

PFAS and Immunomodulation Review and Update

Executive summary

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals considered to be contaminants of emerging concern due to their potential adverse effects on human health.

In 2016, the Australian Department of Health asked Food Standards Australia New Zealand (FSANZ) to establish health-based guidance values (HBGVs) for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). Tolerable daily intakes (TDIs) of 20 ng/kg bw/day for PFOS and 160 ng/kg bw/day for PFOA were established on the basis of reproductive and developmental studies in laboratory animals.

The FSANZ report included a detailed assessment of the immunodulatory effects of PFAS chemicals. It was found that PFAS were a potential immune hazard to humans but the exposure levels required to produce immunomodulation were unknown. Similar conclusions were reached in 2018 by the Australian Expert Health Panel, and in a systematic literature review conducted by the Australian National University.

The objective of this report was to evaluate any new human epidemiological information investigating the relationship between PFAS blood levels and immunomodulatory effects that had not been previously considered as a part of those earlier substantive reviews. Available new studies primarily investigated three different potential immunomodulatory effects of PFAS:

- decreased circulating antibody titres to vaccine-preventable diseases (VPDs)
- increased incidence of infectious diseases
- altered prevalence of hypersensitivity diseases such as asthma and allergies.

Decreased circulating antibody titres to VPDs

A targeted literature search identified four new epidemiological studies investigating associations between PFAS blood levels and circulating antibody titres for measles, rubella, *Haemophilus influenzae*, influenza, diphtheria and tetanus in children or adults. No two of the studies investigated the antibody response to the same vaccine.

No significant association was found between PFAS and response to the influenza vaccine in 78 adults, however the seroconversion rate was low.

National Health and Nutritional Examination Surveys (NHANES) data for 12 to 18 year old youths (n = 1012) showed no significant association between rubella titres and either PFOS or PFOA, or between either PFAS and sex or ethnicity. In adults (581 women, 621 men) there was a significant inverse association between both PFOS and PFOA and rubella IgG titre. When results were stratified for sex, there were no significant negative associations between either PFAS and rubella IgG in women. A significant negative association between rubella titres and PFOA, but not PFOS, was found in men.

In a study of 101 German children, there was a statistically significant inverse association between PFOA and antibodies against *Haemophilus influenza*, diphtheria and tetanus. In this study, no association was found for PFOS or other analysed PFAS congeners.

In a child cohort (n = 422) in Guinea-Bissau, doubling of PFOS and perfluorodecanoic acid (PFDA) concentration was associated with a decrease in measles antibody concentration. No significant associations were seen for PFOA or other measured PFAS congeners.

While these studies provide limited evidence of statistical associations, a causal relationship between increased PFAS blood levels and impaired vaccine response cannot be established with reasonable confidence. Evidence for an association between increasing PFAS blood levels and impaired vaccine response is insufficient for quantitative risk assessment on the basis of substantial uncertainties and limitations including:

- the small number of studies and participants, and mostly cross-sectional design of studies such that conclusions around causality should be drawn with caution
- limited dose-response information with most studies investigating a narrow range of blood levels associated with background levels of PFAS exposure
- inconsistency in antibody response to vaccines between different PFAS congeners which cannot be explained by study design
- potential for confounding by other known environmental immunotoxicants such as polychlorinated biphenyls
- uncertainty about the clinical relevance, if any, of the observed statistical associations to susceptibility to infectious disease.

Increased incidence of infectious diseases and altered prevalence of hypersensitivity diseases such as asthma and allergies

Eight new studies investigated whether blood levels of PFAS are associated with increased susceptibility to infectious diseases in children. Five new studies examined the association between blood PFAS levels and hypersensitivity responses including atopic dermatitis (eczema), allergies and asthma. Some numerical associations were observed, however the evidence was often inconsistent and contradictory for different PFAS congeners. On the basis of the available information it cannot be ruled out with reasonable confidence that any statistical associations may have been due to confounding, bias or chance.

Conclusions

In summary, new epidemiological studies provide some evidence of statistical associations between PFAS blood levels and impaired vaccine response, increased susceptibility to infectious disease and hypersensitivity responses. However the data are insufficient to establish causal relationships and it cannot be ruled out with reasonable confidence that the observed statistical associations may have been due to confounding, bias or chance. On the basis of the uncertainties and limitations in the evidence base, immunomodulation is not currently considered suitable as a critical endpoint for quantitative risk assessment for PFAS.

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Abbreviations

ADONA	Ammonium 4,8-dioxa-3H-perfluorononanoate
AOR	Adjusted overall risk
ATSDR	Agency for Toxic Substances and Disease Registry
BMI	Body mass index
CA16	Cocksackie virus A 16
CI	Confidence interval
EFSA	European Food Safety Authority
EV71	Enterovirus 71
G-CSF	Granulocyte colony-stimulating factor
GI	Gastrointestinal infection
GM-CSF	granulocyte-monocyte colony-stimulating factor
HAI	Haemagglutinin-inhibition test
HBGV	Health-based guidance value
HFMD	Hand, foot and mouth disease
IFN- α 2	Interferon-alpha2
IFN- γ	Interferon gamma
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IHC	Immunohistochemical staining
IL-1B	Interleukin-1B
IL-6	Interleukin-6
IL-12P70	Interleukin-12P70
IP-10	interferon- γ -inducible protein 10
IQR	Interquartile range
LOD	Limit of detection
LOQ	Limit of quantification
LRTI	Lower respiratory tract infection
MCP-1	Monocyte chemoattractant protein-1
mIgA	Mucosal Immunoglobulin A
MIP-1a	Macrophage inflammatory protein-1a
NHANES	National Health and Nutrition Examination Survey
NOAEC	No Observed Adverse Effect Concentration
OR	Overall risk
PBPK	Physiologically based pharmacokinetic
PCB	Polychlorinated biphenyl
PFAS	Per- and polyfluoroalkyl substances
PFBS	Perfluorobutane sulfonic acid
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHpS	Perfluoroheptanesulfonic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFOSA	Perfluorooctanesulfonamide
PFTeDA	Perfluorotetradecanoic acid
PFTrDA	Perfluorotridecanoic acid

PUnDA	Perfluoroundecanoic acid
RR	Relative risk
RSV	Respiratory syncytial virus
RTI	Respiratory tract infection
TDI	Tolerable daily intake
TNF- α	Tumour necrosis factor- α
URTI	Upper respiratory tract infection
VPD	Vaccine-preventable disease

Introduction

FSANZ was commissioned by the Department of Health in 2016 to conduct Hazard Assessments for three perfluoroalkylated substances (PFAS): perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexane sulfonate (PFHxS). Tolerable daily intakes (TDIs) of 20 ng/kg bw/day for PFOS and 160 ng/kg bw/day for PFOA were established on the basis of reproductive and developmental studies in laboratory animals. There was insufficient information to establish a TDI for PFHxS. In the absence of a TDI, FSANZ considered that using the TDI for PFOS was likely to be protective of public health.

In terms of immunomodulatory effects, FSANZ considered that PFOS and PFOA were a potential immune hazard to humans but the exposure levels required to produce immunomodulation were unknown. Adverse effects on the immune system in animals were only observed at very high doses, relative to those to which human populations are exposed. Furthermore, there was a lack of convincing evidence that such immunomodulation, if it were to occur, was likely to result in clinically relevant outcomes. It was concluded that it was difficult to envisage how the available epidemiology information could be used quantitatively in risk assessment (Drew and Hagan, 2016).

In a subsequent comprehensive review, The Expert Health Panel for PFAS (the Panel) identified that there were few human studies on PFAS and immunological effects, that there was a lack of consistency between studies, and that there is a substantial risk that many findings are due to bias or chance. There was also a strong potential for confounding by other persistent organic pollutants with immune effects. The Panel observed that the strongest evidence for a link between PFAS and clinically-important immunological effects was for impaired vaccine response, but that the human dose-response threshold for potential immune effects was very poorly characterised, and the overall human evidence was weak. It was concluded that while PFAS are likely to alter the function of the immune system, it was unclear if this occurs at current exposures or has any clinically important consequences (Expert Health Panel for PFAS (2018)).

As a part of the PFAS Health Study, Kirk et al (2018) conducted a systematic literature investigating the effect of PFAS exposure on the immune system in children and adults. The main study findings are summarised below and in Table 1.

- For diphtheria vaccine there was limited evidence¹ for an association between PFOA, PFOS, PFHxS and PFDA, noting that three of the four papers were on the same cohort in the Faroe Islands.
- For response to rubella vaccine, the evidence for an association was limited for PFOA and PFOS, and inadequate for PFHxS and perfluorononanoic acid (PFNA).

¹ Limited evidence of a health effect: A positive (direct) or negative (inverse) association has been observed between exposure to PFAS and the health effect in humans for which a causal interpretation is considered to be possible or probable, but chance, bias or confounding could not be ruled out with reasonable confidence.

- For all other vaccines (tetanus, measles, mumps and influenza) the evidence for adverse effects of any PFAS congener was inadequate².
- With regard to associations between PFAS exposure and adverse health outcomes, the evidence for all health outcomes considered (hospitalisations due to infection, middle ear infection, gastroenteritis and colds/influenza) was inadequate.
- The evidence for adverse effects of PFAS on all allergy and hypersensitivity endpoints, including asthma, allergies (including food allergies), plant sensitivity, shrimp allergy, cockroach sensitivity, mould sensitivity, allergic rhinoconjunctivitis, wheezing and eczema) was inadequate.

Table 1: Evaluations by Kirk et al (2018) of immunological health effects of PFAS

Health Effect	PFAS exposure measured	Evaluation of evidence	Papers evaluated
<i>Impaired response to vaccinations</i>			
Diphtheria	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHPA, PFUnDA, PFDODA	Limited evidence for PFOA, PFOS, PFHxs, PFDA	Grandjean et al (2012, 2016, Mogensen et al 2015 (same cohort as Grandjean et al). Kielsen et al (2016)
Tetanus	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHPA, PFUnDA, PFDODA	Inadequate evidence	Grandjean et al (2012, 2016)) Mogensen et al (2015) (same cohort as Grandjean et al) Granum et al (2013) Kielsen et al (2016)
Measles	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence	Granum et al (2013) Stein et al (2016a)
Mumps	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence	Granum et al (2013) Stein et al 2016a)
Rubella	PFOA, PFOS, PFHxS, PFNA	Limited evidence for PFOA and PFOS; inadequate for PFHxS and PFNA	Granum et al (2013) Stein et al 2016a)
Influenza	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence	Looker et al (2014) Stein et al (2016b)
<i>Increased susceptibility to infectious disease</i>			
Hospitalization due to infection	PFOA, PFOS	Inadequate evidence	Fei et al (2010)
Middle ear infection	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence	Okada et al (2012) Granum et al (2013)
Gastroenteritis	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence	Granum et al (2013)

² Inadequate evidence of a health effect: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between PFAS exposure and the health effect in humans.

Colds and influenza	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence	Granum et al (2013) Looker et al (255)
<i>Asthma and allergic diseases</i>			
Asthma	PFOA, PFOS, PFHxS, PFNA, PFDA, PFTeDA, PFDoDA, PFHxA, PFHpA, PFBS	Inadequate evidence	Dong et al (2013) Zhu et al (261) Humblet et al (2014) Stein et al (2016) Steenland et al (2015)
Total allergies	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoDA, PFTTrDA, PFUnDA	Inadequate evidence	Goudarzi et al (2016) Okada et al (2012, 2014) Stein et al (2016)
Total food allergies	PFOA, PFOS	Inadequate evidence	Okada et al (2012)
Shrimp allergy	PFOA, PFOS, PFNA	Inadequate evidence	Stein et al (2016a)
Plant sensitivity	PFOA, PFOS, PFNA	Inadequate evidence	Stein et al (2016a)
Cockroach sensitivity	PFOA, PFOS, PFNA	Inadequate evidence	Stein et al (2016a)
Mould sensitivity	PFOA, PFOS, PFNA	Inadequate evidence	Stein et al (2016a)
Allergic rhinoconjunctivitis	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoDA, PFTTrDA, PFUnDA	Inadequate evidence	Goudarzi et al (2016)
Wheezing	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoDA, PFTTrDA, PFUnDA	Inadequate evidence	Granum et al (2013) Okada et al (2012) Goudarzi et al (2016) Humblet et al (2014)
Eczema	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoDA, PFTTrDA, PFUnDA	Inadequate evidence	(Goudarzi et al (2016) Okada et al (2012) Okada et al (2014) Granum et al (2013)
<i>Autoimmune diseases</i>			
Crohn's disease	PFOA	Inadequate evidence	Steenland et al (2013, 2015)
Multiple sclerosis	PFOA	Inadequate evidence	Steenland et al (2013, 2015)
Lupus	PFOA	Inadequate evidence	Steenland et al (2013, 2015)
Rheumatoid arthritis	PFOA	Inadequate evidence	Steenland et al (2013, 2015)
Ulcerative colitis	PFOA	Inadequate evidence	Steenland et al (2013, 2015)
Table adapted from Kirk et al (2018). See Abbreviations for PFAS congener names.			

A number of studies on the immunomodulation potential of PFAS have become available since the previous FSANZ assessment, the Expert Health Panel for PFAS and Kirk et al (2018) reports. The objective of this report was to evaluate new information investigating the

relationship between PFAS blood levels and immunomodulatory effects to determine whether immunomodulation may be a suitable endpoint to derive a point of departure to support quantitative risk assessment of PFAS chemicals.

Literature search methodology

In August and September 2021, FSANZ conducted specific searches of PubMed, EBSCO and Google Scholar using the following search term combinations:

- *PFAS and immun-*
- *PFAS and vaccin-*
- *PFAS and (antibody or antibodies)*
- *Perfluoro and immun-*
- *Perfluoro and vaccin-*
- *Perfluoro and (antibody or antibodies)*

For PubMed and EBSCO searches, which allow a time period to be specified for searches, the time period was limited to papers published from 2016 inclusive. For PubMed searches, the filter “humans” was applied, to exclude papers describing studies in other species and *in vitro* studies. The literature search identified three new epidemiological studies of associations between PFAS exposure and circulating antibody titres for rubella, *Haemophilus influenzae*, diphtheria and tetanus in children, and one study of response to an influenza vaccine in adults. Eight new studies were found that investigated whether levels of PFAS are associated with increased susceptibility to infectious diseases in children. Five studies examined the association between PFAS exposure and hypersensitivity responses including atopic dermatitis (eczema), allergies and asthma.

A targeted search for new studies was considered justified on the basis that systematic reviews of the literature have previously been conducted in extensive earlier reviews.

Epidemiological studies on PFAS and immunomodulation

Studies are separated into those investigating immunosuppression, and those investigating immunostimulation. Within those categories, studies are reviewed in chronological order of publication, and within a year, alphabetically by first author.

STUDIES OF IMMUNOSUPPRESSION

Studies in children

Goudarzi et al. (2017) Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4 years of age.

This study was conducted as part of the Hokkaido Study on Environment and Children’s Health, a prospective ongoing birth cohort study that began in 2003 with the recruitment of pregnant women. A total of 1558 mother-child pairs were included in the investigation of PFAS and prevalence of infectious diseases. Maternal PFAS exposure was determined from maternal plasma samples obtained between 28 and 32 weeks of pregnancy. Infectious diseases in children were measured as mothers’ recollections of doctor’s diagnosis of otitis

media, pneumonia, varicella and/or respiratory syncytial virus (RSV) infection. Medical records were not obtained.

Levels of 11 PFAS were determined in plasma. Detection rates exceeded 97% for all but two PFAS (PFHxS and perfluorododecanoic acid (PFDoDa)). The PFAS present at the highest levels were, in descending order, PFOS (mean 4.92 ng/mL), PFOA (2.01 ng/mL), perfluoroundecanoic acid (PFUnDA) (1.43 ng/mL) and PFNA (1.18 ng/mL).

A total of 67.1% of the children had a history of at least one of the diseases. The prevalence of infectious diseases was not significantly different between girls and boys. The association between prenatal PFAS exposure and childhood diseases was assessed using logistic regression models. PFOS exposure in the highest quartile was associated with significantly increased odds ratios for total infectious diseases when compared to the lowest quartile (Q4 vs. Q1 OR: 1.61; 95% CI: 1.18, 2.21; p for trend=0.008) . After sex stratification, the p-value for the trend was significant only for girls. There was also a positive association between infectious diseases and PFHxS for girls (Q4 vs. Q1 OR: 1.55, 95% CI: 0.976, 2.45; p for trend=0.045) , but not boys. There was no association between exposure and infectious diseases for any other individual PFAS. Distribution of maternal plasma PFAS concentrations was included in a table but the units were not reported.

Reviewer comments

Strengths of this study

- A reasonable number of subjects were included in this study.
- The study is longitudinal in nature.

Limitations of the study

- Reliance on PFAS levels in maternal sera.
- Reliance on mothers' recollections of medical diagnoses over a four-year period, rather than using medical records, introduces the possibility of recall error.
- Postnatal exposure of children to PFAS or other immunosuppressant xenobiotics is not considered.
- The study as reported lacks information on risk factors that might have increased the likelihood that children would catch infectious diseases, such as attendance at nursery facilities, or existence of older siblings who could introduce infections caught at school.
- The magnitude of odds ratios was small.
- The majority of endpoints did not show any evidence of association.
- The sex-related difference with regard to PFHxS is not explained and suggests that the association were due to chance.

Zeng et al (2019). Prenatal PFAS exposure and antibody responses to two HFMD viruses in 3 month old infants.

Hand, Foot and Mouth Disease (HFMD) is a common and highly infectious disease. The most vulnerable population is children under the age of 5 years. The principal aetiological agents of HFMD are Enterovirus 71 (EV71) and cocksackievirus A 16 (CA16). Maternal

antibodies transported across the placenta provide some degree of immunity to children up to 12 months of age. Innate and memory immune responses in humans to the two viruses depend on T_H1 and T_H2 CD4⁺ helper T cell subsets. It is thought that the modulating role of interleukin-35 in the ratio between regulatory T cells and T helper 17 cells may be important in the pathogenesis of HFMD caused by EV17, and that PFAS may exert immunotoxic effects through interference with interleukin expression. The authors therefore hypothesized that prenatal PFAS exposure might modify the antibody-mediated immune response to HFMD.

The samples and data for the study originated from the Guangzhou Birth Cohort. A total of 411 women with singleton pregnancies were recruited for the study. Demographic information was collected from the women by questionnaire. Umbilical cord blood was collected at birth, and peripheral blood was also collected from babies at 3 months of age. None of the children were vaccinated against HFMD. Cord blood measurements of PFAS concentrations and HFMD antibody titres were available from 201 infants, and blood samples from 3 months of age were also available for 180 infants. Analysis was for a total of 17 PFAS.

The predominant PFAS in cord blood was PFHxS (mean 3.96 ng/mL), followed by PFOS (mean 3.17 ng/mL) and PFOA (mean 1.22 ng/mL). A consistent inverse correlation was found between cord blood PFAS and antibodies to EV71 and CA16. A doubling of the total PFAS concentration in cord blood was associated with significant increase in likelihood of the antibody titre for CA16 (OR: 2.74 (95% confidence interval (CI): 1.33, 5.61) and/or EV17 (OR = 4.55, 95% CI: 1.45, 4.28) being below the clinically protective level ($\geq 1:8$ titers) at 3 months of age. The association was stronger for boys than for girls.

Reviewer comments

Strengths of the study

- The blood samples are from the subjects themselves, rather than relying on extrapolation from maternal sera.

Limitations of the study

- This was a small study.
- Breast milk PFAS of the mothers was not measured, although breast milk is known to be an important exposure pathway for PFAS in infants. However the authors noted that breastmilk PFAS is likely to be highly correlated with cord blood PFAS.
- Other persistent environmental agents that are associated with immunosuppression in children, such as polychlorinated biphenyls (PCBs) were not assayed.
- The study lacks information on factors that could have impaired placental transfer of antibodies, including lack of maternal immunity to the viruses in question, premature delivery, placental pathology etc.
- There is a lack of information on whether children had older siblings who could have had HFMD, and increased their mother's immunity through exposure.

Abraham et al (2020) Plasma PFAS and vaccine response in healthy 1 year old children.

The subjects for this cross-sectional study were 101 healthy children who had been born within a four-week period, comprising 21 formula-fed children (10 boys and 11 girls) and 80 children who had been breastfed for at least 4 months (mean 5.5 months; 41 boys and 39 girls). The study was conducted using samples and data collected as part of a study on persistent organic pollutants conducted in the late 1990s. The PFAS analysed in plasma of children aged between 341 and 369 days (mean 351 days) were PFOA, PFOS, PFHxS, PFNA, PFDoDA, perfluorohexanoic acid (PFHxA), perfluorobutane sulfonic acid (PFBS), PFDA and 3H-perfluoro-3((3-methoxy-propoxy) propanoate (ADONA).

The childrens' blood was also analysed for vaccine-induced antibodies to *Haemophilus influenzae* type b (IgG), diphtheria (IgG) and tetanus (IgG and IgG1). Preliminary analysis showed that the time since the last vaccination had a strong influence on antibody levels, so for subsequent analysis, adjustment was made for the time since last vaccination. Blood samples were also subject to clinical pathology assessments, which in addition to routine haematology and clinical chemistry, included several markers of thyroid status, as well as differential analysis of immunoglobulin classes and lymphocyte subpopulations.

PFOS and PFOA were quantifiable in all the children, and PFHxA and PFNA were quantifiable in most children. The other five PFASs were predominantly or exclusively below the LOQ. Mean PFOS concentration in formula-fed children was 6.8 ± 3.4 µg/L plasma and in breast-fed children, 15.2 ± 6.9 µg/L plasma. Corresponding values for PFOA were 3.8 ± 1.1 µg/L and 16.8 ± 6.6 µg/L respectively; for PFHxA, 1.7 ± 1.1 µg/L and 2.1 ± 1.3 µg/L; and for PFNA, 0.2 ± 0.1 µg/L and 0.6 ± 0.2 µg/L.

A statistically significant inverse association was found between PFOA and antibodies against *Haemophilus influenzae* type b ($r = -0.32$), diphtheria ($r = -0.23$) and tetanus (IgG1 only) ($r = -0.25$). When subjects were stratified according to PFOA concentration, comparison of the highest and lowest quintiles showed that PFOA was associated with antibody levels, on a logarithmic scale, that were 86% lower for *Haemophilus influenzae* type b, 53% lower for diphtheria and lower 54% for tetanus. By estimating the PFOA concentration above which the antibody titres showed a downward trend on a population basis, the authors derived No Observed Adverse Effect Concentrations (NOAECs) for plasma PFOA on antibodies against *Haemophilus influenzae* type b, diphtheria and tetanus of 12.2, 16.2 and 16.9 µg/L respectively.

FSANZ notes that substantial interindividual variability in response is evident on visual inspection of the data.

PFOA level was also reportedly inversely associated with production of interferon gamma (IFN γ) by *ex vivo* lymphocytes after stimulation with diphtheria and tetanus toxoids. However, the source of the lymphocytes is not clear from the paper, since the children were originally sampled in the late 1990s.

Reviewer comments

Strengths of the study

- The children are very close in age.
- The investigations of immune parameters were relatively thorough.

- Some other persistent organic pollutants were considered.
- Differences between breastfed and formula-fed children were considered.
- Because the samples were collected in the 1990s, higher PFAS levels were present than in more recent studies.

Limitations of the study

- The cohort size was very small, only 101 children overall.
- There is substantial interindividual variability in response.
- There is a lack of information on whether the decreases in antibody concentrations are clinically relevant. That is; PFOA may cause antibody titres to fall below effective levels sooner than they naturally would have, but if the recommended vaccine schedule is followed, antibody titres might remain sufficient to protect against disease, particularly in formula-fed infants.
- The question of the stability of antibodies in samples stored for decades is not addressed.

Huang et al (2020). Prenatal exposure to PFASs and respiratory tract infections in preschool children.

The participants in this study were the Shanghai Prenatal Cohort, which is an ongoing prospective study of mother-child pairs. Women were recruited during pregnancy between 2011 and 2013. Of the 1269 mother-child pairs enrolled in the Cohort, 344 children were included in the current study, on the basis that data on concentrations of PFAS, serum IgG and serum IgE in cord blood, as well as medical records on respiratory tract infections (RTI) diagnosed by paediatricians were available.

Analysis for 10 PFAS was conducted, of which eight were detected in more than 90% of cord blood samples. PFOA was the most abundant PFAS (mean concentration 6.68 ng/mL cord plasma), followed in descending order of abundance by PFOS (mean 2.44 ng/mL), PFNA (mean 0.63 ng/mL), PFUnDA (mean 0.39 ng/mL), PFDA (mean 0.35 ng/mL), PFHxS (mean 0.16 ng/mL), PFDoDA (mean 0.09 ng/mL), and PFBS (mean 0.05 ng/mL).

Statistically significant associations identified were a positive association between cord blood PFBS concentration and incidence of respiratory tract infection (RTI) episodes in the first 5 years of life ($\beta = 6.05$, 95% CI (0.84, 11.26)), and a negative association between PFBS concentration and IgG concentration in blood at 5 years old. A one-unit logarithm-transformed PFBS concentration increase in cord blood was associated with 1.56 (95% CI (0.27, 2.85)) and 2.30 (95% CI (0.48, 4.11)) more episodes of RTI in children aged 2 and 4 years, respectively. A one-unit logarithm-transformed PFBS concentration increase in cord blood was associated with a 0.82-unit (95% CI (-1.67, - 0.01)) logarithm-transformed IgG concentration decrease in the serum at children aged 5 years. When the data were analysed by sex of the child, there were no significant associations between PFAS concentrations and RTI incidence in boys, but the significant association between prenatal PFBS exposure and RTI incidence persisted in girls ($\beta = 7.20$, (95% CI (-0.05, 14.36))). No statistically significant associations between RTIs and other PFAS congeners were found.

Reviewer comments

Strengths of the study

- Medical records were used, rather than reliance on parental diagnoses and/or parental recall.

Limitations of the study

- Small cohort size
- Associations only observed for PFBS, for which estimates of effect were extrapolated from very low median concentrations in serum.
- Lack of information on whether children were breastfed.
- Lack of detail about the pathogens responsible for the RTIs, if identified.
- Lack of information on maternal antibodies to common respiratory tract pathogens.
- Extrapolation from prenatal exposure over a long postnatal interval (5 years).
- Lack of consideration of confounding factors such as exposure to other immunotoxic xenobiotics, and of potential sources of infection such as older siblings or attendance at preschool.
- No explanation is suggested for the sex difference.

Kvalem et al (2020) PFAS and respiratory infections, allergy and asthma in children.

This study used both cross-sectional and longitudinal data from the prospective birth cohort Environment and Childhood Asthma (ECA) study in Oslo. Data in this study included information and measurements collected at 2, 10 and 16 years after birth. A total of 378 children from the original 3754 had PFAS measurements at 10 years, as well as anthropometry, skin prick test, blood sampling for allergic sensitisation, spirometry including methacholine challenge, and parental interview at 10 and 16 years. In addition, the spirometry at 10 years included treadmill test, and the children were interviewed at 16 years.

Analysis of blood samples at 10 years of age was for 19 PFAS, but for 10 PFAS, all samples were below LOQ and so they were not considered further. The nine PFAS included for statistical analysis were above LOQ in at least 70% of samples and were perfluorooctane sulfonamide (PFOSA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, PFHxS, perfluoroheptane sulfonic acid (PFHpS) and PFOS. Health outcomes were lung function, asthma, atopic dermatitis, rhinitis, allergic sensitisation, common cold and lower respiratory tract infection (LRTI).

PFOS was the most abundant PFAS (mean 20.9 ng/mL serum), followed by PFOA (mean 4.62 ng/mL serum) and PFHxS (mean 3.33 ng/mL serum). The mean serum concentrations of the remaining PFAS were < 1.0 ng/mL serum. Serum concentrations of PFAS tended to be higher in boys than girls and the difference reached statistical significance for PFOA (mean 4.90 ng/mL in boys vs. 4.32 ng/mL in girls), PFNA (mean 0.69 vs. 0.57 ng/mL respectively), PFDA (mean 0.21 vs. 0.17 ng/mL respectively), PFUnDA (mean 0.20 vs. 0.17 ng/mL respectively), PFHpS (mean 0.43 vs. 0.32 ng/mL respectively) and PFOS (mean 22.8 vs 19.0 ng/mL).

In the cross-sectional analysis at 10 years of age, there was a significant positive association between PFHpA and asthma in girls (RR 1.31 (95% CI: 1.08;1.60)). In the longitudinal data,

there was an inverse association between atopic dermatitis and PFNA (RR 0.51 (0.35;0.73) per interquartile range (IQR) of 0.28 ng/mL), PFDA (RR 0.64 (0.42;0.98) per IQR of 0.14 ng/mL) and PFUnDA (RR 0.45 (0.29;0.69) per IQR of 0.12 ng/mL) in girls, but not in boys. There was also an inverse association between atopic dermatitis and PFHxS in all participants (RR 0.79 (0.34;0.99) per IQR of 8.6 ng/mL) and in boys (RR 0.71 (0.52;0.99) per IQR of 1.26 ng/mL). PFNA and PFHpS were positively associated with rhinitis in girls (RR (95% CI): 1.56 (1.18 ; 2.06) per IQR of 0.28 ng/mL and RR 1.36 (1.28;1.45) per IQR of 0.16 ng/mL, respectively) at age 16, whereas PFOA was positively associated with rhinitis in all participants (RR 1.08 (1.01;1.14) per IQR of 1.77 ng/mL). No associations were found between PFASs and lung function. In longitudinal data, PFDA was inversely associated with common cold (all participants; OR (95% CI): 1–2 times last 12 months 0.78 (0.55;1.09) and ≥ 3 times last 12 months OR 0.56 (0.37;0.84) per IQR of 0.13 ng/mL), but PFHpA, PFOA, PFHpS and PFOS were positively associated with LRTI (RR (95% CI): 1.28 (1.08;1.51) per IQR of 0.13 ng PFHpA/mL), (RR 1.10 (1.02;1.19) per IQR of 1.77 ng PFOA/mL), RR 1.12 (1.09;1.16) per IQR of 0.20 ng PFHpS/mL) and RR 1.34 (1.17;1.55) per IQR of 9.23 ng PFOS/mL).

Reviewer comments

Strengths of this study

- Thorough clinical characterisations of lung function and diagnostic criteria for asthma.
- The authors carried out Bonferroni adjustment for multiple statistical tests.

Limitations of the study

- Small numbers of participants.
- Blood sampling for PFAS at a single time-point.
- Reliance on recall over periods of years.
- Lack of consideration of other xenobiotics that can alter immune response

Timmermann et al (2020) PFASs, vaccine responses and morbidity in children in Guinea-Bissau.

This study was based on a subset of data from a randomised controlled trial of measles vaccination conducted in Guinea-Bissau, which compared response to two doses of measles vaccine (at 4-7 months and 9 months) to that following a single dose of measles vaccine at 9 months. The measured outcome was mortality up to 3 years of age. Children were recruited, and those in the intervention group given the first vaccination, at 4-7 months of age. All children were then vaccinated at 9 months of age and mothers were interviewed about the child's health, as well as giving information on breastfeeding and the child's consumption of solid foods. Antibodies against measles were measured in a subgroup of 422 children at recruitment, at 9 months and at 2 years of age. Maternal blood samples were collected at recruitment for this cohort. Sufficient serum to also measure PFAS was available for 237 children, comprising 135 from the intervention (two measles vaccinations) group and 102 from the control (one measles vaccination) group. The PFAS analysed in sera were PFOS, PFOA, PFNA, PFDA, PFHxS and PFUnDA. One child had a serum PFUnDA concentration below the LOQ, but with that exception, all children had measurable concentrations of all six

PFAS. Group mean values for PFOS, PFOA, PFNA, PFDA, PFHxS and PFUnDA were 0.77, 0.68, 0.21, 0.19, 0.10 and 0.12 ng/mL serum respectively.

The protective serum concentration of measles antibodies is not known for certain, but a value of 120 mIU/mL was used. At recruitment, 74% of children had antibody levels below that value. At the 9-month time-point, 99% of children in the control group, and 10% of those in the intervention group, had antibody levels below 120 mIU/mL. At the 2-year visit, 98% of the children in the control group and 1% of children in the intervention group had antibody levels below 120 mIU/mL. Girls tended to have higher antibody concentrations than boys.

The authors concluded that in children vaccinated at 4 to 7 months of age, a doubling of serum PFOS was associated with a mean 21% decrease (95% CI: 2, 37%) in antibody concentration at 9 months of age, and a doubling of PFDA concentrations was associated with a mean 25% decrease (95% CI: 1, 43%) at 9 months of age. However these associations were not observed at 2 years of age. In the control group, doubling of PFOS concentration was associated with a mean 27% decrease (95% CI: 4, 44%) in measles antibody at the 9-month visit, at which time they received measles vaccination. At the 2-year visit, measles antibody concentrations were 22% higher in association with a doubling of PFOS in boys (95%CI: -11, 66%) in the control group, but were 28% lower in girls (95%CI: -48, -1%) ($p_{\text{interaction}} = 0.02$) in the same group.

When the associations between PFAS and any morbidity at inclusion (4-7 months of age) and at 9 months of age, most (35 of 48) analyses showed increased odds of morbidity with increased PFAS concentration at inclusion, but only a few of the associations were statistically significant. The strongest results were for PFHxS and PFOA in relation to coughing and to any morbidity. At 9 months, ORs for coughing in association with doubling of PFHxS and PFOA were 2.15 (95% CI: 1.17, 3.97) and 1.87 (95% CI: 1.02, 3.45) respectively. ORs for any morbidity were 1.82 (95% CI: 1.06, 3.11) and 2.02 (95% CI: 1.20, 3.41), respectively.

Reviewer comments

Strengths of the study:

- The study was a randomised controlled trial.
- Vaccination records were robust, because vaccination was part of the study design.
- Breastfeeding was included in the data collected.
- There was consideration of potential confounders.

Limitations of the study

- The number of study subjects is relatively small.
- Reported odds ratios are also small.
- "Coughing" is a very nonspecific symptom.
- Morbidity data relied on parental recall rather than medical records.
- Lack of information on exposures to other xenobiotics that could affect immune response.
- The concentrations of PFAS were very low.

Dalsager et al (2021). Prenatal exposure to PFAS and hospitalisation for infectious diseases in childhood.

Data and samples for this study were obtained as part of the Odense Child Cohort study, an ongoing cohort study of children in Odense, Denmark. Of the mother-child pairs in the Odense Child Cohort study, 1,503 were included in this study. Serum samples of pregnant women were analysed for PFOS, PFOA, PFHxS, PFDA and PFNA. Data on hospital admissions of the children were obtained from the Danish National Patient Register. Admissions were included in the data analysed in this study only if the primary reason for admission was an infectious disease. For the purposes of statistical analyses, admissions were grouped into upper respiratory tract infections (URTI), LRTI, gastrointestinal infections (GI) and other infections.

Mean levels of PFOS, PFOA, PFHxS, PFNA and PFDA in maternal sera were 7.52, 1.68, 0.36, 0.64 and 0.29 ng/mL respectively. Mean duration of follow-up of children from birth was 3.6 years, at which time 394 children (26%) had been hospitalised at least once for infectious disease. A doubling of maternal serum PFOS was significantly associated with a 23% increase in risk of hospitalisation for any infection (adjusted Hazard Ratio (HR): 1.23 (95% CI: 1.05, 1.44)), with a larger HR for boys than for girls. The adjusted HR for PFOA was 1.13 (95% CI: 0.97, 1.29) whereas those of other PFAS were close to 1. In contrast to the HRs for PFOS, the HR for PFDA was larger for girls (HR 1.25 (95% CI: 1.00, 1.57)) than for boys (HR 0.97 (95% CI: 0.83, 1.13)). Every doubling of maternal PFOS increased the risk of admission for LRTI by 54% (adjusted HR: 1.54 (1.11, 2.15)) whereas the corresponding increase for PFOA was 27% (adjusted HR: 1.27 (1.01, 1.59)). On the other hand, risk of admission for GI decreased with increasing prenatal exposure to all five PFAS (for example; PFOA, HR: 0.55 (0.32, 0.95)). Adjustment for breastfeeding and for prematurity had little effect on the outcomes.

Reviewer comments

Strengths of the study

- The number of subjects was relatively large.
- The study used medical records rather than parental recall.
- There was prospective follow-up of study subjects.
- The authors considered and investigated the effects of potential confounders, such as breastfeeding and prematurity.

Limitations of the study

- Extrapolation from maternal sera rather than sera from the children themselves
- There are other factors besides severity of infection that affect the decision to admit a child to hospital, such as social determinants.
- The results were inconsistent across different PFAS.
- The sex-related differences are not discussed.
- The study as reported does not appear to have taken into account sources of infections, such as older siblings and whether or not the child attended a childcare facility or kindergarten.

- Some types of infections were uncommon; for example there were only 40 GI events. This leads to substantial uncertainty.
- The most common group of infections was “other infections”, with 275 episodes. This is a very non-specific category.
- There was no analysis or consideration of other xenobiotics that can affect immune response.

Studies in adults

Stein et al (2016b) PFAS and immune response to FluMist vaccination

FluMist is an intranasal vaccine using a live attenuated influenza virus. In this study, the immune response to FluMist vaccination was examined in relation to serum concentrations of four PFAS in 78 healthy adults (age range 21-49) who were vaccinated in the 2010-2011 influenza season.

Analysis was conducted for eight PFAS but only four were found in sera of all study participants; PFOS (mean 5.22 (95% CI: 4.52, 6.02) ng/mL), PFOA (mean 2.28 (95% CI: 2.03, 2.56) ng/mL), PFHxS (mean 1.1 (95% CI: 0.897, 1.34) ng/mL), and PFNA (mean 0.77 (95% CI: 0.673, 0.881) ng/mL).

A pre-vaccination blood sample was collected immediately before participants were vaccinated. They returned for a follow-up visit on Day 3 and on Day 30, and provided nasal washes and blood samples on both follow-up visits. Most participants had high pre-vaccination titres to H3N2 flu strain, one of the components in the vaccines, so the study authors concentrated on response to H1N1. The response was measured by haemagglutinin-inhibition test (HAI) and immunohistochemical staining (IHC) using canine kidney cell monolayers infected with H1N1. Cytokines and chemokines were assayed in serum and nasal secretions. Granulocyte colony-stimulating factor (G-CSF), interferon- γ -inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor- α (TNF- α), IFN- α 2, IFN- γ , macrophage inflammatory protein-1a (MIP-1a), granulocyte-monocyte colony-stimulating factor (GM-CSF), interleukin-1B (IL-1B), interleukin-6 (IL-6), and interleukin-12P70 (IL-12P70) were measured in serum. IP-10, MCP-1, G-CSF and IFN- α 2 were measured in nasal secretions. Cytokines for which less than 90% of samples were above the limit of detection (LOD) were not used for statistical analysis. They were GM-CSF, IL-1B, IL-6 and IL-12P70 in serum and G-CSF and IFN- α 2 in nasal secretions. Localised mucosal response was assayed by measuring haemagglutinin-specific mucosal immunoglobulin A (mIgA) in nasal secretions.

Seroconversion after vaccination was low, at 9% measured by HAI and 25% by IHC. Most immune cytokine markers showed no significant changes following FluMist administration, but IP-10 was significantly higher after vaccination, in both serum and nasal secretions. Associations between seroconversion and PFAS concentrations were not statistically significant. There were no readily discernible or consistent patterns between PFAS concentrations and baseline cytokine, chemokine or mIgA concentrations, or between PFAS concentrations and post-vaccination changes in those markers.

Reviewer comments

Strengths of the study

- Measurement of multiple parameters of immune system function.

Limitations of this study include:

- The sample size was very small.
- There was a low seroconversion rate in response to FluMist vaccination.
- Other xenobiotics that can affect immune response were not considered

Pilkerton et al (2018) PFAS and rubella immunity

Information analysed in this study was for individuals over the age of 12, and was obtained from the National Health and Nutritional Examination Surveys (NHANES) for 1999-2000 and 2003-2004. The NHANES data are cross-sectional. The sample examined in this study consisted of NHANES participants 12 years and older for whom blood PFAS levels, rubella IgG titres and ethnicity were available, and who were not pregnant. The only PFAS considered in the analyses were PFOA and PFOS. Body mass index (BMI) was included as a covariate because it may affect serum PFAS. Parity was considered for women because pregnancy and lactation represent routes by which PFAS may be removed from the body.

The analyzed sample contained 581 adult women, 621 adult men, and 1012 youth (12 to 18 years).

Average PFOA concentration was approximately 6 ng/mL (standard error \pm 0.3) in men, 4.3 ng/mL in women and 4.8 ng/mL in youth. Sex differences in adults were significant. The average PFOS concentration in men was 28.1 ng/mL (standard error \pm 0.7) in men, 22.1 ng/mL in women and 25.1 ng/mL in youth.

In youth there was no significant association between rubella titres and either PFOS or PFOA, or between either PFAS and sex or ethnicity. However in adults there was a significant inverse association between both PFOS (Quartile F value 3.44, $Pr > F$ 0.0295) and PFOA (Quartile F value 6.60, $Pr > F$ 0.006) and rubella IgG titre. When results were stratified for sex, there were no significant negative associations between either PFAS and rubella IgG in women. A significant negative association between rubella titres and PFOA, but not PFOS, was found in men.

The authors commented that the differences between men and women are not surprising because the women had lower PFAS concentrations overall than the men, and it is well established that women mount a more robust immune response to antigens than men do. Increased antibody responses in women, relative to men, have been reported for vaccinations against mumps, smallpox, influenza and rubella.

Reviewer comments

Strengths of the study

- The number of people included in the study was fairly large.
- Factors that may affect serum PFAS, such as BMI, and parity in women, were included as covariates.

Limitations of the study

- NHANES data are cross-sectional, with different participants in each survey.
- There is a lack of information on whether participants had been vaccinated against rubella, and if so, at what age.
- There is a lack of information concerning whether the participants had been exposed to wild-type rubella.
- There is a lack of information on exposure to other xenobiotics that can affect immune response.

STUDIES OF IMMUNOSTIMULATION

Studies in children

Chen et al (2018) Prenatal exposure to PFAS and childhood atopic dermatitis

This prospective study began with the recruitment of 1056 pregnant women over three years. Prenatal information was obtained by questionnaires and medical records, and cord blood was collected at delivery. Infants were subject to follow-up at 6, 12 and 24 months. Atopic dermatitis was diagnosed by two dermatologists independently. A total of 687 infants had cord blood PFAS concentration data available and were followed up for 24 months, and of those infants, 173 (25.2%) were diagnosed with atopic dermatitis (eczema). Atopic dermatitis in infants is a type of hypersensitivity and indicative of a propensity to allergies such as hay fever and asthma.

Of ten PFAS for which cord blood analysis was conducted, PFOS, PFNA, PFHxS were found in all samples, while PFOA, perfluorodecanoate (PFDA), PFDoDa, PFUnDa and perfluorobutane sulphonate (PFBS) were found in >90% of samples. PFOSA and PFHpA were found in <30% of samples and were not included in statistical analyses. The median (Q1-Q3) values of PFOA, PFOS, PFNA, PFDA, PFUnDA, PFDoDA, PFHxS and PFBS were 6.98 (95% CI: 4.94–9.55), 2.48 (95% CI: 1.82–3.24), 0.65 (95% CI: 0.50–0.83), 0.36 (95% CI: 0.23–0.54), 0.40 (95% CI: 0.29–0.53), 0.09 (95% CI: 0.07–0.13), 0.16 (95% CI: 0.13–0.20), 0.05 (95% CI: 0.04–0.06) ng/mL, respectively. Most PFAS concentrations were slightly but not significantly higher in female than male infants.

After adjustment for confounders, the highest quartiles of PFOA, PFNA, PFDA and PFHxS were associated with atopic dermatitis in girls, with adjusted overall risk (AOR) being 2.52 (95% CI: 1.12–5.68), 2.14 (95% CI: 0.97–4.74), 2.14 (95% CI: 1.00–4.57), and 2.30 (95% CI: 1.03–5.15), respectively. Additionally, the second quartile of PFDoDA was associated with a 3.2-fold increase in atopic dermatitis risk (3.24, (95% CI: 1.44–7.27)) in girls. No statistically significant associations between any PFAS and atopic dermatitis were found in boys.

Reviewers comments

Strengths of the study

- The study was prospective in design.

- Diagnoses were confirmed by dermatologists, rather than relying on parental diagnosis and/or recall.
- The cohort of children is fairly large.
- Follow-up was conducted several times.
- Breastfeeding, a source of postnatal exposure to PFAS to children, was included in the statistical analysis.

Limitations of the study

- Exposure was assessed only on the basis of cord blood, without subsequent blood collections to assess postnatal exposure.
- There is a lack of information concerning allergies and hypersensitivity reactions in parents or siblings, to identify any genetic tendency to hypersensitivity reactions, or any other risk factors for hypersensitivity reactions.
- Differences between the sexes are not explained.

STUDIES OF BOTH IMMUNOSUPPRESSION AND IMMUNOSTIMULATION

Studies in children

Impinen et al (2019) Maternal levels of PFAS during pregnancy and childhood allergy and asthma

Data analysed in this study were obtained from the prospective Norwegian Mother and Child Cohort Study (MoBa). Analysis of maternal plasma collected mid-pregnancy (median of 18 weeks gestation) from 1943 women was conducted for 19 PFAS. Six PFAS were selected on the basis that at least 80% of measurements were above the limit of quantification (LOQ). The selected PFAS were PFOS, PFOA, PFHxS, PFNA, PFUnDA and PFHpS.

Data on child health were collected by questionnaire when the child was 0.5, 1.5, 3 and 7 years old. The 3 year questionnaire was returned by 1270 mothers and the 7 year questionnaire was returned by 972 mothers. In the questionnaires, parents reported if doctors had diagnosed asthma, atopic eczema, food allergy, or inhalation allergy in the children. In addition, parent-reported symptoms included night cough, hives, urticaria, pruritic rash, wheezing or tightness in the chest, and itchy/weepy eyes and/or runny nose without a cold. At the 3-year time-point, parental reports of infections such as colds, throat infections, ear infections, gastrointestinal infections and urinary tract infections were also recorded.

Median concentrations of PFOS, PFOA, PFHxS, PFNA, PFUnDA and PFHpS in maternal plasma were 12.87, 2.54, 0.65, 0.45, 0.20 and 0.15 ng/mL, respectively.

A statistically significant inverse relationship between maternal PFUnDA and atopic eczema was found in girls (OR 0.60 (95% CI: 0.44, 0.82)), but not boys. Between birth and 3 years of age, common cold was inversely associated with maternal PFOS (RR 0.94 (95% CI: 0.91, 0.97)) and PFOA (RR 0.94 (95% CI: 0.91, 0.98)), again only in girls. Bronchitis and pneumonia were positively associated with PFOS (RR 1.20 (95% CI: 1.07, 1.34)), PFOA, PFHxS and PFHpS (RR 1.15 (95% CI: 1.05, 1.25)) in the same age group, although the associations with PFOA (RR 1.32 (95% CI: 1.09, 1.59)) and PFHxS (RR 1.29 (95% CI: 1.12, 1.48)) were significant only for girls. Streptococcal throat infection in the first three years was

positively associated with PFNA in both sexes (RR 1.25 (95% CI: 1.00,1.57)), but with PFOA only in boys (RR 1.42 (95% CI: 1.12,1.79)) and with PFUnDA only in girls (RR 1.47 (95% CI: 1.14, 1.90)). Pseudocroup was positively associated with PFOA (RR 1.22 (95% CI: 1.07,1.38)) and PFHxS (RR 1.20 (95% CI: 1.11, 1.30)) but inversely associated with PFUnDA (RR 0.86 (95% CI: 0.78, 0.95)). Ear infection in the first 3 years was positively associated with PFHxS (RR 1.09 (95% CI:1.04, 1.14)) but inversely associated with PFOS (RR 0.88 (95% CI: 0.82,0.94)) and PFUnDA (RR 0.90 (95% CI: 0.84, 0.96)). There was no association between maternal PFAS during pregnancy and ear infections reported in the 7 year questionnaire, which covered the ages of 6 to 7 years. There was a positive association between gastrointestinal infections in the first three years and maternal PFNA, but only in girls (RR 1.15 (95% CI: 1.06, 1.24)). The text of the paper states that gastrointestinal infections between 6 and 7 years were positively associated with PFOA and PFHxS in both sexes, but the numerical data do not show a significant association. Urinary tract infections were inversely associated with PFOS (RR 0.70 (95% CI: 0.61, 0.80)), PFOA (RR 0.73 (95% CI: 0.62, 0.86)) and PFHpS (RR 0.85 (95% CI: 0.75, 0.96)) in girls up to the age of 3, and inversely associated with PFHxS (RR 0.79 (95% CI: 0.66, 0.95)) in girls aged 6 to 7.

Most mothers had no previous deliveries, and 97% of babies were breastfed, the majority for at least 7 months. There was a significant association between nursery attendance and colds, streptococcal throat infection, pseudocroup and gastrointestinal infections in children up to the age of 3 (p values of 0.001, 0.003, 0.001 and 0.005 respectively). When results were stratified for nursery attendance, the association between PFOA and the common cold disappeared. Significant associations between streptococcal throat infections and PFNA and PFUnDA were found only in the nursery group, whereas an inverse relationship between streptococcal throat infection and PFOS was found in the no nursery group. For pseudocroup, there was a positive association with PFOA and PFHxS in children not attending nursery, but an inverse association between pseudocroup and PFUnDA in children attending nursery. For diarrhoeal disease, there was an inverse association with PFOS in children not attending nursery, but a positive association with PFNA in children attending nursery.

Reviewer comments

Strengths of the study

- This study improves on some others by considering exposure to infectious agents through nursery attendance, and the results show that this is an important factor that should not be omitted.

Limitations of the study

- Reliance on parental recall of doctors' diagnoses, and on parental diagnoses not verified by medical examination
- There is a lack of information on other risk factors for hypersensitivity responses
- Inconsistencies between different PFAS, and findings of both positive and negative associations, are not explained.

Impinen et al. (2018). Prenatal PFAS exposure, respiratory tract infections, allergy and asthma in children

Data were obtained from the general population birth cohort in the prospective 'Environment and Childhood Asthma' study conducted in Oslo. Cord blood from 641 healthy neonates was analysed for concentrations of 19 PFAS. Available data included lung function at birth for 802 children, follow-up parental interviews and skin prick tests at 2 years of age, and clinical examinations at 10 years of age. The clinical examinations included skin prick test and spirometry, and parents were interviewed.

Cord blood concentrations of only six PFAS were sufficient for statistical analysis. These were PFOS, PFOA, PFOSA, PFHxS, PFNA, and PFUnDA. The highest median concentrations were of PFOS (5.2 ng/mL) and PFOA (1.6 ng/mL). Median concentrations of the remaining four PFAS were 0.4, 0.3, 0.2 and 0.1 ng/mL for PFOSA, PFHxS, PFNA and PFUnDA respectively

Associations were found between LRTIs in the first 10 years of life and cord blood concentration of PFOS, PFOA, PFOSA, PFNA and PFUnDA, with β values of 0.50, 0.28, 0.10, 0.09 and 0.18 respectively. The p values were < 0.0001 for PFOS, PFOA, PFOSA and PFUnDA, and 0.013 for PFNA. An association between common colds in the first two years of life and cord blood concentration of PFUnDA (β 0.11; $p < 0.0001$) was also identified. However, no associations were found between cord blood concentrations of any PFAS and lung function at birth, asthma, allergic rhinitis, allergic sensitisation or allergic disease.

Reviewers comments

Strengths of the study

- A moderately large cohort of subjects.
- Assessment of multiple hypersensitivity responses.
- Parental history of allergic diseases, and household smoking, were included in demographic data.
- Demographic data also included parity of mothers and whether children were breastfed.
- Statistics were adjusted to account for multiple statistical comparisons made.

Limitations of the study

- potential for recall error due to reliance on parental interviews at the 2 and 10 year time-points.
- The authors' explanation of biological mechanisms is weak.
- Prenatal exposure to 10 years of age is a very long interval over which to extrapolate, with potential recall issues for parents, and with apparently no postnatal measurements of exposure to PFAS or other potentially immunomodulating xenobiotics during that interval.

Bamai et al (2020). PFASs and childhood allergies and infectious diseases in children up to age 7.

This study is a follow-up study to that of Goudarzi et al (2017) and likewise based on data from the Hokkaido Study on Environment and Children's Health. Of the original >20,000 children recruited, maternal prenatal PFAS measurements, results from questionnaires

completed when the children were 7 years of age, and results from at least one of the questionnaires sent when children were 1, 2 or 4 years old were available for 2206 children. Eleven PFAS were measured; PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), PFOA, PFHxS, PFNA and PFOS. Outcome assessments included wheeze, asthma, rhino-conjunctivitis (separated into colds/flu and allergic rhino-conjunctivitis), atopic dermatitis, chickenpox, otitis media, pneumonia, and RSV infection.

The PFAS present at the highest levels in maternal blood were PFOS (mean 4.92 ng/mL), PFOA (2.01 ng/mL), perfluoroundecanoic acid (PFUnDa) (1.43 ng/mL) and PFNA (1.18 ng/mL).

An inverse association with allergic rhino-conjunctivitis and prenatal exposure to PFNA (RR 0.83 (0.69, 0.99)) or PFDA (RR 0.82 (0.72, 0.94)). There were also inverse associations between eczema and prenatal exposures to PFOA (RR 0.85 (0.77, 0.94)), PFUnDA (RR 0.86 (0.78, 0.95)), PFDoDA (RR 0.88 (0.78, 0.98)), PFTrDA (0.89 (0.80, 0.99)) and PFOS (RR 0.86 (0.76, 0.98)). Dose-response-relationships were evident in inverse associations between maternal PFDA or PFUnDA and allergic rhino-conjunctivitis, and in inverse associations between PFOA, PFUnDA, PFTrDA or PFOS and risk of eczema. Prenatal exposure to PFDoDA, was inversely associated with doctor-diagnosed chickenpox (OR 0.85 (0.72, 1.00)), PFTrDA was inversely associated with otitis media (OR 0.84 (0.72, 0.98)), and PFOS was inversely associated with RSV infection (OR 0.72 (0.56, 0.91)). However prenatal exposure to PFOA was positively associated with an increased risk of pneumonia (OR 1.17 (1.01, 1.37)). After stratification for the presence of siblings, prenatal exposure to PFDA and PFOS were associated with reduction in doctor-diagnosed chickenpox (OR 0.79 (0.52, 1.21)) and RSV infection (OR 0.92 (0.63, 1.35)) respectively. The positive association between PFOA and increased risk of pneumonia, and the inverse association between PFDoDA and risk of chickenpox also remained after stratification for the presence of siblings. Among children with no siblings, there were significant positive associations between PFDA and pneumonia (OR 1.64 (1.01, 2.66)), and between PFOA and RSV infection (OR 1.58 (1.13, 2.22)).

Reviewer comments

Strengths of the study

- The study population was relatively large.
- The presence of siblings, potential vectors of infections, was included in analyses.

Weaknesses of this study .

- Wide confidence intervals for some outcomes indicate that there was significant uncertainty.
- Reliance on parents' memories for questionnaires, rather than medical records, introduces the possibility of recall error.
- There is a lack of information on exposures to other risk factors for allergic responses, as well as other immunomodulating xenobiotics .

Other national or international assessments

The German Human Biomonitoring Commission established values for monitoring of PFOA and PFOS in 2021 (Hölzer et al 2021; Schümann et al 2021). HBM-1 values were defined as concentrations in human biological material below which no adverse health effects are expected, and therefore there is no need for action, and were set at 2 ng PFOA/mL human blood plasma, and 5 ng PFOS/mL human blood plasma. HBM-1 values were derived from a range of reported adverse associations of these PFASs in epidemiological studies, including time to pregnancy, gestational diabetes, birth weight, lipid metabolism, response to vaccines, age at menarche, thyroid function and age at menopause (Hölzer et al 2021). HBM-II values were defined as the concentration in human biological material which, when exceeded, may lead to health impairment. For women of childbearing age, HBM-II values of 5 ng PFOA/mL for human blood plasma, and 10 ng PFOS/mL human blood plasma, were set. For all other subpopulations, the HBM-II values were 10 ng PFOA/mL human blood plasma and 20 ng PFOS/mL human blood plasma. The Commission concluded that data on reduced antibody formation were insufficient for derivation of HBM-II values, citing the small number of studies, partially inconsistent results, and difficulties in extrapolation from decreased antibody titres to risk of infectious diseases (Schümann et al 2021).

The only international food regulatory agency to derive a health-based guidance value for PFAS based on immunotoxicity studies in humans is EFSA (2020b). The EFSA health-based guidance value is derived from the studies of Grandjean et al (2012) and Abraham et al (2020). The Grandjean et al (2012) study showed an association between low antibody titres in response to diphtheria vaccine in children of 7 years of age and prenatal exposure to PFOS and PFOA. The Abraham et al (2020) study showed an association between low antibody titres in response to vaccinations against tetanus, diphtheria and *Haemophilus influenza* and PFOA, but not PFOS, exposure.

EFSA derived a HBGV for the sum of exposure to PFOS, PFOA, PFHxS and PFNA. A BMDL₁₀ of 17.5 ng/mL serum at the age of 1 year was calculated from the Abraham et al (2020) study, and used to estimate the daily intake of PFAS by the breastfeeding mother that would result in this level in the infants, using a PBPK model and assuming that breastfeeding continued for 12 months. It was calculated that the maternal exposure to the sum of the four PFAS would be 0.63 ng/kg bw/day, and that the tolerable weekly intake (TWI) should be $7 \times 0.63 = 4.4$ ng/kg bw. The report notes limitations associated with the use of this endpoint because the number of data points in the key study were small ($n = 101$), especially at higher serum concentrations, and there was a relatively large inter-individual variability in the response. These factors introduce a level of uncertainty in the dose response curve shape and therefore identification of a BMDL.

Other submitters' comments³ on the Draft EFSA Opinion (EFSA 2020a) included:

³ Submissions were received from national authorities (Federal Food Safety and Veterinary Office(CH), German Environment Agency, Food, Nutrition and Health Unit (IT), UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, Swedish Environmental Protection Agency, Norwegian Scientific Committee for Food and Environment, German Federal Institute for Risk Assessment, Ministerium für Ländlichen

- The associations in the studies considered pivotal by the EFSA CONTAM Panel are weak, and cross-sectional studies cannot demonstrate causation.
- Vaccination response in humans is known to be highly variable, and the decline in antibodies after vaccination is not well defined, but cannot be assumed to be linear.
- The mechanism/s by which PFAS affect the immune system are poorly understood.
- It is not appropriate to apply PBPK models validated for adults to data obtained from breastfed infants or small children.
- It is not appropriate to derive a TWI for adults from data from breastfed infants.
- Other authoritative bodies have identified different critical effects for the individual PFAS.
- It is clear from the available data that the potencies of the four PFAS differ.

The Agency for Toxic Substances and Disease Registry (ATSDR) published a draft Toxicological Profile for Perfluoroalkyls in 2018, followed by a final version in May 2021. Data from animal studies were used to derive minimal risk levels (MRLs) of 3×10^{-6} mg/kg/day for PFOA and PFNA, 2×10^{-6} mg/kg/day for PFOS and 2×10^{-5} mg/kg/day for PFHxS. Points of departure (PODs) were based on developmental effects for PFOA, PFOS and PFNA, and endocrine (thyroid) effects for PFHxS. The ATSDR reviewed evidence of associations between PFAS and immunomodulation endpoints including decreased antibody response to vaccines, infectious disease resistance, hypersensitivity, and autoimmunity. The ATSDR considered that the evidence for an association between perfluoroalkyl exposure and decreased antibody response to vaccines was the strongest of these endpoints, but did not consider the data sufficient to establish cause-and-effect relationships, and highlighted the significant inter-individual variability in the association between impaired vaccine response and PFAS blood concentration.

Discussion

Epidemiological studies have investigated three different potential effects of PFAS on immunomodulation;

- decreased circulating antibody titres to VPDs.
- increased incidence of infectious diseases.
- altered prevalence of hypersensitivity diseases such as asthma and allergies.

Raum und Verbraucherschutz Baden Württemberg DE)); universities/public research institutes (Center for Research on Ingredient Safety (Michigan State University), Karolinska Institutet (SE), National Institute for Public Health and the Environment (NL); the private sector (Daikin Chemical Europe GmbH, American Chemistry Council, The 3M Company) organisations classified as 'other' (the European Human Biomonitoring Initiative, the Flemish Family Organisation, FoodDrink Europe, Mamma No Pfas, Federation of the European Cutlery, Flatware, Hollowware and Cookware Industries), one non-government organisation (Fidra) and six individuals including Anthony Tweedale, Philippe Grandjean and Esben Budtz-Jørgensen (commenting jointly), Carlotta Bargossa, Linda Birnbaum and Rolf Soer.

Reduced titres of circulating antibodies to VPDs

This review identified a small number of observational studies investigating an association between PFAS and decreased circulating antibody titres to vaccine-preventable diseases or antibody response that had not been previously considered by FSANZ, the Expert Panel or Kirk et al (2018) (Table 2). The study of Zheng et al (2019) also measured antibody titres, although they were presumed to represent maternal antibodies transferred prenatally.

Stein et al (2016b) found no statistically significant associations between seroconversion and PFAS concentrations following administration of an intranasal vaccine using a live attenuated influenza virus. It is noted however that seroconversion rates were low.

Pilkerton et al (2018) found no significant association between rubella titres and either PFOS or PFOA, or between either PFAS and sex or ethnicity, in youth. In adults there was a significant inverse association between both PFOS (Quartile F value 3.44, Pr>F 0.0295) and PFOA (Quartile F value 6.60, Pr>F 0.006) and rubella IgG titre. When results were stratified for sex, there were no significant negative associations between either PFAS and rubella IgG in women. A significant negative association between rubella titres and PFOA, but not PFOS, was found in men.

In a study of 101 children, Abraham et al (2020) found a statistically significant inverse association between PFOA and antibodies against *Haemophilus influenza* type b ($r = -0.32$), diphtheria ($r = -0.23$) and tetanus (IgG1 only) ($r = -0.25$). When subjects were stratified according to PFOA concentration, comparison of the highest and lowest quintiles showed that PFOA was associated with antibody levels, on a logarithmic scale, that were 86% lower for *Haemophilus influenza* type b, 53% lower for diphtheria and lower 54% for tetanus. PFOS was not associated with statistically significant associations for antibodies against *Haemophilus influenza*, diphtheria or tetanus.

Timmermann et al (2020) reported that at 9 months, doubling of PFOS was associated with a 21% decrease in measles antibody concentration, and a doubling of PFDA was associated with a 25% decrease. However these associations were not observed at 2 years of age. In the control group, doubling of PFOS concentration was associated with a 27% decrease in measles antibody. In the control group at the 2-year visit, measles antibodies increased 22% with a doubling of PFOS in boys, but was 28% lower in girls. No significant associations with antibody levels against measles were observed for PFOA. It is noteworthy that blood levels of PFAS were very low in this study, because it was based in Africa.

Table 2: Results of studies that examined PFAS effects on antibody titres induced by vaccination

Study	Antigen	Subpopulations	PFAS					
			PFOS	PFOA	PFHxS	PFNA	PFDA	PFUnDa
Stein et al (2016b)	H1N1 influenza		-	-	-	-		
Pilkerton et al (2018)	Rubella	Teens	-	-				
		Adult men	-	inv.				
		Adult women	-	-				
Abraham et al (2020)	<i>Haemophilus influenza</i>		-	inv.	-	-		
	Diphtheria		-	inv.	-	-		
	Tetanus		-	inv.	-	-		

Timmermann et al (2020)	Measles	Control group (1 vaccination) at 9 months	inv.	-	-	-	-	-
		Intervention group (2 vaccinations) at 9 months	inv.	-	-	-	inv.	-
		Control group (1 vaccination) at 2 years	-	-	-	-	-	-
		Intervention group (2 vaccinations) at 2 years	-	-	-	-	-	-
inv.= significant inverse association - = no significant association detected Shaded box = not analysed or not subject to statistical analysis								

Consistent with earlier observations, the associations between increased PFAS blood levels and antibody titres in these studies were generally weak, and partially inconsistent findings were observed for PFOS, PFOA and other PFAS chemicals for the same antigen (Table 2). While these studies provide limited evidence of statistical associations, a causal relationship between increased PFAS blood levels and impaired vaccine response cannot be established with reasonable confidence. The evidence for an association between increasing PFAS blood levels and impaired vaccine response is insufficient for quantitative risk assessment on the basis of substantial uncertainties and limitations including:

- the small number of studies and participants, and mostly cross-sectional design of studies such that conclusions around causality should be drawn with caution.
- limited dose-response information with most studies investigating a narrow range of blood levels associated with background levels of PFAS exposure.
- inconsistency in antibody response to vaccines between different PFAS congeners which cannot be explained by study design.
- potential for confounding by other known environmental immunotoxicants such as PCBs for which inverse associations with blood serum antibody concentrations against tetanus and diphtheria have previously been reported in the child populations living in the Faroe islands (Heilmann et al. 2010).
- uncertainty about the clinical relevance, if any, of the observed statistical associations to susceptibility to infectious disease.

This conclusion is consistent with the recent decisions of the German Human Biomonitoring Commission (Hölzer et al 2021; Schümann et al 2021), ATSDR (2018, 2021), and a number of earlier opinions from national agencies and bodies (Danish EPA 2016; Expert Health Panel for PFAS 2018; Kirk et al 2018; UK COT 2006; US EPA 2016). Only EFSA (2020b) has so far considered the data on antibody titres from human studies suitable for use in deriving a HBGV and quantitative risk assessment.

Increased rates of infectious diseases

A number of new studies investigated whether levels of PFAS are associated with increased susceptibility to infectious diseases in children. These studies include Goudarzi et al (2017),

Impinen et al (2018, 2019), Bamai et al (2020), Huang et al (2020), Kvaalem et al (2020), Timmermann et al (2020) and Dalsager et al (2020). Results are summarized in Table 3.

Side-by-side comparison of the studies in Table 3 shows that results are inconsistent between studies. The possibility that the statistically significant associations are due to confounding factors, chance or bias cannot be ruled out. The frequency of inverse associations (i.e., higher levels of PFAS were associated with lower incidences of illnesses) is not consistent with the hypothesis that PFAS are associated with immune suppression in children, raising instead the possibility that many or most of the associations occurred by chance or were due to unrecognized confounding.

In terms of dose-response, Timmermann et al (2021) reported a positive association between PFOS and incidence of infectious disease, but Huang et al (2020) and Bamai et al (2020) found no corresponding association, although the mean serum PFOS concentration in their studies was an order of magnitude higher than in the Timmermann study. Huang et al (2020) found no association between PFOA and incidence of infectious diseases but Impinen et al (2018, 2019), Bamai et al (2020) and Dalsager et al (2021) all found associations, even though the mean serum PFOA concentration in their studies was approximately one-third that measured by Huang et al (2020).

In a trial of ammonium perfluorooctanoate as a chemotherapeutic agent in 49 cancer patients, involving weekly doses of the test substance at 50 to 1200 mg for 6 weeks, there were no clinical observations that suggested altered immune responses, even though for parts of the trial the plasma PFOA level exceeded 1000 μM (approximately 420,000 $\mu\text{g/L}$). Some of the participants in the study were observed for 12 weeks (Convertino et al. 2018).

Overall these conclusions are consistent with Kirk et al (2018) who found that the evidence for an association between PFAS exposure and the incidence of infectious disease is inadequate. This is also consistent with the conclusions of the ATSDR that the evidence for increased susceptibility to infectious disease is inconsistent for some PFAS and absent for others. EFSA (2021) consider an association between PFAS and increased susceptibility is plausible, but that the existing evidence is insufficient.

Table 3: Results of studies that compared PFAS concentrations and childhood infectious diseases																
Study	Diseases	Sub-populations	PFAS													
			PFOS	PFOA	PFHxS	PFHxA	PFHpA	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA	PFTeDA	PFOSA	PFHpS	PFBS
Goudarzi et al (2017)	Otitis media, pneumonia, varicella, RSV	Girls	✓	-	✓	-	-	-	-	-	-	-	-	-		
		Boys	-	-	-	-	-	-	-	-	-	-	-	-		
Impinen et al (2018)	Lower respiratory tract infections		✓	✓	-			✓		✓				✓		
Impinen et al (2019)	Cold	Girls	✓	✓	-			-		-					-	
		Boys	-	-	-			-		-					-	
	Throat infection	Girls	-	-	-			✓		✓					-	
		Boys	-	✓	-			✓		-					-	
	Pseudocroup	Girls	-	✓	✓			-		Inv.					-	
		Boys	-	✓	✓			-		Inv.					-	
	Ear infection	Girls	-	-	✓			-		-					-	
		Boys	-	-	✓			-		-					-	
	Gastrointestinal infection	Girls	-	-	-			✓		-					-	
		Boys	-	✓	✓			-		-					-	
Urinary tract infection	Girls	-	✓	✓			-		-					-		
	Boys	-	-	-			-		-					-		
Bronchitis pneumonia	Girls	-	-	-										✓		
	Boys	✓	-	-										✓		
Bamai et al (2020)	Chickenpox		-	-	-	-	-	-	✓	-	Inv.	-	-	-	-	
	Otitis media		-	-	-	-	-	-	-	-	-	Inv.	-	-	-	
	RSV		Inv.	✓	-	-	-	-	-	-	-	-	-	-	-	
	pneumonia		-	✓	-	-	-	-	✓	-	-	-	-	-	-	
Huang et al (2020)	Respiratory tract infections	Girls	-	-	-		-	-	-		-			-		✓
		Boys	-	-	-		-	-	-		-			-		-

✓ = positive association. Inv. = inverse association. - = no association. Shaded cell means PFAS was not analysed or statistical comparison not made.

Table 3 continued: Results of studies that compared PFAS concentrations and childhood infectious diseases

Study	Diseases	Sub-populations	PFAS													
			PFOS	PFOA	PFHxS	PFHxA	PFHpA	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA	PFTeDA	PFOSA	PFHpS	PFBS
Kvalem et al (2020)	Common cold	Girls	-	-	-		-	-	Inv.	-				-	-	
		Boys	-	-	-		-	-	Inv.	-				-	-	
	Lower respiratory tract infections	Girls	✓	✓	-		✓	-	-	-				-	✓	
		Boys	✓	✓	-		✓	-	-	-				-	✓	
Timmermann et al (2020)	Diarrhoea	Girls	-	-	-			-	Inv.	-						
		Boys	-	-	-			-	✓	-						
	All morbidity	Girls	Inv.	-	-			-	-	Inv.						
		Boys	✓	-	-			-	-	✓						
Dalsager et al (2020)	Hospitalization	Girls	✓	✓	-			-	-							
		Boys	✓	✓	-			-	-							
	Gastro-intestinal infection	Girls	Inv.	Inv.	Inv.			Inv.	Inv.							
		Boys	Inv.	Inv.	Inv.			Inv.	Inv.							

✓ = positive association. Inv. = inverse association. - = no association. Shaded cell means PFAS was not analysed or statistical comparison not made.

Effects on atopic dermatitis, allergy and asthma

Studies examining the association between PFAS exposure and hypersensitivity responses including atopic dermatitis (eczema), allergies and asthma, include those of Chen et al (2018), Impinen et al (2018, 2019), Bamai et al (2020), and Kvalem et al (2020). Results are compared and contrasted in Table 4.

Results of different studies are inconsistent and in some cases contradictory. For example, Chen et al (2018) found a positive association between prenatal exposure to PFHxS and atopic dermatitis, whereas Kvalem et al (2020) found an inverse association between PFHxS and atopic dermatitis in children. Similarly Chen et al (2018) found a positive association between prenatal exposure to PFDA and atopic dermatitis, whereas Kvalem et al (2020) found an inverse association between PFDA and atopic dermatitis in girls, and no association in boys.

EFSA (2021) concluded that the epidemiological evidence does not provide sufficient evidence of associations between PFAS and hypersensitivity reactions such as asthma and allergies. The ATSDR (2021) found that evidence for associations between PFOA, PFOS, PFHxS, PFNA, PFDA, PFBS, and PFDoDA and increased risk of asthma is marginal. They noted the small number of studies and the presence of conflicting study results.

Table 4: Results of studies that compared PFAS concentrations and childhood hypersensitivity diseases																
Study	Diseases	Sub-populations	PFAS													
			PFOS	PFOA	PFHxS	PFHxA	PFHpA	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA	PFTeDA	PFOSA	PFHpS	PFBS
Chen et al (2018)	Atopic dermatitis		-	✓	✓	-		-	✓	-	✓					-
			-	-	-	-		-	-	-	-					-
Impinen et al (2018)	Asthma		-	-	-			-		-				-		
	Allergic rhinitis		-	-	-			-		-				-		
	Allergic sensitisation		-	-	-			-		-				-		
Impinen et al (2019)	Atopic dermatitis	Girls	-	-	-			-		Inv.					-	
		Boys	-	-	-			-		-					-	
	Asthma/wheeze	Girls	-	-	-			-		-					-	
		Boys	-	-	-			-		-					-	
Bamai et al (2020)	Allergic rhinoconjunctivitis		-	-	-	-	-	-	Inv.	Inv.	-	-	-		-	
	Eczema		Inv.	Inv.	-	-	-	Inv.	Inv.	Inv.	-	-			-	
Kvalem et al (2020)	Atopic dermatitis	Girls	-	-	Inv.		-	Inv.	Inv.	Inv.				-	-	
		Boys	-	-	Inv.		-	-	-	-				-	-	
	Asthma	Girls	-	-	-		✓	-	-	-				-	-	
		Boys	-	-	-		-	-	-	-				-	-	

✓ = positive association. Inv. = inverse association. - = no association. Shaded cell means PFAS was not analysed or statistical comparison not made.
 *may be allergic rather than infectious

Conclusions and recommendations

New epidemiological studies provide limited evidence of statistical associations between PFAS blood levels and impaired vaccine response, increased susceptibility to infectious disease and hypersensitivity responses. However the data are insufficient to establish causal relationships and it cannot be ruled out with reasonable confidence that the observed statistical associations may have been due to confounding, bias or chance. On the basis of the uncertainties and limitations in the evidence base, immunomodulation is not currently considered suitable as a critical endpoint for quantitative risk assessment of PFAS.

Further epidemiological studies that would increase confidence in the data set would include:

- Prospective rather than cross-sectional studies
- Studies examining antibody response to the same vaccine, across different populations, and correcting for potentially confounding variables, including other environmental chemicals with immunological properties
- More studies examining a range of immunological endpoints rather than measuring only antibody titres
- Studies examining more specific infectious diseases, with medical records confirming the diagnosis, and not confined to diseases requiring hospitalization.

Elucidation of the biological basis of immunomodulation by PFAS, through the use of *in vitro* approaches and animal models, is also needed.

References

Abraham K, Mielke H, Fromm H, Völkel W, Menzel J, Peiser M, Zepp F, Willich SN and Weikert C (2020) Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. *Archives of Toxicology* 94: 2131-2147

Agency for Toxic Substances and Disease Registry (ATSDR) 2018 Toxicological Profile for Perfluoroalkyls Draft for Public Comment. <https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf>

Agency for Toxic Substances and Disease Registry (ATSDR) 2021 Toxicological profile for Perfluoroalkyls. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. DOI: 10.15620/cdc:59198
<https://www.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=1117&tid=237>

Bamai YA, Goudarzi H, Araki A, Okada E, Kashino I, Miyashita C and Kishi R (2020) Effect of prenatal exposure to per- and polyfluoroalkyl substances on childhood allergies and common infectious diseases in children up to age 7 years: the Hokkaido study on environment and children's health. *Environment International* 143(2020) 105979
<https://doi.org/10.1016/j.envint.2020.105979>

Chen Q, Huang R, Hua L, Guo Y, Huang L, Zhao Y, Wang X and Zhang J (2018). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: a prospective birth cohort study. *Environmental Health* 17:8
<https://ehjournal.biomedcentral.com/articles/10.1186/s12940-018-0352-7>

Convertino M, Church TR, Olsen GW, Liu Y, Doyle E, Elcombe CR, Barnett AL, Samuel LM, MacPherson IR and Evans TRJ (2018) Stochastic pharmacokinetic-pharmacodynamic modelling for assessing the systemic health risk of perfluorooctanoate (PFOA). *Toxicological Sciences* 163(1): 293-306 <https://academic.oup.com/toxsci/article/163/1/293/4865972>

Dalsager L, Christensen N, Halekoh U, Timmermann CAG, Nielsen F, Kyhl HB, Husby S, Grandjean P, Jensen TK and Andersen HR (2021). Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: A study among 1,503 children from the Odense Child Cohort. *Environment International* 149: 106395
<https://doi.org/10.1016/j.envint.2021.106395>

Danish EPA (2015) Perfluoroalkylated substances: PFOA, PFOS and PFOSA: Evaluation of health hazards and proposal of a health-based quality criterion for drinking water, soil and ground water. Environment project No. 1665, 2015. Danish Ministry of the Environment, Environmental Protection Agency. <http://www2.mst.dk/Udgiv/publications/2015/04/978-87-93283-01-5.pdf>

Dong GH, Tung KY, Tsai CH, Liu MM, Wang D, Liu W, Jin Y-H, Hsieh W-S, Lee YL and Chen P-C (2013). Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. *Environmental Health Perspectives* 121(4): 507-13

Drew R and Hagen T (2016) Immunomodulation by PFASs: A brief literature review. ToxConsult document ToxCR300816-RF

ECHA (2009) Guidance in a nutshell: Chemical safety assessment.

EFSA Panel on Contaminants in the Food Chain (CONTAM) 2018 Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Journal 16(12): 5194 <https://doi.org/10.2903/j.efsa.2018.5194>

EFSA 2020a. Outcome of a public consultation on the draft risk assessment of perfluoroalkyl substances in food. <https://doi.org/10.2903/sp.efsa.2020.EN-1931>

EFSA Panel on Contaminants in the Food Chain (CONTAM) 2020b Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal 18(9): 6223 <https://doi.org/10.2903/j.efsa.2020.6223>

Expert Health Panel for Per- and Poly-Fluoroalkyl Substances (PFAS) (2018) [https://www1.health.gov.au/internet/main/publishing.nsf/Content/C9734ED6BE238EC0CA2581BD00052C03/\\$File/expert-panel-report.pdf](https://www1.health.gov.au/internet/main/publishing.nsf/Content/C9734ED6BE238EC0CA2581BD00052C03/$File/expert-panel-report.pdf) Accessed 4 June 2021

FAO/WHO (2009) Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria 240.

Fei C, McLaughlin JK, Tarone RE and Olsen J (2007). Perfluorinated chemicals and fetal growth: A study within the Danish National Birth Cohort. Environmental Health Perspectives 115(11): 1677-82.

Fenton SE, Ducatman A, Boobis A, DeWitt JC, Lau C, Ng C, Smith JS and Roberts SM (2021) Per- and polyfluoroalkyl substance toxicity and human health review: Current state of knowledge and strategies for informing future research. Environmental Toxicology and Chemistry 40(3): 606-630 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7906952/>

Goudarzi H, Miyashita C, Okada E, Kashino I, Chen C-J, Ito S, Araki A, Kobayashi S, Matsuura H and Kishi R (2017) Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4 years of age. Environment International 104: 132-138

Grandjean P, Andersen EW, Budtz-Jorgensen E, Nielsen F, Molbak K, Weihe P and Heilmann C, 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. The Journal of the American Medical Association, 307, 391–397. <https://doi.org/10.1001/jama.2011.2034>

Hölzer J, Lilienthal H and Schümann M (2021). Human Biomonitoring (HBM)-I values for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) – Description, derivation and discussion. Regulatory Toxicology and Pharmacology 121:104862 <https://doi.org/10.1016/j.yrtph.2021.104862>

Huang H, Yu K, Zeng X, Chen Q, Liu Q, Zhao Y, Zhang J, Zhang X and Huang L (2020) Association between prenatal exposure to perfluoroalkyl substances and respiratory tract infections in preschool children. Environmental Research 191: 110156 <https://doi.org/10.1016/j.envres.2020.110156>

Humblet O, Diaz-Ramirez LG, Balmes JR, Pinney SM, and Hiatt RA (2014). Perfluoroalkyl chemicals and asthma among children 12-19 years of age: NHANES (1999-2008). *Environmental Health Perspectives* 122(10): 1129-33

Impinen A, Nygaard UC, Lødrup Carlsen KC, Mowinckel Pc, Carlsen KH, Haug LS and Granum B (2018) Prenatal exposure to perfluoroalkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. *Environmental Research* 160: 518-523

Impinen A, Longnecker MP, Nygaard UC, London SJ, Ferguson KK and Haug LS (2019). Maternal levels of perfluoroalkyl substances (PFASs) during pregnancy and childhood allergy and asthma related outcomes and infections in the Norwegian Mother and Child (MoBa) cohort. *Environment International* 124: 462-472

Kielsen K, Shamim Z, , Ryder LP, Nielsen F, Grandjean P, Budtz-Jørgensen E and Heilmann C (2016). Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. *Journal of Immunotoxicology* 13(2): 270-3.

Kirk M, Smurthwaite K, Bräunig J, Trevenar S, D'Este C, Lucas R, Lal A, Korda R, Clements A, Mueller J and Armstrong B (2018). The PFAS Health Study: Systematic Literature Review https://rsph.anu.edu.au/files/PFAS%20Health%20Study%20Systematic%20Review_1.pdf

Kvalem HE, Nygaard UC, Lødrup Carlsen KC, Carlsen KH, Haug LS and Granum B (2020). Perfluoroalkyl substances, airways infections, allergy and asthma related health outcomes – implications of gender, exposure period and study design. *Environment International* 134: 105259 <https://doi.org/10.1016/j.envint.2019.105259>

Looker C, Luster MI, Calafat AM, Johnson VJ, Burlison GR, Burlison FG and Fletcher T (2014). Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. *Toxicological Sciences* 138(1): 76-88.

Pilkerton CS, Hobbs GR, Lilly C and Knox SS (2018). Rubella immunity and serum perfluoroalkyl substances: Sex and analytic strategy. *PLOS One* <https://doi.org/10.1371/journal.pone.0203330>

Schümann M, Lilienthal H and Hölzer J (2021). Human biomonitoring (HBM)-II values for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) – Description, derivation and discussion. *Regulatory Toxicology and Pharmacology* 121: 104868 <https://doi.org/10.1016/j.yrtph.2021.104868>

Steenland K, Zhao L, Winquist A and Parks C (2013). Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the Mid-Ohio Valley. *Environmental Health Perspectives* 121(8):900-5.

Steenland K, Zhao L and Winquist A (2015). A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). *Occupational and Environmental Medicine* 72(5):373-80

Stein CR, McGovern KJ, Pajak AM, Maglione PJ and Wolff MS (2016a) Perfluoroalkyl and Polyfluoroalkyl Substances and Indicators of Immune Function in Children Aged 12 – 19 years: NHANES. *Pediatric Research* 79(2): 348–357. doi:10.1038/pr.2015.213

Stein CR, Ge Y, Wolff MS, Ye X, Calafat AM, Kraus T and Moran TM (2016b). Perfluoroalkyl substance serum concentrations and immune response to FluMist vaccination among healthy adults. *Environmental Research* 149: 171-178

Timmermann CA, Jensen KJ, Nielsen F, Budz-Jorgensen E, van der Klis F, Benn CS, Grandjean P and Fisker AB (2020). Serum perfluoroalkyl substances, vaccine responses, and morbidity in a cohort of Guinea-Bissau children. *Environmental Health Perspectives* 128(8) <https://doi.org/10.1289/EHP6517>

United Kingdom Committee on Toxicity (2006) COT Statement on the Tolerable Daily Intake for Perfluorooctanoic Acid.

<https://cot.food.gov.uk/sites/default/files/cot/cotstatementpfoa200902.pdf>

US EPA (2002) A review of the reference dose and reference concentration processes. EPA/630/P-02/002F

US EPA (2016) Health Effects Support Document for Perfluorooctanoic Acid (PFOA). EPA 822-R-16-003. https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_hesd_final_508.pdf

Zeng X-W, Bloom MS, Dharmage SC, Lodge CJ, Chen D, Li S, Guo Y, Roponen M, Jalava P, Hirvonen M-R, Ma H, Hao Y-T, Chen W, Fu C and Dong G-H (2019). Prenatal exposure to perfluoroalkyl substances is associated with lower hand, foot and mouth disease viruses antibody response in infancy: Findings from the Guangzhou Birth Cohort Study. *Science of the Total Environment* 663: 60-67

Zhu Y, Qin XD, Zeng XW, Paul G, Morawska L, Su MW, Tsai C-H, Wang S-Q, Lee YL and Dong G-H (2016). Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: By gender and asthma status. *Science of the Total Environment* 559: 166-73