothes, personal care products, disinfectants) sports, hobbies, contact with animals, smoking and consumption of alcohol, health status and disease experience, occurrence of asthma or eczema or allergies among relatives.

For mothers and other women, info was recorded on menstrual cycle and parity. Participants also completed food frequency questionnaires in order to assess the consumption of food items as described by Colles et al. [8]. The consumption of locally produced food was also recorded. Additionally, a short questionnaire on recent exposure during the past 3 days (to solvents, insecticides, herbicides, tobacco smoke, air refreshers, sprays for impermeabilizing clothes, personal care products, disinfectants) was filled out [34, 35].

Asthma/allergy questionnaire data

The participants filled out a questionnaire on asthma and allergy outcomes (see Additional file 1: Table S1), derived from the questionnaires of the International Study of Asthma and Allergies in Childhood (ISAAC) [36] and European Community Respiratory Health Survey (ECRHS) [37]. Asthma ever was defined as answering yes on either the question of being doctor diagnosed, and/or having suffered an attack of asthma, and/or having suffered from wheezing without a cold, and/or having suffered from shortness of breath and/or having used asthma medication. Asthma doctor diagnosed, was defined as answering yes on the question of being doctor diagnosed. Current asthma was defined as answering yes on either the question of having suffered from an attack of asthma and/or having suffered from wheezing without a cold, and/or having suffered from shortness of breath and/or having taken asthma medication during the last 12 months. Allergic rhinitis was defined as: having any form of allergic rhinitis, and/or using medication against allergic rhinitis (nasal sprays, nose drops, tablets). Eczema was scored positive in case the subject reported the use of eczema medication. Allergy was asked specifically as having allergy for food, medications, insects, metals, personal care products, household or maintenance products or animals. In FLEHS-2 these factors were grouped as systemic allergy (allergy to food, medical drugs and insect bites), allergy to animals and contact allergy (to metals, personal care products, household or maintenance products). In FLEHS-3 these factors were grouped as systemic allergy (allergy to food, medical drugs and insect bites), allergy to animals, product allergy (allergy to personal care products, household or maintenance products) and allergy to metals.

Measurement of poly- and perfluoroalkyl substances (PFAS)

Measurement of PFOS, PFHxS, PFOA, PFNA and PFBS in peripheral serum and cord plasma was described in detail by van Larebeke et al. [30]. Briefly, using procedures as described by Kato et al. [38], the analytical method consisted of an offline protein precipitation with acetonitrile, followed by separation by HPLC and MS/MS detection (Applied Biosystems API3000 triple quadrupole mass spectrometer (Foster City, CA, USA) in negative ionization MS/MS mode with multiple reaction monitoring. The limit of quantification (LOQ) was determined as twice the LOD and was equal to 0.3 µg/L for PFOS and PFOA in FLEHS-2 (2007–2011) and in FLEHS-3 (2012–2015) LOQ was 0.2 µg/L for PFOS, PFOA, PFHxS and PFBS, and 0.1 µg/L for PFNA. To assess the comparability of measurements performed during FLEHS-2 (2007–2011) with those performed during FLEHS-3 (2012–2015), PFOS and PFOA levels were re-measured in 3 samples from the biobank of FLEHS-2 together with the samples of FLEHS-3. Deviation percentages ranged from − 7.8 to − 29.4% for PFOS and + 5.0% to + 21.5% for PFOA.

Statistical analysis

The statistical analysis was performed as described in detail by van Larebeke et al. [30] Associations with biological or health effects were only assessed for PFASs for which measurable quantities were observed for the vast majority of participating subjects. Therefore, it was deemed acceptable to replace values below the LOQ by LOQ/2. Associations between internal exposure to PFAS compounds and parameters of health effects were assessed using multiple logistic regression, taking into account predetermined confounding factors. Confounders included in all studies smoking and self-assessed familial occurrence of the disease. Potential covariates selected on the basis of the literature and mechanistic considerations, including age when not selected as a confounding factor, were included in the starting multiple regression models when they showed, in simple regression, an association ($p<0.25$) with a dependent variable of interest. Confounders and potential covariates are listed in Additional file 1: Tables S2, S3, S4, S5 and S6. Confounders and covariates were included as continuous variables whenever continuous data were available. Stepwise multiple logistic regression analyses were done using R version 3.3.0 and RStudio version 1.0.136, and logistf for Firth logistic regression for associations where exposure variables showed quasi separation in the normal logistic regression. In the models the PFAS concentrations were included on the original scale, without transformation. To limit the number of independent variables in the final models, confounding factors were removed from the model if they had a p-value > 0.5 and subsequently covariates were removed if they had a p value > 0.05, as adjustment for confounders is more important than adjustment for covariates. For each association the confounding factors and significant covariates included in the final model are mentioned in Additional file 1: Table S7. For logistic regression odds ratios (OR) were calculated. Odds ratios and their 95% confidence intervals were reported for interquartile (IQR) increases in exposure.

Estimates for IQR differences in exposure were either calculated as 'ratios' or 'differences' in the levels of effect markers, respectively, in case of ln-transformed or non-transformed effect markers, used in the regression models. Associations with a p value < 0.05 or a p value < 0.1 are, respectively, designated as significant or marginally significant.

Regression analyses are sensitive to influential cases, whose deletion from the dataset would noticeably change the result of the calculation. Exclusion of influential cases was performed as described and discussed in detail by van Larebeke et al. [30]. We present results after exclusion of influential cases. For most associations there were no or only one influential case, the maximum number of influential cases was 4 (this occurred in two associations as shown in Additional file 1: Table S7). Additional file 1: Table S7 shows results in which all data were included, i.e. also the influential cases.

Results

Response

In the FLEHS-2 study on adolescents, 921 adolescents were contacted by letter, 349 of them (37.9%) answered, 22.5% agreed to participate and finally 21.6% (114 boys and 85 girls) of the participants completed the study. The level of education of the participating adolescents and the socioeconomic status of their families was similar to that in a study focused on the Flemish reference population.

In the FLEHS-2 study on adults (50–65 years old), 2098 adults were contacted through e-mail by the human resources department of the provincial administration for which they were working. An answer was obtained from 636 persons (30.4%), 473 persons (22.5%) agreed to participate and finally 204 of them were selected to participate taking into account gender and level of employment.

In the FLEHS-2 study on mothers, 220 cord blood samples were taken, but it was not possible to accurately assess the response percentage as it was not possible to determine in how many deliveries cord blood could possibly have been taken. It was estimated that in 83% of the deliveries occurring in the participating maternity units between August 2008 and July 2009 no cord blood sample was taken because the delivery occurred outside the working hours of staff essential to the project. In a subset of 1174 deliveries in which cord blood could have been taken 146 (12.4%) cord blood samples were indeed taken after the delivery. In a subset of 170 deliveries in which a cord blood sample was taken after the delivery 131 (77%) mothers agreed to participate, 21 (12.4%) refused to participate, 3 (1.7%) interrupted their participation because of the length of the Questionnaire, 2 (1.2%) agreed to contribute to a mixed sample and 13 (7.6%) could not participate because they did not fulfil the inclusion criteria.

In the FLEHS-3 study on adults (20–40 years old), participants were recruited through General Practitioners. Half of the contacted General Practitioners refused to collaborate, mainly due to lack of time. 1369 persons were invited to participate and 248 (18.1%) of them agreed to participate. Thirty persons could not participate because they were not available on the day on which the sampling would occur. Among the 218 remaining persons 205 were selected (taking into account gender, area of residence and socioeconomic status) to participate.

In the FLEHS-3 study on mothers, the aim was to recruit 250 mothers as a representative sample of the Flemish population and additionally 80 mothers with a lower socioeconomic status. Finally, for 269 participating mothers cord blood was available, 102 of whom were part of a household in which no one received a higher education. It was not possible to assess the response in the study as a whole, but it was possible to make an estimate based on a sample. In this sample, cord blood was drawn in 18.7% of deliveries, and in 5.5% of the deliveries for which cord blood was taken the mothers refused to participate.

Characteristics of the participants

Personal characteristics of participants including some aspects of reproductive life, their dietary consumption, information concerning life style factors including use of personal care products and socioeconomic status have been published in the paper by Colles et al. [8]. These data are summarized in Additional file 1: Table S8.

Internal exposure to PFAS

PFAS concentrations in cord plasma or serum of the subjects participating in the FLEHS-2 and FLEHS-3 studies have been published in detail by Colles et al. [8] and are summarized in Additional file 1: Table S9. At least 99.5% of the PFOA or PFOS values were above LOQ in the FLEHS-2 and FLEHS-3 studies. The same was true for PFHxS and PFNA measured in adults of FLEHS-3. In cord plasma of mothers, PFHxS and PFNA were only analysed since the FLEHS-3 birth cohort. There, the rate of detection was 84% for PFHxS and 89.6% for PFNA. For PFBS measured in adults and in cord plasma of mothers of FLEHS-3, the detection rate was below 5% and these data are therefore not retained in the current analysis.

Immune system-related disease outcomes

The immune-related disease outcomes are summarized in Table 2. Per study population doctor-diagnosed asthma was reported by 7–10% of the participants in the different study populations and doctor-diagnosed allergic rhinitis was reported by 18.1–34.3% of the participants. Eczema occurred in the different study populations in 8.4–15.8% of the studied individuals. Allergies were common and were reported by about 30% of the total of all the 1092 participants in the 5 studies.

Associations between PFAS and asthma, allergic rhinitis, allergies and eczema

Odds ratios per sub-study were calculated to investigate associations between internal exposure to PFAS and the immune system-related diseases asthma, allergic rhinitis, eczema and other allergic conditions. All studied associations (after removal of any influential cases) are shown in Figs. 2, 3, 4. Statistical data with 95% confidence intervals, also including data comprising all cases, can be found in Additional file 1: Table S7. Below the main results after removal of any influential cases are summarized.

Asthma

For mothers in FLEHS-2 and FLEHS-3, PFAS exposure was inversely associated with asthma in all of the, respectively, 6 and 12 studied asthma outcomes (Fig. 2). For mothers in FLEHS-2, 4 of the 6 inverse associations were significant; for these 4 associations the decreases in odds associated with an interquartile increase in exposure ranged from 36.7% (PFOS vs. asthma ever, $p=0.042$) to 46.9% (PFOS vs. asthma current, $p=0.024$) (Fig. 2). Of the 12 inverse associations for mothers in FLEHS 3, 6 were significant and 2 were marginally significant. For these 8 associations the decreases in odds associated with an interquartile increase in exposure ranged from 42.0%

Table 2 Descriptive statistics of percentage of participants with immune-related disease outcomes in the different FLEHS-2 and FLEHS-3 studies

Immune outcome (%)	2008–2009 FLEHS-2 mothers $N=240$ (18–42 years)	2010–2011 FLEHS-2 adolescents $N=197$ (14–15 years)	2008–2009 FLEHS-2 adults $N=197$ (20–40 years)	2013–2014 FLEHS-3 mothers $N=268$ (18–44 years)	2014 FLEHS-3 adults $N=206$ (50–65 years)
Doctor diagnosed asthma	8.8	9.1	8.3	9.6	7.3
Current asthma	17.3	14.4	16.5	15.7	17.2
Asthma ever	25.7	20.3	20.6	24.4	25.3
Doctor diagnosed allergic rhinitis	18.1	21.2	34.3	27.2	24.0
Allergic rhinitis	24.3	28.4	36.0	31.2	28.2
Eczema	13.1	15.8	14.2	8.4	12.9
Allergy to animals	9.2	8.3	10.6	14.7	7.6
Systemic allergy	24.7	21.6	41.9	28.1	30.7
Contact allergy	30.1	18.2	30.3	17.1 (products)[a] 13.1 (metal)[a]	11.5 (products)[a] 10.7 (metal)[a]

Where contact allergy = allergy to metals, personal care products, household or maintenance products. Systemic allergy = allergy to food, medical drugs and insect bites

[a] In the FLEHS-3 mothers the grouping was done as two separate groups: product allergy (personal care products, household or maintenance products) and allergy to metals

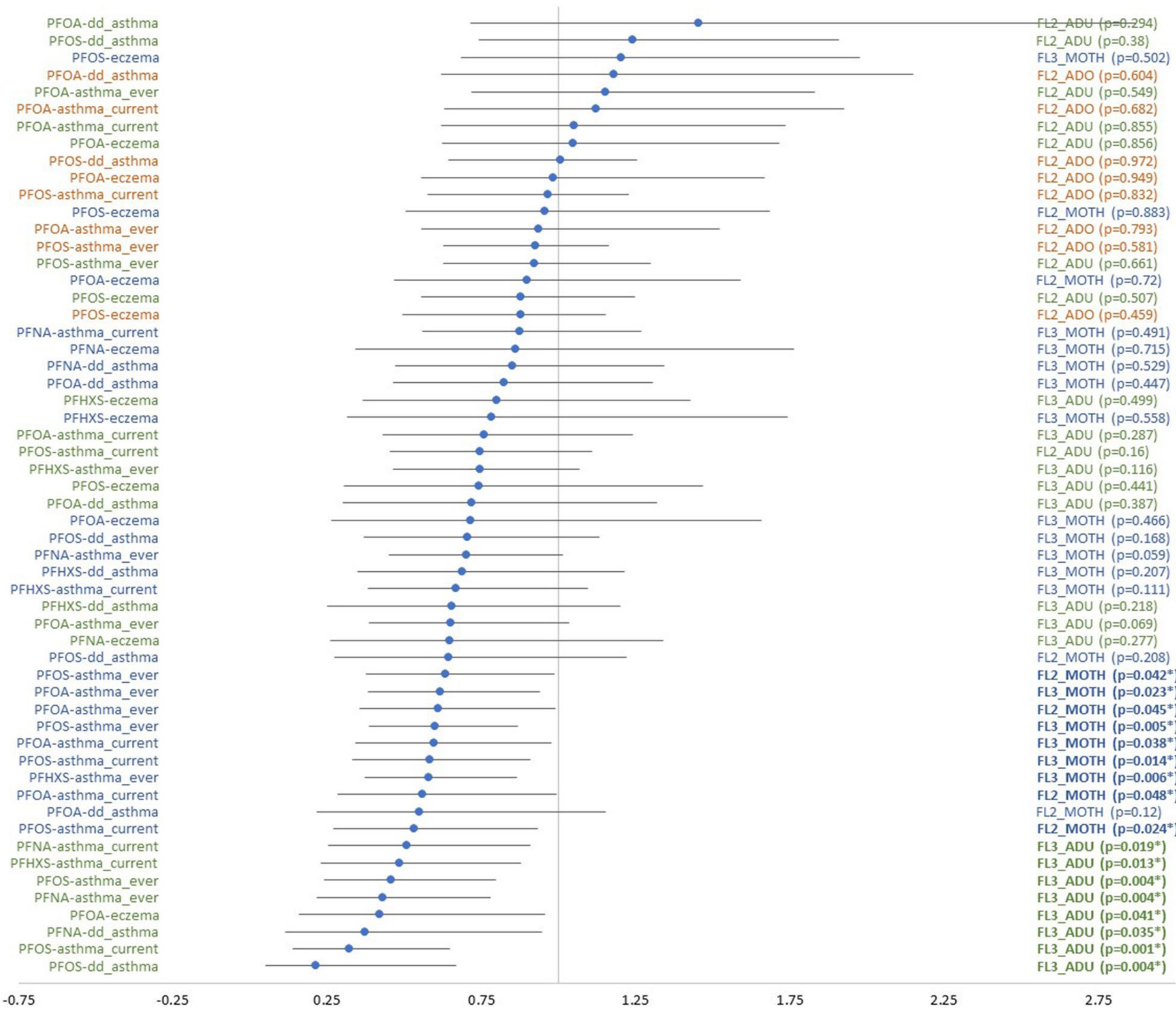

Fig. 2 Forest plot of the odds ratios (ORs) of the asthma outcomes and eczema in relation to the measured PFAS in the different FLEHS studies (with *FL2* FLEHS-2, *FL3* FLEHS-3, *ADO* adolescents, *ADU* adults, *MOTH* mothers). The OR (95% confidence interval) is expressed for an interquartile increase in exposure. Confounders and covariates that were included at the start of the stepwise multiple regressions are mentioned in Additional file 1: Tables S2, S3, S4, S5 and S6

(PFHxS vs current asthma, $p = 0.006$) to 30.0% (PFNA vs current asthma, $p = 0.059$) (Fig. 2).

For adolescents in FLEHS-2, 3 of the 6 associations of PFAS with asthma outcomes were inverse, 3 were positive, none of these associations were significant (Fig. 2).

For adults in FLEHS-2, 4 of the 6 associations with asthma outcomes were positive, 2 were inverse, none of these associations were significant (Fig 2).

For adults in FLEHS-3, all of the 12 associations of PFOS, PFOA, PFNA or PFHxS with the asthma outcomes were inverse. Of these 12 inverse associations, 7 were significant and 1 was marginally significant. For these 8 associations decreases in odds for an interquartile increase in PFAS ranged from 35.0% (PFOA vs. asthma ever, $p = 0.069$) to 78.7% (PFOS vs. doctor-diagnosed asthma, $p = 0.004$) (Fig. 2).

Eczema

Concerning eczema, 12 of the 14 associations studied were inverse (Fig. 2). One association was significant, i.e. in adults in FLEHS-3, a significant decrease in odds for eczema of 58.1% ($p = 0.041$) was observed for an interquartile increase in PFOA concentration.

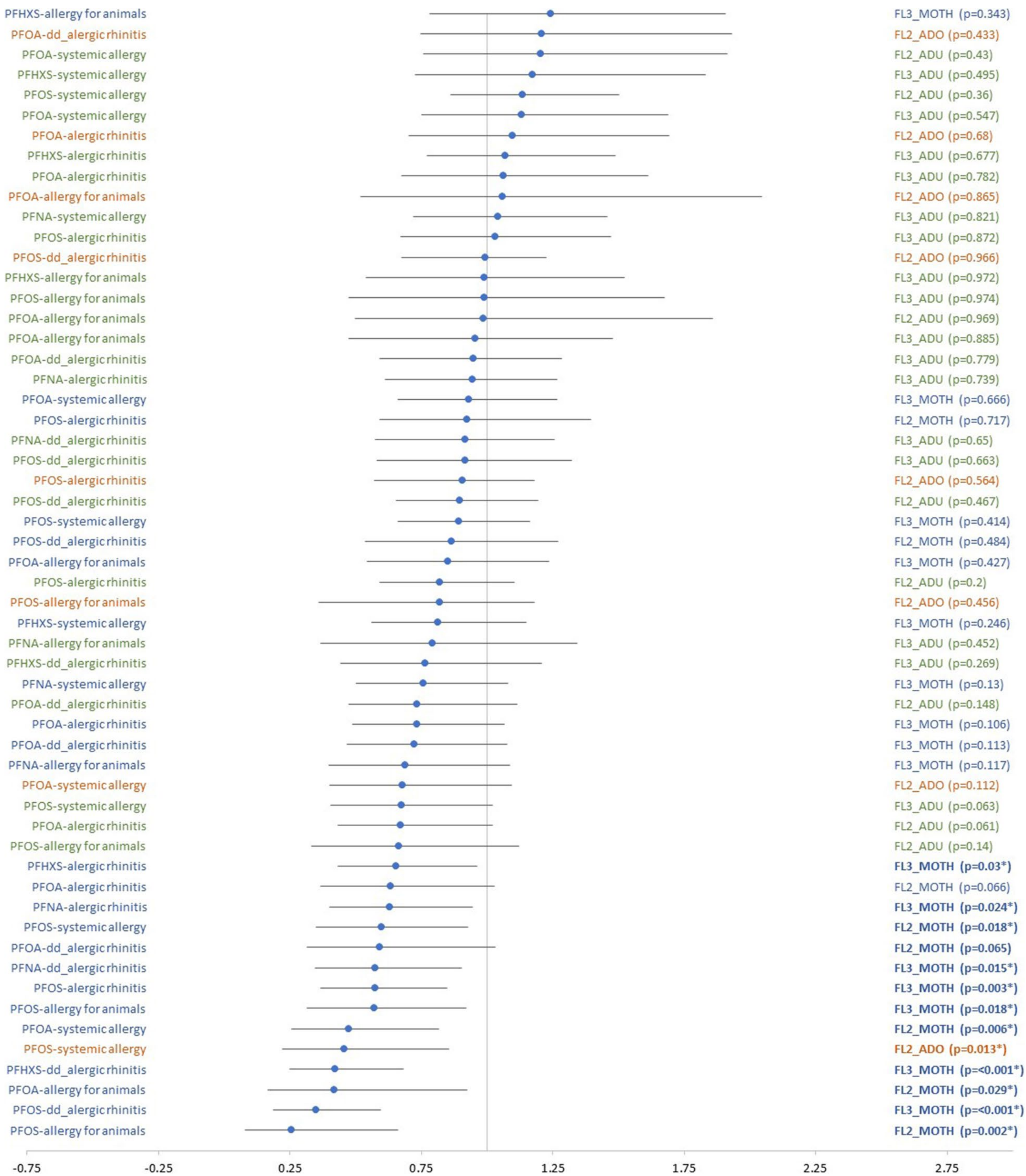

Fig. 3 Forest plot of the odds ratios (ORs) of the allergy outcomes: allergic rhinitis, allergy to animals and systemic allergy in relation to the measured PFAS in the different FLEHS studies (with *FL2* FLEHS-2, *FL3* FLEHS-3, *ADO* adolescents, *ADU* adults, *MOTH* mothers). The OR (95% confidence interval) is expressed for an interquartile increase in exposure. Confounders and covariates that were included at the start of the stepwise multiple regressions are mentioned in Additional file 1: Tables S2, S3, S4, S5 and S6

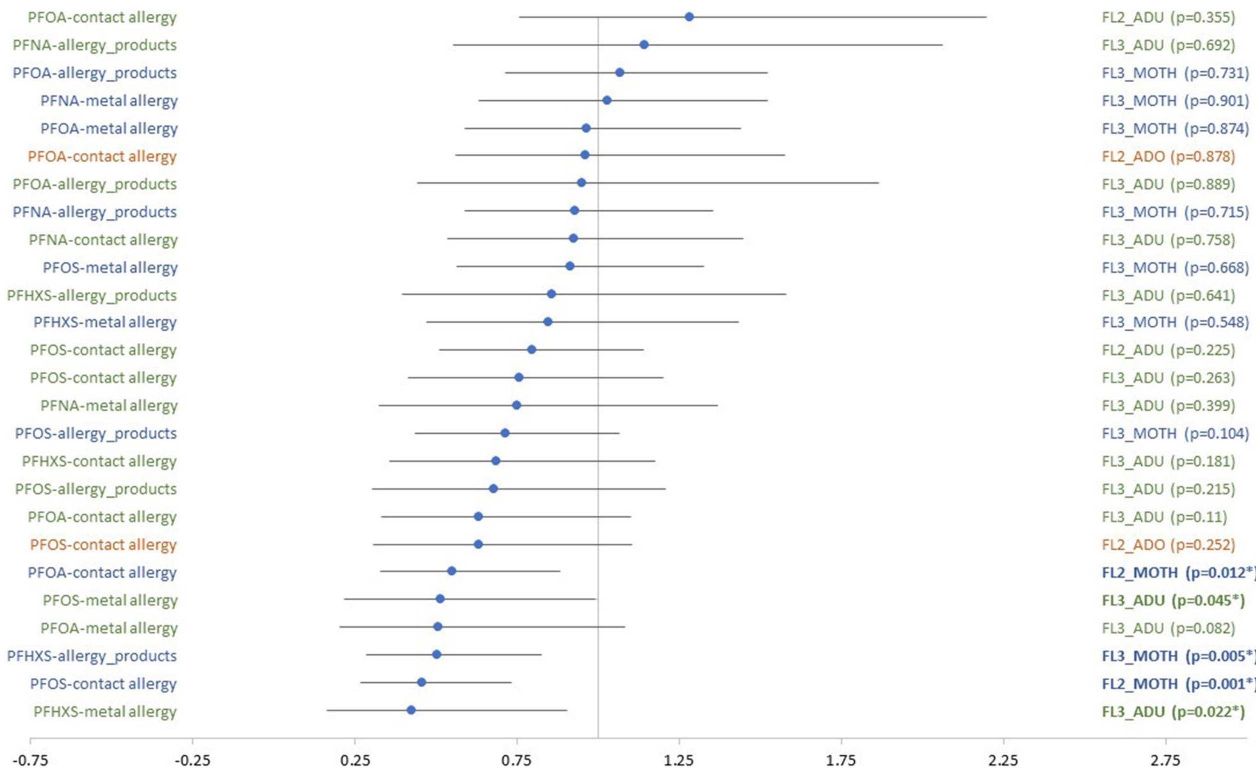

Fig. 4 Forest plot of the odds ratios (ORs) of the allergy outcomes: contact allergy (only in FLEHS-2), allergy to metals (only in FLEHS-3), product allergy (only in FLEHS-3) in relation to the measured PFAS in the different FLEHS studies (with *FL2* FLEHS-2, *FL3* FLEHS-3, *ADO* adolescents, *ADU* adults, *MOTH* mothers). The OR (95% confidence interval) is expressed for an interquartile increase in exposure. Confounders and covariates that were included at the start of the stepwise multiple regressions are mentioned in Additional file 1: Tables S2, S3, S4, S5 and S6

Allergic rhinitis

Concerning allergic rhinitis, for mothers all of the, respectively, 4 and 8 studied associations in FLEHS-2 and FLEHS-3 with allergic rhinitis outcomes were inverse (Fig. 3). In FLEHS-2 two of these inverse associations (both associations of PFOA with allergic rhinitis outcomes) were marginally significant (Fig. 3). In FLEHS-3 six of the 8 inverse associations for mothers were significant with decreases in odds associated with an interquartile increase in exposure ranging from 34.6% (PFHxS vs allergic rhinitis, $p = 0.03$) to 65.2% (PFOS vs doctor-diagnosed allergic rhinitis, $p < 0.001$) (Fig. 3).

For adolescents in FLEHS-2, 2 of the 4 associations of PFAS with allergic rhinitis outcomes were inverse, 2 were positive, none of these associations were significant (Fig. 3).

For adults in FLEHS-2, all 4 associations of PFAS with allergic rhinitis outcomes were inverse, one of these marginally significant with a decrease in odds associated with an interquartile increase in exposure to PFOA of 32.8% ($p = 0.061$) (Fig. 3).

For adults in FLEHS-3, 5 of the 8 associations of PFAS with allergic rhinitis outcomes were inverse, 3 were positive, none of these associations were significant (Fig. 3).

Allergies other than allergic rhinitis

Concerning allergies (other than allergic rhinitis), for mothers in FLEHS-2, all of 6 associations with allergy outcomes were significantly inverse with decreases in odds associated with an interquartile increase in exposure ranging from 40.0% (PFOS vs. systemic allergy, $p = 0.018$) to 74.3% (PFOS vs. allergy to animals, $p = 0.002$) (Figs. 3, 4).

For mothers in FLEHS 3, 13 of the 16 associations were inverse, 3 were non-significantly positive. Of the 13 inverse associations, 2 were significant: an interquartile increase in exposure to PFHxS and PFOS were, respectively, associated with a 49.7% decrease in the odds of product allergy ($p = 0.005$) and a 43.1% decrease in the odds of allergy to animals (Figs. 3, 4).

For adolescents in FLEHS-2, 5 of the 6 associations with allergy outcomes were inverse and 1 non-significantly positive. Of the 5 inverse associations 1 was significant with a decrease in odds of systemic allergy of 54.5%

($p = 0.013$) for an interquartile increase in PFOS concentration (Figs. 3, 4).

For adults in FLEHS-2, 3 of the 6 studied associations were inverse, 3 were positive and none of these associations were significant (Figs. 3, 4).

For adults in FLEHS-3, 12 of the 16 associations were inverse and 4 were non-significantly positive (Figs. 3, 4). Of the 12 inverse associations, 3 were significant, with decreases in odds associated with an interquartile increase in exposure ranging from 48.7% (PFOS vs. metal allergy, $p = 0.045$) to 57.7% (PFHxS vs. metal allergy, $p = 0.022$) (Fig. 4). One inverse association was marginally significant with a decrease in odds for systemic allergy of 32.4% ($p = 0.063$) for an interquartile increase in PFOS concentration (Fig. 3).

Discussion

This study is based on self-reported outcome data using questions from questionnaires that were used in important European and world-wide studies. The subjects included in the study could not present themselves for participation. Adults and mothers were selected to be representative for the general Flemish population, whereas the adolescents were selected to be representative for the Grensland industrial area. The human biomonitoring campaigns on which this study is based were organized to evaluate the internal exposure of the Flemish population to a series of pollutants and to evaluate possible exposure–effect relations. These human biomonitoring campaigns were not intended to study the effects of PFAS in particular nor were they intended to study internal exposure among persons suffering from a particular disease or condition. Selective participation is thus not an issue. The participants filled out the questionnaires before they were informed about their PFAS serum concentrations and other parameters of internal exposure. Recall bias is thus unlikely.

In Flanders the internal exposures to PFAS in the period 2008–2014 were comparable or somewhat lower than in other Western countries [8] with values of the same order as those found in the Netherlands in 2011–2013 (mean PFOS concentration in cord blood of 1.62 µg/L) but clearly lower than those found in Denmark in 1996–2002 or in Canada in 2004–2005 with, respectively, mean PFOS concentrations in cord blood of 11.0 µg/L and 7.19 µg/L as discussed by Colles et al. [8]. Considering time trends, the levels of PFOS and PFOA in FLEHS-2 (2008–2009) and FLEHS-3 (2013–2014) in cord blood decreased by, respectively, 59% (2.66–1.10 µg/L) and 21% (1.51–1.19 µg/L) [39] over that 5-year period.

In mothers and adults participating in the FLEHS studies internal exposure to PFAS was clearly associated with a decrease in the risk of asthma, allergic rhinitis and several types of allergies and to a lesser extent with a decrease in the risk of eczema (Figs. 2, 3, 4). The associations with asthma, eczema and allergic rhinitis outcomes were not clearly inverse for adolescents in FLEHS-2, and for adolescents even the evidence for an inverse association with allergies other than allergic rhinitis was limited. That our observations on adolescents apparently differ from those we made on mothers and adults is difficult to explain but might be partly explained by the somewhat smaller interquartile range in PFAS serum concentrations observed for these adolescents and possibly also by the fact that the adolescent cohort was not representative for Flanders as a whole but was selected in the context of an industrial hot spot with higher concentrations of environmental pollutants, some of which might contribute to immune system-related diseases.

Inverse associations between internal exposure to PFAS and incidence or prevalence of immune system-related diseases are described by many authors. Our findings were consistent with the Hokkaido study where the risk of eczema and allergic diseases decreased in association with higher maternal levels of some PFAS [23]. In the Hokaido study, prenatal exposure to PFOA, perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA) was inversely associated with rhinoconjunctivitis, while that for PFOA, PFOS, PFUnDA, perfluorododecanoic acid (PFDoDA), and perfluorotridecanoic acid was, in children up to 7 years, inversely associated with eczema [40]. In the Spanish INMA birth cohort study, PFNA concentrations in maternal plasma collected during the 1st trimester of pregnancy (years: 2003–2008) showed a significant inverse association with asthma during childhood. PFOS showed a significant inverse association with eczema whereas higher PFOA concentrations were associated with lower forced vital capacity and lower forced expiratory volume at 4 years but not at 7 years of age [24]. In the Norwegian Mother and Child Cohort Study, Impinen et al. [41] found a statistically significant inverse association between maternal PFUnDA concentrations during pregnancy and ever having atopic eczema in girls. PFUnDA also tended to be inversely associated with both wheeze and asthma.

But there are also reports on positive or complex associations between internal exposure to PFAS and immune system-related diseases. Humblet et al. [42] observed, among children 12–19 years of age, a positive association between serum concentration of PFOA and odds of asthma and an inverse association for PFOS with both asthma and wheezing. Zhou et al. [43] and Dong et al. [25] reported for children a positive association between PFAS and asthma. Jackson-Browne et al. [44] observed a weak association between serum PFAS concentrations and increased asthma prevalence in US children. In a cohort of 675 Norwegian adolescents, aged 13–19 years, both the sum of 18 PFAS and PFOS were positively associated with self-reported doctor-diagnosed asthma [26].

The EFSA scientific opinion [6] summarized all studies and concluded that the available evidence was insufficient to suggest that exposure to PFAS is associated with allergy and asthma in children and adults. On the other hand, they indicated that there is more consistency in the reports on the association between higher internal exposure to PFAS and a weaker immune response to vaccination [45, 46] or an increased risk of infections [40, 47–51]. It seems reasonable to conclude that the totality of available data indicates that PFAS interfere with the function of the immunological system.

Mechanistic insights explain at least part of the diverse effects of PFAS on the immune system. There is convincing evidence indicating that endocrine disrupting compounds (EDCs), such as PFAS, can disturb immune regulation by their action on different levels of the immune regulatory network, including cellular and humoral response, survival, maturation, and cytokine synthesis of immune cells [52]. This means, EDCs can lead to allergic and autoimmune diseases and may attenuate immunity against infection [53, 54], but EDCs such as PFAS were also associated with a decrease in the risk of asthma and allergy-related conditions as shown in this and other studies (see above). Contrasting observations considering asthma, allergy and infections might be explained by the immunomodulatory and immune-toxic effects of PFAS as pointed out by Kishi et al. [23]. Using in vitro tests to study the effect of PFOA and PFOS on the release of the IL-6, IL-8, TNF-alpha, IL-4, IL-10 and IFN-gamma cytokines by immune cells, Corsini et al. [55] showed that PFOS as well as PFOA could directly suppress the secretion of some cytokines in some cells, and that PFOA and PFOS have different mechanisms of action. The effect on cytokine release was pre-transcriptional, with a role for PPAR-alpha in PFOA-induced immunotoxicity, while an inhibitory effect on degradation of the I-kappaB inhibitor could explain the immunomodulatory effect of PFOS. In the mouse, PFOA exposure caused decreased spleen and thymus weights, decreased thymocyte and splenocyte counts, decreased immunoglobulin response, and changes in specific populations of lymphocytes in the spleen and thymus [12, 56]. Gestational exposure to PFOS induced a decrease in natural killer cell function and in immunoglobulin M production in mice [52]. The precise nature of the effect of PFAS on immune phenomena might depend on other intervening factors. For example, increased oestrogenic activity might be associated with an increased risk of asthma [54], possibly implying that a decrease in oestradiol serum concentrations (as observed in association with PFAS exposure in male adolescents in our study, manuscript in preparation) could contribute to a decrease in the risk of asthma. Consistent with this, Zhou et al. [43] found stronger associations between PFAS exposure and asthma in children who have increased levels of oestradiol. Integrative omics analyses showed that PFOS could alter the production of interleukins in human lymphocytes [57]. Additionally, PFOS exposure could dysregulate clusters of genes and lipids that play important roles in immune functions, such as lymphocyte differentiation, inflammatory response, and immune response [57]. Overall, in the EFSA scientific opinion [6] effects on the immune system were considered the most critical for risk assessment.

As to strengths and limitations of the study, five cohorts of FLEHS-2 and FLEHS-3 were included consisting of people representative of the general population or a specific industrial area. Selective participation and recall bias were highly unlikely due to the structure of the study. The final models used in the statistical analyses contained a limited number of independent variables. It was possible to show, in cohorts of mothers and adult populations examined at different points in time, a consistent association with immune system-related events. PFAS blood levels were analysed in the same lab during the different surveys performed in a 7 years' time frame. Furthermore, uniform study protocols facilitated comparability of the results.

Weaknesses in our study comprise that we had to rely on questionnaires instead of validated clinical data. Also, internal exposure of mothers to PFAS was assessed through cord plasma measurements and not through maternal serum measurements. To limit the number of independent variables in the final models confounding factors were removed from the model if they had a p-value > 0.5 and subsequently covariates were removed if they had a p value > 0.05. This implies that some residual confounding might subsist. However, in view of the consistency in our findings across many independent observations it is unlikely that the residual confounding might be an important issue. Many statistical associations were tested, increasing the likelihood of chance findings. However, the immunotoxic and endocrine disrupting effects of PFAS are well established and render exposure-effect relations biologically plausible, so, in accordance with the views of the epidemiologist Kenneth Rothman [58], we did not apply corrections for multiple testing. As our studies were cross-sectional they do not directly point to causality. However, by confirming the results of other research, they contribute to the knowledge and evidence concerning the effects of PFAS.

Conclusion

Our observations contribute to the evidence for immune regulation disturbance by PFAS, consistent with what has been reported in the literature. Indeed, the coherent inverse associations with asthma, eczema and allergy outcomes over nearly all studied FLEHS campaigns and for different age groups almost certainly rests on a disturbance of the regulation of immunological mechanisms. Interestingly, this immune-regulating disturbing effect appears to have not only adverse health effects, but can also be associated with inverse associations with certain immune system-related diseases.

Abbreviations

AMGC	Department of Analytical, Environmental and Geochemistry
ECRHS	European Community Respiratory Health Survey
EDCs	Endocrine disrupting compounds
EFSA	European Food Safety Agency
FLEHS	Flemish Environment and Health Study
G-EQUAS	GERMAN EXTERNAL QUALITY ASSESSMENT SCHEME for Analyses in Biological Materials
HPLC	High-performance liquid chromatography
IFN-gamma	Interferon gamma
IL-10	Interleukin 10
IL-4	Interleukin 4
IL-6	Interleukin 6
IL-8	Interleukin 8
IQR	Interquartile
Ln-transformed	Natural logarithm has been taken, with as base the number "e"
LOD	Limit of detection
LOQ	Limit of quantification
MS/MS	Tandem mass spectrometry
OR	Odds ratio
p25	Percentile 25
p75	Percentile 75
p90	Percentile 90
PAHs	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PFAS	Poly- and perfluoroalkyl substances
PFBS	Perfluorobutane sulfonate
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFUnDA	Perfluoroundecanoic acid
PIH	Provincial Institute of Hygiene
PPAR-alpha	Peroxisome proliferator-activated receptor (PPAR)-alpha
PSUs	Primary sampling units
SES	Socioeconomic status
TNF-alpha	Tumour necrosis factor alpha
UZA	Antwerp University Hospital
VITO	Vlaamse In stelling voor Technologisch Onderzoek
VUB	Vrije Universiteit Brussel

Supplementary Information

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Additional file 1: Table S1. Questionnaire on asthma and allergy outcomes. **Table S2.** FLEHS-2 campaign on adolescents list of confounders and potential covariates included in stepwise multiple logistic regressions. **Table S3.** FLESH-2 campaign on mothers list of confounders and potential covariates included in stepwise multiple logistic regressions. **Table S4.** FLEHS-2 campaign on adults list of confounders and potential covariates included in stepwise multiple logistic regressions. **Table S5.** FLESH-3 campaign on mothers list of confounders and potential covariates included in stepwise multiple logistic regressions. **Table S6.** FLESH-3 campaign on adults list of confounders and potential covariates included in stepwise multiple logistic regressions. **Table S7.** Association of immune system-related health effects with blood concentrations of perfluoro compounds. **Table S8.** Characteristics of the study populations for which PFAS measurements were obtained.

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Author contributions

NVL participated in the setting up of the studies, was the major contributor in writing the manuscript and was the spokesperson for the FLEHS 2 studies. GK participated in the setting up of the studies, and in the writing of the manuscript. SD performed the statistical analysis. AC participated in the setting up of the studies and in the practical organization; LB participated in the setting up of the studies and assisted in the statistical analysis. EDH participated in the setting up of the studies and in the organization of the field work. EG participated in the setting up of the studies and assisted in the statistical analysis and in the practical organization. BM participated in the setting up of the studies, in the field work and had a major role in the sociological aspects of the studies. TS performed the chemical analyses on PFAS. SR gave advice concerning the statistical analysis. DC participated in the practical organization of the studies and in the sociological aspects of the studies. TN participated in the setting up of the studies. VN participated in the setting up of the studies, in the organization of the field work and was the spokesperson for the FLEHS-3 studies. WB participated in the setting up of the studies and was responsible for the general coordination of part of the studies. GS participated in the setting up of the studies, was responsible for the general coordination of part of the studies and was coordinator of the field work committee. All authors read and approved the final manuscript.

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Availability of data and materials

The aggregated data are publicly available via the IPCHEM data platform (https://ipchem.jrc.ec.europa.eu/RDSIdiscovery/ipchem/index.html#discovery). The individual records can be requested via the procedures that are available on this portal (https://ipchem.jrc.ec.europa.eu/RDSIdiscovery/ipchem/index.html#showmetadata/FLEHS1REFNB).

Declarations

Ethics approval and consent to participate
In order to participate, all subjects had to give written informed consent. Informed consent was obtained from a parent and/or legal guardian if participants were under 18. The FLEHS studies were approved by the Ethics Committees of the University of Antwerp and the Antwerp University Hospital (UZA), Belgium. The dossier numbers for the different studies were, respectively, UA A08 09 (FLEHS-2, mothers, adults and adolescents of industrial contaminated site) and B300201318591 for FLEHS-3 mothers, and B300201419843 for FLEHS-3 adults. The studies on mothers were also approved by the respective Ethics Committees of each of the participating maternity units. All methods were carried out in accordance with relevant guidelines and regulations. (Declaration of Helsinki).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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