A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and immunological health conditions in humans

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ABSTRACT

Whether perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS), two widely used and biopersistent synthetic chemicals, are immunotoxic in humans is unclear. Accordingly, this article systematically and critically reviews the epidemiologic evidence on the association between exposure to PFOA and PFOS and various immune-related health conditions in humans. Twenty-four epidemiologic studies have reported associations of PFOA and/or PFOS with immune-related health conditions, including ten studies of immune biomarker levels or gene expression patterns, ten studies of atopic or allergic disorders, five studies of infectious diseases, four studies of vaccine responses, and five studies of chronic inflammatory or autoimmune conditions (with several studies evaluating multiple endpoints). Asthma, the most commonly studied condition, was evaluated in seven studies. With few, often methodologically limited studies of any particular health condition, generally inconsistent results, and an inability to exclude confounding, bias, or chance as an explanation for observed associations, the available epidemiologic evidence is insufficient to reach a conclusion about a causal relationship between exposure to PFOA and PFOS and any immune-related health condition in humans. When interpreting such studies, an immunodeficiency should not be presumed to exist when there is no evidence of a clinical abnormality. Large, prospective studies with repeated exposure assessment in independent populations are needed to confirm some suggestive studies with certain endpoints.

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Supplemental data for this article can be accessed at http://dx.doi.org/10.3109/10408444.2015.1122573.

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Introduction

Perfluorooctanoate (PFOA; C_{20}F_{39}COO^-) and perfluorooctanesulfonate (PFOS; C_{18}F_{37}SO_3^-), two of several perfluoroalkyl and polyfluoroalkyl substances (PFASs), were used widely in industrial and commercial surfactant and polymer applications beginning in the 1950s (Buck et al. 2011). Because of the ubiquitous use and emission of PFASs, these chemicals are commonly detected in the environment, wildlife, and humans (Buck et al. 2011; Butenhoff et al. 2006; Calafat et al. 2007a; Giesy and Kannan 2001; Kannan et al. 2004). The pervasiveness of PFOA and PFOS and their long clearance half-lives in humans (Olsen et al. 2007) have provoked intense interest in understanding the potential human health impact of long-term exposure to these chemicals. Guided largely by evidence of immunotoxic effects of PFOA and PFOS in cellular and animal experimental systems (Corsini et al. 2014; DeWitt et al. 2012), much epidemiologic research in recent years has focused on possible effects of these chemicals on various immune-related health conditions in humans.

Industrial production and emission of PFOA and PFOS in North America and Europe have ceased (U.S. EPA 2006), and serum levels of both chemicals, especially PFOS, have correspondingly declined over time in these regions (Glynn et al. 2012; Kato et al. 2011; Nost et al. 2014; Olsen et al. 2012; Yeung et al. 2013a, 2013b). However, production of these chemicals or their precursors has increased in parts of Asia (Li et al. 2015; Wang et al. 2014; Xie et al. 2013; Zhang et al. 2012). In view of the ongoing environmental release and persistence of PFOA and PFOS, their widespread detection in humans, and experimental evidence of animal immunotoxicity at high administered doses, their potential effects on the human immune system should be clarified. Therefore, this systematic review was conducted to summarize and evaluate the epidemiologic literature on PFOA and PFOS in relation to human immunological conditions, and to assess whether the evidence is sufficient to demonstrate a causal relationship. As background for the review of epidemiologic studies, this paper begins with a brief, non-systematic review of animal studies of the immunotoxicity of PFOA and PFOS and the possible relevance of their results to humans.

Overview of animal studies on the immunotoxicity of PFOA and PFOS

Experimental studies of the immunotoxicity of PFOA and PFOS have recently been reviewed, with some authors concluding that these compounds can cause immune suppression at doses that are potentially relevant to highly exposed humans and wildlife (Corsini et al. 2014; DeWitt et al. 2012). Earlier studies of high, acutely toxic dietary doses of PFOA up to 75 mg/kg/day resulted in suppression of antigen-specific immunoglobulin M (IgM) antibody production, splenic and thymic atrophy, and altered T-cell phenotypic distribution in male C57BL/6 mice (Yang et al. 2002a, 2000, 2001); however, direct immunotoxic effects could not be distinguished from secondary effects of severe toxicity, effects on other target organs, and stress. In male CD rats, by contrast, no immunotoxic effects of PFOA were observed (Loveless et al. 2008), even at acutely toxic oral doses up to 30 mg/kg/day that resulted in body weight loss, hepatomegaly, and focal liver necrosis, indicating inter-species differences in the immune sensitivity to PFOA exposure. Decreased IgM production in CD-1 mice administered 10 and 20 mg/kg/day PFOA was probably secondary to severe systemic toxicity and stress, as indicated by an approximately 20% loss in body weight and a more than 3-fold increase in liver weight (Loveless et al. 2008).

At PFOA doses that were not acutely toxic, a 15-day exposure study in C57BL/6J and C57BL/6N female mice demonstrated dose-dependent PFOA-mediated suppression of IgM synthesis (which is involved mainly with early, primary immunity), but not IgG synthesis (which is involved mainly in secondary immunity) or delayed-type hypersensitivity responses (Dewitt et al. 2008). The lowest observed adverse effect level (LOAEL) was 3.75 mg/kg/day PFOA from drinking water for 15 days, a dose that led to approximately 74 000 ng/mL PFOA in serum at 1 day post-exposure and 35 000 ng/mL serum PFOA at 15 days post-exposure. The 1-day post-exposure serum concentration is approximately 25–74 times greater than that measured in occupationally highly exposed humans (∼1000–3000 ng/mL) (Olsen et al. 2003; Olsen et al. 2000; Olsen & Zobel 2007; Woskie et al. 2012), 200–2500 times greater than that measured in environmentally exposed individuals living near a PFOA production facility (∼30–350 ng/mL) (Emmett et al. 2006b; Frisbee et al. 2009), and 20 000 times greater than that measured in the general U.S. population (∼3–4 ng/mL) (Kato et al. 2011; Olsen et al. 2012).
By contrast, no effect on antibody responses was found in Sv/129 mice dosed with 30 mg/kg/day PFOA in drinking water for 15 days (DeWitt et al. 2009), suggesting strain differences in susceptibility to PFOA. Such inter-strain variability – or, alternatively, heterogeneity by route of exposure – may underlie the finding that dermal exposure to 0.25–50 mg/kg/day PFOA for 4 days increased total and ovalbumin-specific IgE levels and ovalbumin-specific airway hyperreactivity in female BALB/c mice (Fairley et al. 2007), whereas no effect of oral PFOA exposure on delayed-type hypersensitivity was seen in C57BL/6 mice (DeWitt et al. 2008).

Immunotoxicity studies of PFOS exposure have yielded inconsistent results. As with PFOA, high PFOS doses resulting in acute toxicity (body weight loss and hepatomegaly) in C57BL/6 mice also caused splenic and thymic atrophy and immunosuppression, including depressed natural killer cell activity, lymphocyte proliferation, and T-cell-dependent antibody response (Dong et al. 2009; Zheng et al. 2009). However, such findings cannot reliably be attributed to a direct immunotoxic effect of PFOS.

One study demonstrated suppression of IgM production via both T-cell-dependent and T-cell-independent pathways in B6C3F1 male mice administered 0.05 mg/kg/day PFOS by oral gavage for 28 days (corresponding to a serum concentration of 91.5 ng/mL PFOS) and female mice administered 0.5 mg/kg PFOS (corresponding to a serum concentration of 666 ng/mL PFOS) (Peden-Adams et al. 2008). The serum concentration in female mice is comparable to that measured in occupationally exposed humans (~1000 ng/mL) (Olsen et al. 2003; Olsen & Zobel 2007), while that in male mice is within an order of magnitude of serum concentrations observed in the general U.S. population (~9–30 ng/mL) (Frisbee et al. 2009; Kato et al. 2011; Olsen et al. 2012). Lymphocyte proliferation, natural killer cell activity, and plasma lysozyme activity were not suppressed by PFOS in this study; the latter two parameters were increased in male and female mice, respectively. Another study in female B6C3F1 mice reported impaired immunity to influenza A virus (H1N1) following a 21-day oral gavage exposure to either 0.005 or 0.025 mg/kg/day PFOS (resulting in 189 or 670 ng/mL plasma PFOS, respectively) (Guruge et al. 2009).

By contrast, dietary exposure of male B6C3F1 male mice to 7 mg/kg/day PFOS for 28 days, resulting in a serum concentration of 11 600 ng/mL PFOS (more than 10 times the average in occupationally exposed humans), had no effect on IgG or IgM synthesis (Qazi et al. 2010). This study also found no effect of PFOS on the total number of circulating leukocytes or the number and phenotypic distribution of thymic or splenic cells. Likewise, dietary exposure of male and female Sprague-Dawley rats to 0.14–7.58 mg/kg/day PFOS for 28 days, resulting in serum PFOS levels of 470–29 900 ng/g in males and 950–43 200 ng/g in females, did not result in a dose-related suppression of serum total IgG, IgM, IgA, or IgE levels, specific IgG synthesis, or delayed-type hypersensitivity responses (Lefebvre et al. 2008). Differences in species, strains, and vehicles of administration may play a role in the inconsistency of results.

In a study of B6C3F1 pups born from the pairing of female C57BL/6N mice with male C4H/HeJ mice, where dams were gavaged with 0.1, 1, or 5 mg/kg/day PFOS on gestational days 1–17, natural killer cell activity was suppressed in 8-week-old male offspring at the 1 and 5 mg/kg/day doses and in female offspring at the 5 mg/kg/day dose (Keil et al. 2008). In addition, specific IgM production was suppressed at 5 mg/kg/day (a dose sufficient to cause hepatomegaly at 4 weeks) in males, but not females. Serum PFOS concentrations were not reported in this study.

The immunotoxic mode of action of PFOA and PFOS in animals remains to be elucidated. Ligand activation of the peroxisome proliferator-activated receptor alpha (PPARα), and possibly also the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR), is a key event in some PFOA- and PFOS-mediated toxicities in laboratory animals (Corton et al. 2014; Elcombe et al. 2010, 2012, 2014; Klaunig et al. 2003; Klaunig et al. 2012). Given inter-species differences in receptor specificity, receptor activity, and ligand binding and activation, the relevance of these signal transduction pathways to humans is unclear. For example, most of the mechanistic steps shown to be involved in rodent hepatocarcinogenesis by PPARα and CAR activators probably do not occur in humans (Corton et al. 2014; Elcombe et al. 2014; Klaunig et al. 2003; Klaunig et al. 2012). However, the role of PPARα, CAR, and PXR in mediating PFOA- and PFOS-induced immunotoxicity is not established (DeWitt et al. 2009). Some studies suggest that immunotoxic effects of PFOA may depend on PPARα in some mouse strains but not others (Corsini et al. 2014; Yang et al. 2002b).

Extrapolation of findings from laboratory animals to humans is also hindered by substantial species and sex differences in the pharmacokinetics of PFOA and PFOS (Butenhoff et al. 2006; Kennedy et al. 2004), including clearance half-lives that vary by several orders of magnitude (Chang et al. 2012; Olsen et al. 2007). This variation appears to be at least partly due to differences in renal tubular reabsorption processes (Han et al. 2012). Such discrepancies highlight the importance of using serum PFOA and PFOS concentrations to represent exposure in both animals and humans.
In summary, under experimental conditions, PFOA and PFOS can cause immune suppression in laboratory animals, although results vary by species, strain, sex, and route of exposure, as well as the type of outcome measured. Experimental LOAELs were above concentrations measured in occupationally exposed humans for PFOA, but near or below those measured in occupationally exposed humans for PFOS. In the absence of an established mode of action/adverse outcome pathway, the relevance of these findings to human health outcomes is not yet known. Therefore, epidemiologic studies of PFOA and PFOS should also be assessed for evidence of immunotoxic effects in humans.

**Literature search and data extraction methods**

The overarching causal question of interest for this review is whether PFOA and PFOS are causally related to adverse immunological health conditions in humans. The literature search strategy, which was designed according to recommended best practices (Rhomberg et al. 2013), is described in detail in the Supplemental Materials. Articles eligible for inclusion were original epidemiologic research studies that reported associations between exposure specifically to PFOA and/or PFOS and any health outcome primarily affecting the immune system. Based on a search of Scopus and MEDLINE, 24 relevant studies published as of September 1 2015 were identified for inclusion (Anderson-Mahoney et al. 2008; Ashley-Martin et al. 2015; Costa et al. 2009; Dong et al. 2013; Emmett et al. 2006b; Fei et al. 2010; Grandjean et al. 2012; Granum et al. 2013; Humblet et al. 2014; Innes et al. 2011; Kielsen et al. 2015; Leonard et al. 2008; Lin et al. 2011; Looker et al. 2014; Okada et al. 2012, 2014; Olsen et al. 2003; Osuna et al. 2014; Pennings et al. 2015; Smit et al. 2015; Steenland et al. 2013, 2015; Uhl et al. 2013; Wang et al. 2011).

Details about the methods of each relevant study, including location, dates, design, subjects, comparison populations, exposure and outcome assessment, funding source, and other aspects, were abstracted by E.T.C. and independently checked by L.E. (see Acknowledgments), and are provided in Supplemental Table 1. When appropriate, additional information was collected from online supplements and earlier, more detailed publications. Some unpublished information was obtained through personal communication with the lead author of one study (Granum et al. 2013). Results from each relevant study, including estimates of association and potential confounders included in multivariate models, are provided in Tables 1–5, which summarize results separately for studies of immune biomarkers or gene expression patterns (Table 1), atopic conditions (Table 2), infections (Table 3), vaccine response (Table 4), and autoimmune and inflammatory conditions (Table 5). Results from multivariate adjusted statistical models, if presented, are shown in the tables in lieu of results from unadjusted or minimally adjusted models.

**Methodological issues in epidemiologic studies of PFOA, PFOS, and immune conditions**

Following data extraction, the quality of individual epidemiologic studies was evaluated based on the validity and reliability of outcome assessment and exposure assessment, control of confounding, potential for selection bias, and appropriateness of the statistical approach. The following sections provide a detailed discussion of issues related to each of these methodological features as they pertain to epidemiologic studies of PFOA and/or PFOS exposure and immunological health conditions.

**Outcome assessment**

Numerous and disparate immune-related endpoints were investigated in the epidemiologic studies of PFOA or PFOS and immune conditions included in this review. Methods used to assess these endpoints are fundamental to the validity of study results, as well as to the interpretation of the clinical and public health relevance of findings.

**Clinical perspective on assessment of immune impairment**

The specific evaluation tools that allow clinical immunologists to examine the health of a patient’s immune system vary depending upon the clinical question that is being asked. The first and most important question concerns the overall health of the patient who is being evaluated, with particular emphasis on the function of the immune system; this process is the same for the evaluation of any other organ in the body. The function of the immune system is, grossly, to defend the body from foreign or unwanted substances and to prevent infection. Thus, a clinical immunologist aims to evaluate whether the patient’s frequency or types of infection deviate from those of otherwise comparable individuals.

This concept is so important that the 10 cardinal warning signs of an abnormality in the immune system (i.e. primary immunodeficiency) are centered on frequencies and types of infections (Supplemental Table 2) (Jeffrey Modell Foundation 2013). For the patient who displays any two of these 10 warning signs, the
Table 1. Results of epidemiologic studies of the association between exposure to perfluorooctanoic acid (PFOA) and/or perfluorooctanesulfonate (PFOS) and immune biomarkers or gene expression profiles.

| Reference         | Outcome                  | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|-------------------|--------------------------|-------------------------|--------------------------------------------------|--------------------|
| Olsen et al. 2003 | White blood cell count   | n = 518                 | “There were no significant mean differences...hematology...[data not shown]” | Age, body mass index, current daily alcohol consumption, current daily cigarette use, years worked at Antwerp or Decatur, and type of job (production vs. non-production) |
| Emmett et al. 2006b | White blood cell count | n = 18 (5%) abnormal | PFOA coefficient = 0.00039608, r = 0.09, p = 0.08 No significant correlation in adults aged ≥19 years or children aged <18 years | None |
| Emmett et al. 2006b | Neutrophils, percent    | n = 35 (9%) abnormal    | PFOA coefficient = 0.0004305, r = 0.02, p = 0.71 No significant correlation in adults aged ≥19 years or children aged <18 years | None |
| Emmett et al. 2006b | Neutrophils, absolute   | n = 12 (3%) abnormal    | PFOA coefficient = 0.00025301, r = 0.07, p = 0.17 No significant correlation in adults aged ≥19 years or children aged <18 years | None |
| Emmett et al. 2006b | Lymphocytes, percent    | n = 18 (5%) abnormal    | PFOA coefficient = 0.0006401, r = 0.03, p = 0.54 No significant correlation in adults aged ≥19 years or children aged <18 years | None |
| Emmett et al. 2006b | Lymphocytes, absolute   | n = 3 (1%) abnormal     | PFOA coefficient = 0.00009406, r = 0.05, p = 0.29 No significant correlation in adults aged ≥19 years or children aged <18 years | None |
| Emmett et al. 2006b | Monocytes, percent      | n = 39 (11%) abnormal   | PFOA coefficient = 0.00023119, r = 0.04, p = 0.44 No significant correlation in adults aged ≥19 years or children aged <18 years | None |
| Emmett et al. 2006b | Monocytes, absolute     | n = 7 (2%) abnormal     | PFOA coefficient = 0.00005008, r = 0.13, p = 0.01 No significant correlation in adults aged ≥19 years or children aged <18 years | None |
| Emmett et al. 2006b | Eosinophils, percent    | n = 19 (5%) abnormal    | PFOA coefficient = -0.00006552, r = 0.01, p = 0.82 No significant correlation in adults aged ≥19 years or children aged <18 years | None |
| Emmett et al. 2006b | Eosinophils, absolute   | n = 22 (6%) abnormal    | PFOA coefficient = 0.00000252, r = 0.00, p = 0.90 No significant correlation in adults aged ≥19 years or children aged <18 years | None |
| Emmett et al. 2006b | Basophils, percent      | n = 0 (0%) abnormal     | PFOA coefficient = 0.00000319, r = 0.03, p = 0.59 No significant correlation in adults aged ≥19 years or children aged <18 years | None |
| Emmett et al. 2006b | Basophils, absolute     | n = 0 (0%) abnormal     | PFOA coefficient = 0.00000586, r = 0.05, p = 0.30 No significant correlation in adults aged ≥19 years or children aged <18 years | None |

(continued)
| Reference       | Outcome                                | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors                                                                 |
|-----------------|----------------------------------------|-------------------------|--------------------------------------------------|-------------------------------------------------------------------------------------|
| Costa et al. 2009 | White blood cells ($\times 10^9$/L)    | % outside reference range: | $t$ statistic $= 0.10$, $p \geq 0.05$ vs. matched non-exposed workers | Currently exposed and non-exposed pairs matched by age, job seniority, working hours, residential area, and housing and living conditions |
|                 |                                        | Exposed: 2.6%            | Exposed vs. non-exposed coefficient $= 0.58$     | Regression analysis controlled for age, job seniority, body mass index, and alcohol consumption (+ smoking for comparison of currently vs. never exposed workers; + year of observation for comparison by PFOA level in workers with concurrent PFOA and outcomes) |
|                 |                                        | Non-exposed: 5.6%        | $(-0.19, 1.35)$                                  |                                                                                     |
|                 |                                        |                         | PFOA coefficient $= 0.029$ ($-0.011, 0.071$)     |                                                                                     |
| Costa et al. 2009 | C-reactive protein (mg/L)              | % outside reference range: | PFOA coefficient $= -0.020$ ($-0.268, 0.228$)   | Age, job seniority, body mass index, alcohol consumption, and year of observation    |
|                 |                                        | Exposed: 5.1%            |                                                                                                 |                                                                                     |
|                 |                                        | Non-exposed: NR          |                                                                                                 |                                                                                     |
| Costa et al. 2009 | $\alpha_1$ globulins (%)              | % outside reference range: | $t$ statistic $= 0.59$, $p \geq 0.05$ vs. matched non-exposed workers | Currently exposed and non-exposed pairs matched by age, job seniority, working hours, residential area, and housing and living conditions |
|                 |                                        | Exposed: 0%               | Exposed vs. non-exposed coefficient $= -1.82$  | Regression analysis controlled for age, job seniority, body mass index, and alcohol consumption (+ smoking for comparison of currently vs. never exposed workers; + year of observation for comparison by PFOA level in workers with concurrent PFOA and outcomes) |
|                 |                                        | Non-exposed: 0.9%         | $(-8.18, 4.54)$                                  |                                                                                     |
|                 |                                        |                         | PFOA coefficient $= 0.026$ ($-0.001, 0.053$)    |                                                                                     |
| Costa et al. 2009 | $\alpha_2$ globulins (%)              | % outside reference range: | $t$ statistic $= 0.20$, $p \geq 0.05$ vs. matched non-exposed workers | Currently exposed and non-exposed pairs matched by age, job seniority, working hours, residential area, and housing and living conditions |
|                 |                                        | Exposed: 12.8%            | Exposed vs. non-exposed coefficient $= 0.27$   | Regression analysis controlled for age, job seniority, body mass index, and alcohol consumption (+ smoking for comparison of currently vs. never exposed workers; + year of observation for comparison by PFOA level in workers with concurrent PFOA and outcomes) |
|                 |                                        | Non-exposed: 14.0%        | $(-0.75, 1.28)$                                  |                                                                                     |
|                 |                                        |                         | PFOA coefficient $= 0.026$ ($0.007, 0.045$)     |                                                                                     |
| Costa et al. 2009 | $\beta$ globulins (%)                 | % outside reference range: | $t$ statistic $= 0.49$, $p \geq 0.05$ vs. matched non-exposed workers | Currently exposed and non-exposed pairs matched by age, job seniority, working hours, residential area, and housing and living conditions |
|                 |                                        | Exposed: 0%               | Exposed vs. non-exposed coefficient $= -0.003$ | Regression analysis controlled for age, job seniority, body mass index, and alcohol consumption (+ smoking for comparison of currently vs. never exposed workers; + year of observation for comparison by PFOA level in workers with concurrent PFOA and outcomes) |
|                 |                                        | Non-exposed: 0%           | $(-0.37, 0.36)$                                  |                                                                                     |
|                 |                                        |                         | PFOA coefficient $= 0.011$ ($-0.008, 0.030$)     |                                                                                     |
| Costa et al. 2009 | $\gamma$ globulins (%)                | % outside reference range: | $t$ statistic $= 0.10$, $p \geq 0.05$ vs. matched non-exposed workers | Currently exposed and non-exposed pairs matched by age, job seniority, working hours, residential area, and housing and living conditions |
|                 |                                        | Exposed: 0%               | Exposed vs. non-exposed coefficient $= -0.53$   | Regression analysis controlled for age, job seniority, body mass index, and alcohol consumption (+ smoking for comparison of currently vs. never exposed workers; + year of observation for comparison by PFOA level in workers with concurrent PFOA and outcomes) |
|                 |                                        | Non-exposed: 0%           | $(-2.29, 1.24)$                                  |                                                                                     |
|                 |                                        |                         | PFOA coefficient $= 0.013$ ($-0.005, 0.031$)     |                                                                                     |
| Reference     | Outcome                        | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors                                                                 |
|---------------|--------------------------------|-------------------------|--------------------------------------------------|-------------------------------------------------------------------------------------|
| Costa et al. 2009 | IgG (g/L)                      | % outside reference range: | PFOA coefficient $= -0.017$ ($-0.115, -0.080$) [As reported, 95% confidence interval excludes $-0.017$; upper 95% confidence limit may be 0.080, since $p$-value is reported as non-significant.] | Age, job seniority, body mass index, alcohol consumption, and year of observation |
|               |                                | Exposed: 2.6% & smoking for comparison of currently vs. never exposed workers; year of observation for comparison by PFOA level in workers with concurrent PFOA and outcomes |                                                   |                                                                                     |
|               |                                | Non-exposed: NR          |                                                   |                                                                                     |
| Costa et al. 2009 | IgA (g/L)                      | % outside reference range: | NR                                               | Age, job seniority, body mass index, alcohol consumption, and year of observation |
|               |                                | Exposed: 12.8%           |                                                   |                                                                                     |
|               |                                | Non-exposed: NR          |                                                   |                                                                                     |
| Costa et al. 2009 | IgM (g/L)                      | % outside reference range: | PFOA coefficient $= 0.048$ ($-0.093, 0.190$) | Age, job seniority, body mass index, alcohol consumption, and year of observation |
|               |                                | Exposed: 5.1%            |                                                   |                                                                                     |
| Lin et al. 2011 | Serum C-reactive protein (log mg/L) | NR                      | PFOA percentile:                                 | Age, gender, smoking status, drinking status, household income, systolic blood pressure, waist circumference, homeostasis model assessment of insulin resistance index, total cholesterol, and creatinine |
|               |                                |                         | $<50^\text{th}$ (0.75–237 ng/mL): mean (SE) = 1.38 (0.14) |                                                                                     |
|               |                                |                         | $50^\text{th}$–74th (2.39–5.92 ng/mL): mean (SE) = 1.43 (0.14) |                                                                                     |
|               |                                |                         | $75^\text{th}$–89th (6.01–9.62 ng/mL): mean (SE) = 1.48 (0.18) |                                                                                     |
|               |                                |                         | $\geq 90^\text{th}$ (9.64–28.13 ng/mL): mean (SE) = 1.41 (0.21) |                                                                                     |
|               |                                |                         | P-trend $= 0.982$                               |                                                                                     |
|               |                                |                         | PFOA percentile:                                 |                                                                                     |
|               |                                |                         | $<50^\text{th}$ (0.1–8.92 ng/mL): mean (SE) = 1.41 (0.13) |                                                                                     |
|               |                                |                         | $50^\text{th}$–74th (8.95–14.35 ng/mL): mean (SE) = 1.41 (0.16) |                                                                                     |
|               |                                |                         | $75^\text{th}$–89th (14.92–19.14 ng/mL): mean (SE) = 1.48 (0.17) |                                                                                     |
|               |                                |                         | $\geq 90^\text{th}$ (19.98–67.26 ng/mL): mean (SE) = 1.36 (0.21) |                                                                                     |
|               |                                |                         | P-trend $= 0.957$                               |                                                                                     |
| Wang et al. 2011 | Serum total IgE at age 2 years (kU/L, log-transformed) | $n = 244$ overall | Log PFOA (ng/mL): Overall coefficient $= 0.027$ (SE = 0.244), $p = 0.870$ | Gender, gestational age, parity, maternal age, and prenatal environmental tobacco smoke exposure |
|               |                                | $n = 133$ males         | Boys coefficient $= 0.097$ (SE = 0.345), $p = 0.710$ |                                                                                     |
|               |                                | $n = 111$ females        | Girls coefficient $= 0.001$ (SE = 0.452), $p = 0.998$ |                                                                                     |
| Wang et al. 2011 | Cord serum total IgE (kU/L, log-transformed) | $n = 244$ overall | Log PFOA (ng/mL): Overall coefficient $= 0.134$ (SE = 0.003, 0.458) | Gender, gestational age, parity, maternal age, and prenatal environmental tobacco smoke exposure |
|               |                                | $n = 133$ males         | Boys coefficient $= 0.206$ (SE = 0.047, 0.702) |                                                                                     |
|               |                                | $n = 111$ females        | Girls coefficient $= 0.067$ (SE = 0.231), $p = 0.823$ |                                                                                     |
|               |                                |                         | Log PFOS (ng/mL): Overall coefficient $= 0.161$ (SE = 0.064, 0.642) |                                                                                     |
| Reference                  | Outcome                          | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|----------------------------|----------------------------------|-------------------------|---------------------------------------------------|--------------------|
| Okada et al. 2012          | Cord serum IgE (IU/mL)           |                          | Boys coefficient = 0.175 (0.004, 0.704)            | Maternal age, maternal allergic history, distance from home to highway, infant gender, parity, birth season, and blood sampling period |
|                            |                                  |                          | Girls coefficient = 0.151 (SE = 0.165), p = 0.616 |                     |
|                            |                                  |                          | Log_{10} maternal PFOA (ng/mL):                     |                     |
|                            |                                  |                          | Overall: Linear coefficient = 0.282 (−0.229, 0.792) |                     |
|                            |                                  |                          | Quadratic coefficient = −1.009 (−1.918, −0.101)    |                     |
|                            |                                  |                          | Cubic coefficient = −1.430 (−3.384, 0.524)         |                     |
|                            |                                  |                          | Males: Linear coefficient = −0.315 (−1.114, 0.485) |                     |
|                            |                                  |                          | Quadratic coefficient = 0.227 (−1.584, 2.037)      |                     |
|                            |                                  |                          | Cubic coefficient = 1.277 (−2.191, 4.744)          |                     |
|                            |                                  |                          | Females: Linear coefficient = 0.766 (0.104, 1.428) |                     |
|                            |                                  |                          | Quadratic coefficient = −1.429 (−2.416, −0.422)   |                     |
|                            |                                  |                          | Cubic coefficient = −3.078 (−5.431, −0.726)        |                     |
|                            |                                  |                          | Log_{10} maternal PFOS (ng/mL):                     |                     |
|                            |                                  |                          | Overall: Linear coefficient = 0.282 (−0.229, 0.792) |                     |
|                            |                                  |                          | Quadratic coefficient = −1.009 (−1.918, −0.101)    |                     |
|                            |                                  |                          | Cubic coefficient = −1.430 (−3.384, 0.524)         |                     |
|                            |                                  |                          | Males: Linear coefficient = −0.315 (−1.114, 0.485) |                     |
|                            |                                  |                          | Quadratic coefficient = 0.227 (−1.584, 2.037)      |                     |
|                            |                                  |                          | Cubic coefficient = 1.277 (−2.191, 4.744)          |                     |
|                            |                                  |                          | Females: Linear coefficient = 0.766 (0.104, 1.428) |                     |
|                            |                                  |                          | Quadratic coefficient = −1.429 (−2.416, −0.422)   |                     |
|                            |                                  |                          | Cubic coefficient = −3.078 (−5.431, −0.726)        |                     |
|                            |                                  | n = 231 overall          | Log_{10} maternal PFOA (mg/L):                     |                     |
|                            |                                  | n = 103 males            | Overall OR = 1.1 (95% credible interval = 0.6, 1.8) |                     |
|                            |                                  | n = 128 females          | Boys OR = 1.1 (0.5, 2.0)                           |                     |
|                            |                                  |                          | Girls OR = 1.1 (0.5, 2.1)                          |                     |
|                            |                                  |                          | Log_{10} maternal PFOS (mg/L):                     |                     |
|                            |                                  |                          | Overall OR = 1.1 (95% credible interval = 0.6, 1.9) |                     |
|                            |                                  |                          | Boys OR = 1.1 (0.4, 2.0)                           |                     |
|                            |                                  |                          | Girls OR = 1.1 (0.7, 2.1)                          |                     |
| Ashley-Martin et al. 2015  | Cord plasma thymic stromal lympho-|                          | Log_{10} maternal PFOA (µg/L):                     | Maternal age, infant sex |
|                            | poietin and interleukin-33 ≥ 80th|                          | Overall OR = 1.1 (95% credible interval = 0.6, 1.8) |                     |
|                            | percentile                       |                          | Boys OR = 1.1 (0.5, 2.0)                           |                     |
|                            |                                  | n = 1242 overall         | Girls OR = 1.1 (0.5, 2.1)                          |                     |
|                            |                                  | n = 664 males            | Log_{10} maternal PFOS (µg/L):                     |                     |
|                            |                                  | n = 578 females          | Overall OR = 1.1 (95% credible interval = 0.6, 1.9) |                     |
|                            |                                  |                          | Boys OR = 1.1 (0.4, 2.0)                           |                     |
|                            |                                  |                          | Girls OR = 1.1 (0.7, 2.1)                          |                     |
|                            |                                  | n = 1242 overall         | Log_{10} maternal PFOA (µg/L):                     | Maternal age, infant sex |
|                            |                                  | n = 664 males            | Overall OR = 1.1 (95% credible interval = 0.6, 1.9) |                     |
|                            |                                  | n = 578 females          | Boys OR = 1.2 (0.6, 2.3)                           |                     |
|                            |                                  |                          | Girls OR = 1.0 (0.5, 1.9)                          |                     |
|                            |                                  | n = 1242 overall         | Log_{10} maternal PFOS (µg/L):                     |                     |
|                            |                                  | n = 664 males            | Overall OR = 1.1 (95% credible interval = 0.6, 1.9) |                     |
|                            |                                  | n = 578 females          | Boys OR = 1.2 (0.7, 2.3)                           |                     |
|                            |                                  |                          | Girls OR = 1.0 (0.4, 1.9)                          |                     |
| Pennings et al. 2015       | Gene expression                  | NR                      | Number of correlated genes (p < 0.05):              | None               |
|                            |                                  |                          | PFOA: 453 positively correlated, 490 negatively correlated |
|                            |                                  |                          | PFOS: 636 positively correlated, 671 negatively correlated |

(continued)
evaluation of the immune system is relatively straightforward. Given the specialization of the immune system, the signs can give clues regarding the type of immunodeficiency. For example, common infections of the upper and lower respiratory tract, excessive diarrhea, and to some extent oral infections may point to problems with fighting bacteria, i.e. antibody-mediated immunity. Uncommon opportunistic infections may point to problems with fighting fungal and fungal-like infections, i.e. cell-mediated immunity. Both types of infections may be seen if antibody-mediated and cell-mediated immunity are simultaneously affected, as in severe combined immunodeficiency or severe HIV/AIDS. Thus, the most important part of any evaluation of the immune system is a thorough patient history. This history allows the selection of a series of laboratory evaluations, including a blood draw in most cases, to confirm the diagnosis of immunodeficiency, identify the abnormal part(s) of the immune system, and point to an appropriate therapeutic intervention. Even when the immune abnormality is secondary, as with a malignancy of the immune system or poisoning of the immune system, the signs, symptoms, and evaluation remain the same; that is, a deficit in immunoreactivity leads to infection, which is then evaluated as noted above. Immunodeficiency cannot be presumed to exist in the absence of evidence of such a clinically recognizable abnormality.

The question arises, then, of whether it is appropriate to evaluate the immune system when there is no observable abnormality simply because of an exposure (real or suspected) that has the potential to be immunotoxic. In this situation, a clinical immunologist might do the same series of tests as outlined above, but the question is completely different: instead of asking what part of the immune system is not working properly, based on clinical evidence of an abnormality, the concern is whether there is some part of the immune system that is abnormal, and if so, whether that abnormality will result in disease. From a clinician's perspective, if an abnormality is noted but it does not predict disease, then at best time and money are wasted, and at worst a patient is informed erroneously that he or she is sick or will get sick when this is not true, thereby breaking the rule of "primum non nocere" – above all do no harm.

### Outcome assessment of immune biomarkers

Whereas clinical evaluation of immunodeficiency focuses on the frequencies and types of infection, the epidemiologic studies of PFOA and PFOS included in this review investigated a variety of immune-related clinical conditions, including but not limited to infections, as
Table 2. Results of epidemiologic studies of the association between exposure to perfluorooctanoic acid (PFOA) and/or perfluorooctanesulfonate (PFOS) and atopic or allergic health conditions.

| Reference          | Outcome                      | Number (%) with outcome | Estimate of association (95% confidence interval)                                                                 | Adjustment factors                                      |
|--------------------|------------------------------|-------------------------|------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|
| Anderson-Mahoney et al. 2008 | Asthma (All ages, 18–34 years, 35–49 years, 50–64 years, or 65 + years) | All ages: n = 105 | All ages: standardized prevalence ratio (SPR) = 1.82 (1.47, 2.25) 18–34 years: SPR = 2.97 (p < 0.0001) in males, 1.97 (p < 0.0001) in females 35–49 years: SPR = 1.85 (p = 0.0005) in males, 1.39 (p = 0.0003) in females 50–64 years: SPR = 1.38 (p = 0.002) in males, 1.65 (p < 0.0001) in females 65 + years: SPR = 3.35 (p < 0.0001) in males, 1.69 (p = 0.01) in females | Age and sex |
| Leonard et al. 2008 | Asthma mortality | Observed: 0 deaths Expected in U.S.: 2.1 deaths Expected in West Virginia: 1.8 deaths Expected in DuPont workers: 0.7 deaths | SMR vs. U.S. = 0 (0, 1.76) SMR vs. West Virginia = 0 (0, 2.05) SMR vs. DuPont workers = 0 (0, 5.27) | Age, sex, and calendar period |
| Wang et al. 2011 | Atopic dermatitis | n = 43 (17.6%) | Cord serum PFOA:  
Q1 (<0.085 ng/mL): referent  
Q2 (0.085–0.57 ng/mL): OR = 0.84 (0.28, 2.48)  
Q3 (0.57–1.085 ng/mL): OR = 1.03 (0.42, 2.56)  
Q4 (>1.085 ng/mL): OR = 0.58 (0.22, 1.58)  
Cord serum PFOS:  
Q1 (<1.25 ng/mL): referent  
Q2 (1.25–1.835 ng/mL): OR = 0.68 (0.20, 2.30)  
Q3 (1.835–2.775 ng/mL): OR = 2.34 (0.86, 6.41)  
Q4 (>2.775 ng/mL): OR = 2.19 (0.78, 6.17)  | Gender, gestational age, maternal age, maternal history of atopy, duration of breastfeeding, and prenatal environmental tobacco smoke exposure |
| Okada et al. 2012 | Food allergy | n = 57 (16.6%) | Per 10-fold increase in PFOA:  
Overall OR = 1.67 (0.52, 5.37)  
Males OR = 0.87 (0.16, 4.89)  
Females OR = 2.37 (0.50, 17.10)  
Per 10-fold increase in maternal PFOS:  
Overall OR = 3.72 (0.81, 17.10)  
Males OR = 5.42 (0.62, 47.20)  
Females OR = 2.75 (0.31, 24.80)  | Maternal age, maternal educational level, pre-pregnancy body mass index, paternal allergy, parity, infant gender, breastfeeding duration, environmental tobacco smoke exposure, day care attendance, and blood sampling period |
| Okada et al. 2012 | Eczema | n = 37 (10.8%) | Per 10-fold increase in PFOA:  
Overall OR = 0.96 (0.23, 4.02)  
Males OR = 1.12 (0.15, 8.42)  
Females OR = 0.88 (0.09, 7.70)  
Per 10-fold increase in maternal PFOS:  
Overall OR = 0.87 (0.15, 5.08)  
Males OR = 0.62 (0.06, 6.67)  
Females OR = 1.24 (0.08, 19.30)  | Maternal age, maternal educational level, pre-pregnancy body mass index, paternal allergy, parity, infant gender, breastfeeding duration, environmental tobacco smoke exposure, day care attendance, and blood sampling period |

"When total dioxins and total polychlorinated biphenyls were evaluated as potential confounders, no significant association remained between [perfluorinated compound] concentrations and infant allergies and infectious diseases during the first 18 months of life (data not shown)."
Table 2. Continued

| Reference         | Outcome               | Number (% with outcome) | Estimate of association (95% confidence interval) | Adjustment factors                                                                 |
|-------------------|-----------------------|-------------------------|----------------------------------------------------|-------------------------------------------------------------------------------------|
| Okada et al. 2012 | Wheezing              | \( n = 33 \) (9.6%)    | Per 10-fold increase in PFOA:                      | Maternal age, maternal educational level, pre-pregnancy body mass index, parental allergy, parity, infant gender, breastfeeding duration, environmental tobacco smoke exposure, day care attendance, and blood sampling period |
|                   |                       |                         | Overall OR = 1.27 (0.27, 6.05)                     |                                                                                     |
|                   |                       |                         | Males OR = 2.72 (0.25, 29.90)                      |                                                                                     |
|                   |                       |                         | Females OR = 1.31 (0.10, 18.00)                    |                                                                                     |
|                   |                       |                         | Per 10-fold increase in PFOS:                      |                                                                                     |
|                   |                       |                         | Overall OR = 2.68 (0.39, 18.30)                    |                                                                                     |
|                   |                       |                         | Males OR = 12.98 (0.80, 212.00)                    |                                                                                     |
|                   |                       |                         | Females OR = 0.61 (0.03, 11.50)                    |                                                                                     |
|                   |                       |                         | No significant differences by blood sampling period |                                                                                     |
|                   |                       |                         | (data not reported)                                |                                                                                     |
| Dong et al. 2013  | Asthma                |                         |                                                    |                                                                                    |
|                   |                       |                         | PFOA:                                              |                                                                                    |
|                   |                       |                         | Q1: Referent                                        |                                                                                    |
|                   |                       |                         | Q2: OR = 1.58 (0.89, 2.80)                         |                                                                                    |
|                   |                       |                         | Q3: OR = 2.67 (1.49, 4.79)                         |                                                                                    |
|                   |                       |                         | Q4: OR = 4.05 (2.21, 7.42)                         |                                                                                    |
|                   |                       |                         | P-trend = 0.001                                    |                                                                                    |
|                   |                       |                         | PFOS:                                              |                                                                                    |
|                   |                       |                         | Q1: Referent                                        |                                                                                    |
|                   |                       |                         | Q2: OR = 1.96 (1.11, 3.47)                         |                                                                                    |
|                   |                       |                         | Q3: OR = 1.32 (0.75, 2.32)                         |                                                                                    |
|                   |                       |                         | Q4: OR = 2.63 (1.48, 4.69)                         |                                                                                    |
|                   |                       |                         | P-trend = 0.003                                    |                                                                                    |
| Dong et al. 2013  | Asthma severity score | \( n = 231 \) asthma cases |                                                    |                                                                                    |
|                   |                       |                         | PFOA:                                              |                                                                                    |
|                   |                       |                         | Q1: mean = 3.63 (2.66, 4.60)                       |                                                                                    |
|                   |                       |                         | Q2: mean = 3.99 (3.02, 4.96)                       |                                                                                    |
|                   |                       |                         | Q3: mean = 4.39 (3.40, 5.38)                       |                                                                                    |
|                   |                       |                         | Q4: mean = 4.57 (3.59, 5.55)                       |                                                                                    |
|                   |                       |                         | P-trend = 0.119                                    |                                                                                    |
|                   |                       |                         | PFOS:                                              |                                                                                    |
|                   |                       |                         | Q1: mean = 3.33 (2.36, 4.31)                       |                                                                                    |
|                   |                       |                         | Q2: mean = 4.18 (3.19, 5.17)                       |                                                                                    |
|                   |                       |                         | Q3: mean = 4.49 (3.52, 5.45)                       |                                                                                    |
|                   |                       |                         | Q4: mean = 4.57 (3.61, 5.54)                       |                                                                                    |
|                   |                       |                         | P-trend = 0.045                                    |                                                                                    |
| Dong et al. 2013  | Asthma control in previous 4 weeks | \( n = 231 \) asthma cases |                                                    |                                                                                    |
|                   |                       |                         | PFOA:                                              |                                                                                    |
|                   |                       |                         | Q1: mean = 22.02 (21.22, 22.82)                     |                                                                                    |
|                   |                       |                         | Q2: mean = 22.14 (21.33, 22.96)                     |                                                                                    |
|                   |                       |                         | Q3: mean = 22.76 (21.96, 23.56)                     |                                                                                    |
|                   |                       |                         | Q4: mean = 22.65 (21.84, 23.45)                     |                                                                                    |
|                   |                       |                         | P-trend = 0.168                                    |                                                                                    |
|                   |                       |                         | PFOS:                                              |                                                                                    |
|                   |                       |                         | Q1: mean = 22.51 (21.71, 23.32)                     |                                                                                    |
|                   |                       |                         | Q2: mean = 22.72 (21.92, 23.52)                     |                                                                                    |
| Reference            | Outcome                      | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|----------------------|------------------------------|-------------------------|--------------------------------------------------|-------------------|
| Granum et al. 2013   | Eczema and itchiness         | n = 21 (25.9%) in 3rd year; 16 (30.2%) with blood | Q3: mean = 22.13 (21.30, 22.94) Q4: mean = 22.21 (21.41, 23.02) P-trend = 0.450 | None               |
|                      |                              | n = 32 (45.7%) in all 3 years | Per 1-ng/mL increase in PFOA: 3rd year OR = 0.56 (0.37, 0.84) All 3 years OR = 0.42 (0.13, 1.30) Per 1-ng/mL increase in PFOS: 3rd year OR = 0.93 (0.72, 1.18) All 3 years OR = 0.94 (0.75, 1.19) |                  |
| Granum et al. 2013   | Wheezing                     | n = 18 (21.2%) in 3rd year; 11 (19.6%) with blood | Per 1-ng/mL increase in PFOA: 3rd year OR = 1.07 (0.33, 3.49) All 3 years OR = 1.92 (0.66, 5.58) Per 1-ng/mL increase in PFOS: 3rd year OR = 1.10 (0.88, 1.37) All 3 years OR = 1.11 (0.89, 1.37) | None               |
| Granum et al. 2013   | Asthma                       | n = 11 (14.5%) in all 3 years | Per 1-ng/mL increase in PFOA: All 3 years OR = 3.56 (0.84, 15.02) Per 1-ng/mL increase in PFOS: All 3 years OR = 1.22 (0.89, 1.656) | None               |
| Granum et al. 2013   | Atopic eczema                | n = 14 (18.4%) in all 3 years | Per 1-ng/mL increase in PFOA: All 3 years OR = 0.96 (0.73, 1.27) | None               |
| Humblet et al. 2014  | Asthma, ever                 | n = 318                  | Per 2-fold increase in PFOA: OR = 1.18 (1.08, 1.39) OR = 1.11 (0.87, 1.42) using survey weights OR = 1.13 (0.99, 1.29) imputing missing data Per 1-ng/mL increase in PFOA: OR = 1.06 (1.00, 1.11) | Age, sex, smoking, race/ethnicity, survey cycle, poverty-income ratio, and health insurance type |
| Humblet et al. 2014  | Asthma, ever                 | n = 318                  | Per 2-fold increase in PFOS: OR = 0.88 (0.74, 1.04) OR = 0.84 (0.66, 1.08) using survey weights OR = 0.86 (0.75, 0.98) imputing missing data Per 1-ng/mL increase in PFOS: OR = 0.99 (0.98, 1.00) | Age, sex, smoking, race/ethnicity, survey cycle, poverty-income ratio, and health insurance type |
| Humblet et al. 2014  | Asthma, current              | n = 191                  | Per 2-fold increase in PFOA: OR = 1.12 (0.92, 1.36) OR = 1.15 (0.86, 1.55) using survey weights OR = 1.06 (0.91, 1.25) imputing missing data Per 1-ng/mL increase in PFOA: OR = 1.03 (0.97, 1.10) | Age, sex, smoking, race/ethnicity, survey cycle, poverty-income ratio, and health insurance type |
| Humblet et al. 2014  | Asthma, current              | n = 191                  | Per 2-fold increase in PFOS: OR = 0.88 (0.72, 1.08) OR = 0.91 (0.68, 1.21) using survey weights | Age, sex, smoking, race/ethnicity, survey cycle, poverty-income ratio, and health insurance type |
| Reference       | Outcome                                      | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors                                      |
|-----------------|----------------------------------------------|-------------------------|--------------------------------------------------|------------------------------------------------------|
| Humblet et al. 2014 | Wheezing in last 12 months                  | n = 217                 | OR = 0.89 (0.75, 1.05) imputing missing data      | Age, sex, smoking, race/ethnicity, survey cycle, poverty-income ratio, and health insurance type |
|                 | Per 1-ng/mL increase in PFOS:               |                         | OR = 0.99 (0.89, 1.01)                            |                                                     |
|                 |                                               |                         | OR = 0.88 (0.69, 1.13) imputing missing data     |                                                     |
|                 |                                               |                         | OR = 1.06 (0.80, 1.41)                             |                                                     |
| Humblet et al. 2014 | Wheezing in last 12 months                  | n = 217                 | Per 2-fold increase in PFOA:                      | Age, sex, smoking, race/ethnicity, survey cycle, poverty-income ratio, and health insurance type |
|                 |                                               |                         | OR = 1.00 (0.88, 1.23)                             |                                                     |
|                 |                                               |                         | OR = 1.14 (0.82, 1.58) using survey weights      |                                                     |
|                 |                                               |                         | OR = 1.01 (0.85, 1.20) imputing missing data     |                                                     |
|                 |                                               |                         | OR = 1.01 (0.89, 1.07)                             |                                                     |
| Okada et al. 2014 | Total allergic diseases (eczema, wheezing, and/or allergic rhinoconjunctivitis symptoms; at 24 months) | PFOS quartiles, overall: | Q1 (≤3.11 ng/mL): n = 197                         | Maternal age, maternal education level, parental allergic history, infant gender, breastfeeding duration, number of siblings, day care attendance, and environmental tobacco smoke exposure in infancy at 24 months |
|                 |                                               |                         | Q2 (3.12–5.02 ng/mL): n = 199                     |                                                     |
|                 |                                               |                         | Q3 (5.03–6.83 ng/mL): n = 171                     |                                                     |
|                 |                                               |                         | Q4 (6.84–30.3 ng/mL): n = 163                     |                                                     |
| Okada et al. 2014 | Total allergic diseases (eczema, wheezing, and/or allergic rhinoconjunctivitis symptoms; at 24 months) | PFOS quartiles, overall: | Q1 (≤3.71 ng/mL): n = 195                         | Maternal age, maternal education level, parental allergic history, infant gender, breastfeeding duration, number of siblings, day care attendance, and environmental tobacco smoke exposure in infancy at 24 months |
|                 |                                               |                         | Q2 (3.72–5.02 ng/mL): n = 185                     |                                                     |
|                 |                                               |                         | Q3 (5.03–6.83 ng/mL): n = 171                     |                                                     |
|                 |                                               |                         | Q4 (6.84–30.3 ng/mL): n = 163                     |                                                     |
Table 2. Continued

| Reference                  | Outcome          | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|----------------------------|------------------|-------------------------|--------------------------------------------------|-------------------|
| Okada et al. 2014          | Eczema (at 24 months) |                         |                                                  |                   |
|                            | PFOA quartiles, overall: |                         |                                                  |                   |
|                            | Q1 (≤3.16 ng/mL): n = 100 |                         |                                                  |                   |
|                            | Q2 (3.82–5.02 ng/mL): n = 99 |                         |                                                  |                   |
|                            | Q3 (5.03–6.83 ng/mL): n = 90 |                         |                                                  |                   |
|                            | Q4 (6.84–30.3 ng/mL): n = 84 |                         |                                                  |                   |
|                            | PFOA quartiles, males: |                         |                                                  |                   |
|                            | Q1: n = 59 |                         |                                                  |                   |
|                            | Q2: n = 53 |                         |                                                  |                   |
|                            | Q3: n = 56 |                         |                                                  |                   |
|                            | Q4: n = 44 |                         |                                                  |                   |
|                            | PFOA quartiles, females: |                         |                                                  |                   |
|                            | Q1: n = 41 |                         |                                                  |                   |
|                            | Q2: n = 50 |                         |                                                  |                   |
|                            | (Males and females do not sum to total) |                         |                                                  |                   |
|                            | Maternal age, maternal education level, parental allergic history, infant gender, breastfeeding duration, number of siblings, and environmental tobacco smoke exposure in infancy at 24 months |                                         |                   |

"At 12 months, no significant association was observed between eczema and [perfluoroalkyl acids], although similar patterns of a decreased risk of eczema were seen (data not shown)."
| Reference          | Outcome | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|--------------------|---------|-------------------------|--------------------------------------------------|-------------------|
| Okada et al. 2014  | Wheezing | n = 397 (19.3%)         | “No significant associations were observed with any maternal [perfluoroalkyl acid] levels at 12 or 24 months (data not shown).” | NR                |
| Smit et al. 2015   | Asthma, ever | Greenland: n = 62 (12.4%) | Per 1-SD increase in PFOA: Greenland OR = 0.79 (0.60, 1.03) | None              |
|                    |         |                         | Ukraine OR = 0.93 (0.47, 1.84)                     |                   |
|                    |         |                         | Combined OR = 0.80 (0.62, 1.04)                    |                   |
|                    |         | Per 1-SD increase in PFOS: GDP | Greenland OR = 0.88 (0.67, 1.15) |                   |
|                    |         |                         | Ukraine OR = 0.75 (0.39, 1.42)                     |                   |
|                    |         |                         | Combined OR = 0.86 (0.67, 1.10)                    |                   |
| Smit et al. 2015   | Eczema, ever | Greenland: n = 59 (12.3%) | Per 1-SD increase in PFOA: Greenland OR = 1.04 (0.78, 1.38) | None              |
|                    |         |                         | Ukraine OR = 0.93 (0.73, 1.19)                     |                   |
|                    |         |                         | Combined OR = 0.97 (0.81, 1.17)                    |                   |
|                    |         | Per 1-SD increase in PFOS: GDP | Greenland OR = 1.02 (0.77, 1.34) |                   |
|                    |         |                         | Ukraine OR = 0.95 (0.74, 1.22)                     |                   |
|                    |         |                         | Combined OR = 0.98 (0.82, 1.18)                    |                   |
| Smit et al. 2015   | Wheeze, ever | Greenland: n = 97 (19.4%) | Per 1-SD increase in PFOA: Greenland OR = 0.88 (0.70, 1.11) | None              |
|                    |         |                         | Ukraine OR = 0.98 (0.70, 1.38)                     |                   |
|                    |         |                         | Combined OR = 0.91 (0.76, 1.10)                    |                   |
|                    |         | Per 1-SD increase in PFOS: GDP | Greenland OR = 0.86 (0.69, 1.08) |                   |
|                    |         |                         | Ukraine OR = 0.77 (0.55, 1.08)                     |                   |
|                    |         |                         | Combined OR = 0.83 (0.69, 1.10)                    |                   |
| Smit et al. 2015   | Eczema, current | Greenland: n = 53 (10.2%) | Per 1-SD increase in PFOA: Greenland OR = 1.04 (0.77, 1.41) | None              |
|                    |         |                         | Ukraine OR = 0.94 (0.62, 1.44)                     |                   |
|                    |         |                         | Combined OR = 1.01 (0.79, 1.29)                    |                   |
|                    |         | Per 1-SD increase in PFOS: GDP | Greenland OR = 0.98 (0.74, 1.30) |                   |
|                    |         |                         | Ukraine OR = 1.23 (0.78, 1.94)                     |                   |
|                    |         |                         | Combined OR = 1.05 (0.82, 1.33)                    |                   |
| Smit et al. 2015   | Wheeze, current | Greenland: n = 27 (5.4%) | Per 1-SD increase in PFOA: Greenland OR = 1.12 (0.74, 1.70) | None              |
|                    |         |                         | Ukraine OR = 0.78 (0.47, 1.29)                     |                   |
|                    |         |                         | Combined OR = 0.97 (0.71, 1.33)                    |                   |
|                    |         | Per 1-SD increase in PFOS: GDP | Greenland OR = 0.91 (0.62, 1.36) |                   |

(continued)
well as non-clinical outcomes. Non-clinical endpoints that were studied include biological markers of immune function, such as levels of leukocytes, immunoglobulins (Ig), and immune-related proteins, measured using standard laboratory hematological tests (Ashley-Martin et al. 2015; Costa et al. 2009; Dong et al. 2013; Emmett et al. 2006b; Granum et al. 2013; Lin et al. 2011; Okada et al. 2012; Olsen et al. 2003; Wang et al. 2011). These are well-established, objectively measured markers of immune function that are unlikely to be subject to systematic bias. Similarly, studies of the immune response to vaccination used standard laboratory assays to measure specific antibody levels (Grandjean et al. 2012; Granum et al. 2013; Kielsen et al. 2015; Looker et al. 2014). Another study measured autoantibody titers as less well-established potential biomarkers of tissue damage (Osuna et al. 2014), and one evaluated gene expression patterns that might be indicative of immunomodulation (Pennings et al. 2015).

However, many standard immune biomarkers, such as leukocyte counts (Saunders 1985; Statland et al. 1978; Winkel et al. 1981) and C-reactive protein (DeGoma et al. 2012; Platz et al. 2010), are known to fluctuate substantially within individuals over time and under different conditions. [Variation in leukocyte counts has also been documented among laboratories, but appears to be less than physiological variability (Mandigo et al. 1995).] Vaccine-induced antibody levels can also vary extensively over time (Rose et al. 2013; Schauer et al. 2003; Skowronski et al. 2008), as can autoantibody levels (Meroni et al. 2014) and gene expression patterns (which vary even among genetically identical cells) (Maheshri & O’Shea 2007; Munsky et al. 2012). Therefore, just as a single exposure measurement may not be sufficiently informative, a one-time measurement of any given immune biomarker level, antibody titer, or gene expression pattern may provide an erroneous depiction of a person’s immune function. Repeated testing is needed to capture immune function more fully. Moreover, some components of the immune system are not readily assayable, either because the appropriate technology has not yet been developed or because some immune cells, such as antigen-presenting cells, do not circulate in the peripheral blood and therefore cannot be sampled by a simple blood draw.

Another issue related to immune biomarkers and antibody levels is whether small but statistically significant changes in these endpoints, when analyzed on a continuous scale, are clinically meaningful, particularly when most or all subjects are within the normal range. Some studies attempted to address this issue by also analyzing outcomes dichotomized relative to standard reference values, with the implication that values
Table 3. Results of epidemiologic studies of the association between exposure to perfluorooctanoic acid (PFOA) and/or perfluorooctanesulfonate (PFOS) and infectious conditions.

| Reference       | Outcome                                      | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors                                                |
|-----------------|----------------------------------------------|-------------------------|---------------------------------------------------|------------------------------------------------------------------|
| Leonard et al. 2008 | Mortality from infectious and parasitic diseases | Observed: 1 death Expected in U.S.: 15.0 deaths Expected in West Virginia: 3.4 deaths Expected in DuPont workers: 1.1 deaths | SMR vs. U.S. = 0.067 (0.002, 0.372) SMR vs. West Virginia = 0.295 (0.007, 1.646) SMR vs. DuPont workers = 0.946 (0.024, 5.269) | Age, sex, and calendar period                                      |
| Fei et al. 2008  | Mortality from influenza and pneumonia         | Observed: 14 deaths Expected in U.S.: 26.5 deaths Expected in West Virginia: 28.2 deaths Expected in DuPont workers: 15.7 deaths | SMR vs. U.S. = 0.529 (0.289, 0.887) SMR vs. West Virginia = 0.497 (0.272, 0.834) SMR vs. DuPont workers = 0.890 (0.487, 1.494) | Age, sex, and calendar period                                      |
| Fei et al. 2010  | Hospitalization for infectious diseases        | PFOA quartiles: Q1 (≤ 3.90 ng/mL): n = 169 Q2 (3.91–5.20 ng/mL): n = 120 Q3 (5.21–6.96 ng/mL): n = 137 Q4 (≥ 6.97 ng/mL): n = 151 | Overall: Q1: referent Q2: RR = 0.71 (0.53, 0.94) Q3: RR = 0.77 (0.59, 1.03) Q4: RR = 0.84 (0.62, 1.13) Per quartile: RR = 0.96 (0.87, 1.06) | Parity, maternal age, pre-pregnancy body mass index, breastfeeding, smoking during pregnancy, socio-occupational status, home density, child age, child gender, sibling age difference, gestational age at blood drawing, birth year, and birth season |
| Fei et al. 2010  | Hospitalization for infectious diseases        | PFOS quartiles: Q1 (16.4–26.0 ng/mL): n = 147 Q2 (26.1–33.3 ng/mL): n = 142 Q3 (33.4–43.2 ng/mL): n = 136 Q4 (≥ 43.3 ng/mL): n = 152 | Overall: Q1: referent Q2: RR = 0.93 (0.71, 1.21) Q3: RR = 0.90 (0.68, 1.18) Q4: RR = 1.00 (0.76, 1.32) Per quartile: RR = 1.00 (0.91, 1.09) | Parity, maternal age, pre-pregnancy body mass index, breastfeeding, smoking during pregnancy, socio-occupational status, home density, child age, child gender, sibling age difference, gestational age at blood drawing, birth year, and birth season |

(continued)
Table 3. Continued

| Reference          | Outcome                | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|--------------------|------------------------|-------------------------|--------------------------------------------------|--------------------|
| Okada et al. 2012  | Otitis media           | n = 61 (17.8%)          | Q4: RR = 1.59 (1.02, 2.49)                        | Maternal age, maternal educational level, parity, infant gender, breastfeeding duration, environmental tobacco smoke exposure, day care attendance, and blood sampling period |
|                    |                        |                         | Per quartile: RR = 1.18 (1.03, 1.36)             | When total dioxins and total [polychlorinated biphenyls] were evaluated as potential confounders, no significant association remained between [perfluorinated compound] concentrations and infant allergies and infectious diseases during the first 18 months of life (data not shown). |
|                    |                        |                         | Boys: Q1: referent                               |                    |
|                    |                        |                         | Q2: RR = 0.80 (0.57, 1.13)                       |                    |
|                    |                        |                         | Q3: RR = 0.61 (0.42, 0.89)                       |                    |
|                    |                        |                         | Q4: RR = 0.77 (0.54, 1.12)                       |                    |
|                    |                        |                         | Per quartile: RR = 0.90 (0.80, 1.02)             |                    |
|                    |                        |                         | Ages 0<1 year: Per-quartile RR = 0.90 (0.80, 1.00) |                    |
|                    |                        |                         | Ages 1<2 years: Per-quartile RR = 0.98 (0.88, 1.09) |                    |
|                    |                        |                         | Ages 2<4 years: Per-quartile RR = 1.13 (1.00, 1.27) |                    |
|                    |                        |                         | Ages ≥4 years: Per-quartile RR = 1.05 (0.94, 1.18) |                    |
|                    |                        |                         | First-born: Per-quartile RR = 1.10 (0.96, 1.27)  |                    |
|                    |                        |                         | Later-born: Per-quartile RR = 0.93 (0.83, 1.05)  |                    |
|                    |                        |                         | Quartile-specific RRs by age or birth order are not shown here |                    |
| Granum et al. 2013 | Common cold, number of episodes | Mean ± SD = 3.0 ± 3.1 in 3rd year; 3.2 ± 3.5 with blood | Per unit increase in PFOA: 3rd year coefficient = 0.42 (0.16, 0.72) | PFOA models: previous breastfeeding, older siblings, maternal allergy, maternal education, child’s gender, child’s birth season, and gross household income (included in backward deletion model); previous breastfeeding and birth season included in final model for PFOA and common cold episodes over all 3 years |
|                    |                        | Mean ± SD = 10.9 ± 6.4 in all 3 years | All 3 years coefficient = 0.42 (0.21, 0.62) |                    |
|                    |                        |                         | Per unit increase in PFOS: 3rd year coefficient = 0.03 (0.00, 0.10) |                    |
|                    |                        |                         | All 3 years coefficient = 0.01 (0.00, 0.05) |                    |
| Granum et al. 2013 | Common cold in 3rd year (yes vs. no) | n = 70 (82.4%) in 3rd year; 45 (83.3%) with blood | Per 1-ng/mL increase in PFOA: 3rd year OR = 1.24 (0.32, 4.83) | None |
|                    |                        | n = 94 (98.9%) in all 3 years | Per 1-ng/mL increase in PFOS: 3rd year OR = 1.13 (0.85, 1.51) |                    |
| Granum et al. 2013 | Gastroenteritis, number of episodes | Mean ± SD = 1.3 ± 1.1 in 3rd year; 1.37 ± 1.1 with blood | Per 1-ng/mL increase in PFOA: 3rd year coefficient = 0.21 (0.02, 0.64) | PFOA model for all 3 years: previous breastfeeding, older siblings, maternal allergy, maternal education, and gross household income (included in backward deletion) |
|                    |                        | Mean ± SD = 3.1 ± 2.3 in all 3 years | All 3 years coefficient = 0.31 (0.002, 0.61) |                    |
Table 3. Continued

| Reference             | Outcome                                     | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|-----------------------|---------------------------------------------|-------------------------|---------------------------------------------------|-------------------|
| Granum et al. 2013    | Gastroenteritis in 3rd year (yes vs. no)    | *n* = 64 (76.2%) in 3rd year; 47 (83.9%) with blood | Per 1-ng/mL increase in PFOS: 3rd year coefficient = 0.06 (−0.03, 0.14)  
All 3 years coefficient = 0.03 (−0.04, 0.10) | PFOA model for 3rd year and PFOS models: none |
|                       |                                             | *n* = 87 (93.5%) in all 3 years | Per 1-ng/mL increase in PFOA: 3rd year OR = 1.16 (0.37, 3.65)  
All 3 years OR = 3.13 (0.37, 26.2) | None |
| Granum et al. 2013    | Otitis media (yes vs. no)                   | *n* = 16 (18.8%) in 3rd year; 10 (17.9%) with blood | Per 1-ng/mL increase in PFOA: 3rd year OR = 0.78 (0.22, 2.77)  
All 3 years OR = 0.76 (0.25, 2.33) | None |
| Looker et al. 2014    | Any “flu” infection in last 12 months       | PFOA quartiles: Q1 (0.25–13.7 ng/mL): *n* = 45  
Q2 (13.8–31.5 ng/mL): *n* = 37  
Q3 (31.6–90 ng/mL): *n* = 44  
Q4 (90.4–2140 ng/mL): *n* = 37 | PFOA quartiles: Q1: referent  
Q2: OR = 1.13 (0.69, 2.00)  
Q3: OR = 1.10 (0.67, 1.81)  
Q4: OR = 0.84 (0.52, 1.36)  
Per unit increase in log10 PFOA: OR = 0.97 (0.58, 1.63) | Age and gender |
| Looker et al. 2014    | Any cold in last 12 months                  | PFOA quartiles: Q1 (0.25–13.7 ng/mL): *n* = 145  
Q2 (13.8–31.5 ng/mL): *n* = 141  
Q3 (31.6–90 ng/mL): *n* = 131  
Q4 (90.4–2140 ng/mL): *n* = 121 | PFOA quartiles: Q1: referent  
Q2: OR = 1.60 (0.99, 2.60)  
Q3: OR = 1.20 (0.75, 1.92)  
Q4: OR = 1.09 (0.67, 1.78)  
Per unit increase in log10 PFOA: OR = 0.83 (0.51, 1.34) | Age and gender |
| Looker et al. 2014    | Any cold or “flu” in last 12 months         | PFOA quartiles: Q1 (0.25–13.7 ng/mL): *n* = 148  
Q2 (13.8–31.5 ng/mL): *n* = 145  
Q3 (31.6–90 ng/mL): *n* = 137  
Q4 (90.4–2140 ng/mL): *n* = 124 | PFOA quartiles: Q1: referent  
Q2: OR = 1.21 (0.73, 2.00)  
Q3: OR = 1.10 (0.67, 1.81)  
Q4: OR = 0.84 (0.52, 1.36)  
Per unit increase in log10 PFOA: OR = 0.85 (0.62, 1.16) | Age and gender |

(continued)
Table 3. Continued

| Reference          | Outcome                                  | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|--------------------|------------------------------------------|-------------------------|--------------------------------------------------|-------------------|
| Looker et al. 2014 | Frequency of colds in last 12 months     |                         |                                                  |                   |
| PFOS quartiles:    |                                          |                         |                                                  |                   |
| Q1 (0.1–5.8 ng/ml): | n = 145                                  |                         |                                                  |                   |
| Q2 (5.9–9.2 ng/ml): | n = 150                                  |                         |                                                  |                   |
| Q3 (9.3–14.5 ng/ml): | n = 137                                  |                         |                                                  |                   |
| Q4 (14.7–42.3 ng/ml): | n = 122                                  |                         |                                                  |                   |
|                   |                                          |                         | OR: 1.15 (0.69, 1.91); p = 0.24                  |                   |
| PFOA quartiles:    |                                          |                         |                                                  |                   |
| Q1: referent       |                                          |                         |                                                  |                   |
| Q2: OR = 0.93 (0.63, 1.38) |                                           |                         |                                                  |                   |
| Q3: OR = 0.95 (0.63, 1.41) |                                           |                         |                                                  |                   |
| Q4: OR = 0.90 (0.60, 1.36); p = 0.97 |                                           |                         |                                                  |                   |
| Per unit increase in log_{10} PFOA: OR = 0.91 (0.70, 1.19) |                       |                         |                                                  |                   |

Columns show first author and year of study reference, outcome of interest, number and proportion of subjects with the outcome, estimates of association with PFOA and/or PFOS levels, and covariates adjusted in multivariable models.

OR: odds ratio; PFOA: perfluorooctanoic acid; PFOS: perfluorooctanesulfonate; Q: quartile; RR: relative risk; SD: standard deviation; SMR: standardized mortality ratio.
Table 4. Results of epidemiologic studies of the association between exposure to perfluorooctanoic acid (PFOA) and/or perfluorooctanesulfonate (PFOS) and vaccine response.

| Reference          | Outcome                                                      | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors                                      |
|--------------------|--------------------------------------------------------------|-------------------------|---------------------------------------------------|--------------------------------------------------------|
| Grandjean et al. 2012 | Serum anti-tetanus toxoid antibody concentration, % difference or % with inadequate concentration (≤0.1 IU/mL) | Maternal PFOA:          | Per 2-fold increase in maternal PFOA:             | Prebooster adjusted for age and sex                     |
|                    |                                                              | Prebooster: n = 509     | Prebooster: 10.5 (−28.2, 11.7)                     | Postbooster adjusted for age, sex, time since vaccination, and booster type |
|                    |                                                              | Postbooster: n = 419    | Postbooster: 14.5 (−10.4, 46.4)                    | Year 7 adjusted for age, sex, and booster type, with or without additional adjustment for child’s specific antibody concentration at age 5 years |
|                    |                                                              | Year 7: n = 424         | Year 7: 7.4 (−17.1, 39.0)                         | No substantial change after additional adjustment for polychlorinated biphenyls in breast milk and age 5 serum |
|                    |                                                              |                           | Year 7, adjusted for age 5: 12.3 (−8.6, 38.1)      |                                                        |
|                    |                                                              | Year 7: OR = 1.21 (0.82, 1.78) |                                                        |                                                        |
|                    |                                                              | Year 7: OR = 0.73 (0.27, 1.95) |                                                        |                                                        |
|                    |                                                              | Per 2-fold increase in age 5 PFOA:   |                                                        |                                                        |
|                    |                                                              | Prebooster: 13.3 (−31.6, 9.9)         |                                                        |                                                        |
|                    |                                                              | Postbooster: −9.7 (−30.7, 17.7)        |                                                        |                                                        |
|                    |                                                              | Year 7: −35.8 (−51.9, −14.2)          |                                                        |                                                        |
|                    |                                                              | Year 7, adjusted for age 5: −28.2 (−42.7, −10.1) |                                                        |                                                        |
|                    |                                                              | Year 7: OR = 1.38 (0.91, 2.10)         |                                                        |                                                        |
|                    |                                                              | Year 7: OR = 4.20 (1.54, 11.44)        |                                                        |                                                        |
| Grandjean et al. 2012 | Serum anti-tetanus toxoid antibody concentration, % difference or % with inadequate concentration (≤0.1 IU/mL) | Age 5 PFOA:              | Per 2-fold increase in age 5 PFOA:                |                                                        |
|                    |                                                              | Prebooster: n = 537       | Year 7 adjusted for age 5: 12.3 (−8.6, 38.1)      |                                                        |
|                    |                                                              | Postbooster: n = 440      | Year 7, adjusted for age 5: −28.2 (−42.7, −10.1)  |                                                        |
|                    |                                                              | Year 7: OR = 1.38 (0.91, 2.10)         |                                                        |                                                        |
|                    |                                                              | Year 7: OR = 4.20 (1.54, 11.44)        |                                                        |                                                        |
| Grandjean et al. 2012 | Serum anti-tetanus toxoid antibody concentration, % difference or % with inadequate concentration (≤0.1 IU/mL) | Age 5 PFOS:              | Per 2-fold increase in age 5 PFOS:                |                                                        |
|                    |                                                              | Prebooster: n = 537       | Year 7 adjusted for age 5: 12.3 (−8.6, 38.1)      |                                                        |
|                    |                                                              | Postbooster: n = 440      | Year 7, adjusted for age 5: −28.2 (−42.7, −10.1)  |                                                        |
|                    |                                                              | Year 7: OR = 1.38 (0.91, 2.10)         |                                                        |                                                        |
|                    |                                                              | Year 7: OR = 4.20 (1.54, 11.44)        |                                                        |                                                        |
| Grandjean et al. 2012 | Serum anti-tetanus toxoid antibody concentration, % difference or % with inadequate concentration (≤0.1 IU/mL) | Age 5 PFOS isomers:      | Per 2-fold increase in maternal n-PFOS (linear):  |                                                        |
|                    |                                                              | Prebooster: n = 509       | Prebooster: 14.7 (−35.0, 12.0)                     |                                                        |
|                    |                                                              | Postbooster: n = 419      | Postbooster: −4.5 (−29.7, 29.9)                    |                                                        |
|                    |                                                              | Year 7: n = 424           | Year 7 adjusted for age 5: 36.1 (−2, 90.6)        |                                                        |
|                    |                                                              |                           | Year 7, adjusted for age 5: 37.1 (5.1, 78.9)      |                                                        |
|                    |                                                              | Per 2-fold increase in age 5 PFOS:     | Prebooster: 6.5 (−15.7, 34.5)                      |                                                        |
|                    |                                                              |                             | Postbooster: 2.8 (−21.7, 34.9)                     |                                                        |
|                    |                                                              | Year 7: 22.2 (−7.8, 62.0)          | Year 7, adjusted for age 5: 11.2 (−11.0, 38.8)    |                                                        |
|                    |                                                              |                             | Year 7, adjusted for age 5: −11.7 (−29.0, 9.9)    |                                                        |
|                    |                                                              |                             | Postbooster: −29.9 (−45.8, −9.2)                   |                                                        |
|                    |                                                              |                             | Year 7: −19.3 (−39.7, 8.0)                        |                                                        |
|                    |                                                              |                             | Year 7, adjusted for age 5: −7.8 (−26.3, 15.5)    |                                                        |
|                    |                                                              |                             | Per 2-fold increase in age 5 br-PFOS (branched)   |                                                        |
|                    |                                                              |                             | Prebooster: −10.4 (−28.3, 12.0)                    |                                                        |
|                    |                                                              |                             | Postbooster: −22.0 (−40.0, 1.3)                    |                                                        |
|                    |                                                              |                             | Year 7: −24.7 (−44.6, 2.5)                        |                                                        |
|                    |                                                              |                             | Year 7, adjusted for age 5: −13.1 (−31.5, 10.3)   |                                                        |
| Grandjean et al. 2012 | Serum anti-diphtheria toxoid antibody concentration, % difference or % | Maternal PFOA:           | Per 2-fold increase in maternal PFOA:             |                                                        |
|                    |                                                              | Prebooster: n = 510       | Prebooster: −16.2 (−34.2, 6.7)                     |                                                        |
|                    |                                                              | Postbooster adjusted for age, sex, time since vaccination, and booster type |                                                        |
### Table 4. Continued

| Reference | Outcome | Number (% with outcome) | Estimate of association (95% confidence interval) | Adjustment factors |
|-----------|---------|-------------------------|---------------------------------------------------|--------------------|
| E. T. Chang et al. | Serum anti-diphtheria toxoid antibody concentration, % difference or % with inadequate concentration (≤0.1 IU/mL) | Maternal PFOS: Prebooster: n = 510 | Per 2-fold increase in maternal PFOS: Prebooster: 38.6 (−5.7, −16.9) Prebooster: −20.6 (−37.5, 0.9) Year 7: −19.7 (−41.8, 10.7) Year 7, adjusted for age 5: −10.0 (−32.6, 20.0) Year 5: OR = 2.48 (1.55, 3.97) Year 7: OR = 2.33 (0.88, 6.14) Per 2-fold increase in age 5 PFOS: Prebooster: −16.0 (−34.9, 8.3) Postbooster: −15.5 (−31.5, 4.3) Year 7: −27.6 (−45.8, −3.3) Year 7, adjusted for age 5: −20.6 (−38.2, 2.1) Year 5: OR = 1.60 (110.16, 2.34) Year 7: OR = 2.38 (0.89, 6.35) | Maternal PFOS isomers: Prebooster: n = 537 Postbooster: n = 440 Year 7: n = 408 Year 7, adjusted for age 5: n = 403 Year 5 PFOS isomers: Prebooster: n = 537 Postbooster: n = 440 Year 7: n = 408 Year 7, adjusted for age 5: n = 403 Per 2-fold increase in normal n-PFOS (linear): Prebooster: −42.6 (−57.3, −22.7) Postbooster: −22.6 (−38.3, −2.2) Year 7: −22.9 (−43.8, 5.8) Year 7, adjusted for age 5: −10.6 (−32.7, 18.8) Prebooster: −16.8 (−35.6, 7.5) Postbooster: −11.8 (−28.4, 8.7) Year 7: −5.7 (−27.7, 22.9) Year 7, adjusted for age 5: −5.8 (−25.3, 18.9) Per 2-fold increase in normal n-PFOS (linear): Prebooster: −17.5 (−35.2, 5.1) Postbooster: −16.1 (−31.3, 2.4) Year 7: −24.2 (−42.1, −0.8) Year 7, adjusted for age 5: −16.4 (−33.8, 5.5) Per 2-fold increase in normal n-PFOS (linear): Prebooster: −8.1 (−28.2, 17.7) Postbooster: −10.9 (−27.3, 9.2) Year 7: −21.1 (−40.6, 49) Year 7, adjusted for age 5: −18.3 (−36.1, 4.5) Per 1-ng/mL increase in PFOA: coefficient = −0.40 (−0.64, −0.17) | Prebooster adjusted for age and sex, time since vaccination, and booster type Postbooster adjusted for age, sex, time since vaccination, and booster type Year 7 adjusted for age, sex, and booster type, with or without additional adjustment for child’s specific antibody concentration at age 5 years Prebooster adjusted for age and sex, time since vaccination, and booster type Year 7 adjusted for age, sex, and booster type, with or without additional adjustment for child’s specific antibody concentration at age 5 years No substantial change after additional adjustment for polychlorinated biphenyls in breast milk and age 5 serum
| E. T. Chang et al. | Serum anti-diphtheria toxoid antibody concentration, % difference or % with inadequate concentration (≤0.1 IU/mL) | Maternal PFOA: Prebooster: n = 537 Postbooster: n = 440 Year 7: n = 424 Year 7, adjusted for age 5: n = 382 Age 5 PFOA: Prebooster: n = 537 Postbooster: n = 440 Year 7: n = 408 Year 7, adjusted for age 5: n = 403 | Per 2-fold increase in age 5 PFOA: Prebooster: −6.8 (−28.3, 21.0) Postbooster: −6.1 (−23.6, 15.5) Year 7: −25.2 (−42.9, −2.0) Year 7, adjusted for age 5: −23.4 (−39.3, −3.4) Year 5: OR = 1.19 (0.81, 1.73) Year 7: OR = 3.27 (1.43, 7.51) | Prebooster adjusted for age and sex, time since vaccination, and booster type Year 7 adjusted for age, sex, and booster type, with or without additional adjustment for child’s specific antibody concentration at age 5 years No substantial change after additional adjustment for polychlorinated biphenyls in breast milk and age 5 serum
| Grandjean et al. 2012 | Serum anti-diphtheria toxoid antibody concentration, % difference or % with inadequate concentration (≤0.1 IU/mL) | Maternal PFOS: Prebooster: n = 510 Postbooster: n = 419 Year 7: n = 424 Year 7, adjusted for age 5: n = 382 Maternal PFOS isomers: Prebooster: n = 537 Postbooster: n = 440 Year 7: n = 408 Year 7, adjusted for age 5: n = 403 Age 5 PFOS: Prebooster: n = 537 Postbooster: n = 440 Year 7: n = 408 Year 7, adjusted for age 5: n = 403 | Per 2-fold increase in maternal PFOS: Prebooster: −38.6 (−54.7, −16.9) Prebooster: −20.6 (−37.5, 0.9) Year 7: −19.7 (−41.8, 10.7) Year 7, adjusted for age 5: −10.0 (−32.6, 20.0) Year 5: OR = 2.48 (1.55, 3.97) Year 7: OR = 2.33 (0.88, 6.14) Per 2-fold increase in age 5 PFOS: Prebooster: −16.0 (−34.9, 8.3) Postbooster: −15.5 (−31.5, 4.3) Year 7: −27.6 (−45.8, −3.3) Year 7, adjusted for age 5: −20.6 (−38.2, 2.1) Year 5: OR = 1.60 (110.16, 2.34) Year 7: OR = 2.38 (0.89, 6.35) | Prebooster adjusted for age and sex, time since vaccination, and booster type Year 7 adjusted for age, sex, and booster type, with or without additional adjustment for child’s specific antibody concentration at age 5 years No substantial change after additional adjustment for polychlorinated biphenyls in breast milk and age 5 serum
| Granum et al. 2013 | Serum anti-rubella antibody concentration (optical density) | n = 50 | Maternal allergy, paternal allergy, maternal education, child’s gender, and age at 3- | Maternal allergy, paternal allergy, maternal education, child’s gender, and age at 3-
Table 4. Continued

| Reference          | Outcome                                                                 | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|--------------------|--------------------------------------------------------------------------|-------------------------|--------------------------------------------------|-------------------|
| Granum et al. 2013 | Serum anti-measles antibody concentration (optical density)               | n = 50                  | Per 1-ng/mL increase in PFOS: coefficient = −0.08 (−0.14, −0.02) | None |
|                    |                                                                          |                         | Per 1-ng/mL increase in PFOA: coefficient = −0.13 (−0.35, 0.09)         | year follow-up (included in backward deletion model) |
| Granum et al. 2013 | Serum anti-Haemophilus influenza type b antibody concentration (μg/mL) | n = 51                  | Per 1-ng/mL increase in PFOS: coefficient = −0.05 (−0.10, 0.01)             | None |
| Granum et al. 2013 | Serum anti-tetanus toxoid antibody concentration (IU/mL)                 | n = 49                  | Per 1-ng/mL increase in PFOA: coefficient = −0.01 (−0.09, 0.10)             | None |
| Granum et al. 2013 | Serum anti-influenza type B antibody: GMT increase, log10 titer increase, log10 titer ratio, seroconversion, or seroprotection |                          | Per unit increase in log10 PFOA: Log10 titer increase: coefficient = −0.02 (−0.13, 0.09) | Log10 models adjusted for age, gender, residential mobility, and history of previous influenza vaccination |
| Looker et al. 2014 | Serum anti-influenza type B antibody: GMT increase, log10 titer increase, log10 titer ratio, seroconversion, or seroprotection | Q1 (0.25–13.7 ng/mL): n = 103 | Q1: GMT rise = 49.46 (38.14, 64.12) | Results stratified by demographic characteristics, comorbidity, or medication use are not shown here |
|                    |                                                                          | Q2 (13.8–31.5 ng/mL): n = 103 | Q2: GMT rise = 46.00 (35.28, 59.97) | Log10 models adjusted for age, gender, residential mobility, and history of previous influenza vaccination |
|                    |                                                                          | Q3 (31.6–90 ng/mL): n = 103 | Q3: GMT rise = 43.55 (33.08, 57.33) | Results stratified by demographic characteristics, comorbidity, or medication use are not shown here |
|                    |                                                                          | Q4 (90.4–2140 ng/mL): n = 102 | Q4: GMT rise = 20.90 (16.58, 28.24); P < 0.05 | Log10 models adjusted for age, gender, residential mobility, and history of previous influenza vaccination |
| Looker et al. 2014 | Serum anti-influenza type B antibody: GMT increase, log10 titer increase, log10 titer ratio, seroconversion, or seroprotection | Q1: n = 61/100          | Q1 vs. Q1: GMT rise = 42.25 (33.41, 53.42) | Log10 models adjusted for age, gender, residential mobility, and history of previous influenza vaccination |
|                    |                                                                          | Q2: n = 54/97           | Q2 vs. Q1: GMT rise = 41.48 (30.73, 55.99) | Results stratified by demographic characteristics, comorbidity, or medication use are not shown here |
|                    |                                                                          | Q3: n = 61/100          | Q3 vs. Q1: GMT rise = 41.13 (31.65, 53.44) | Log10 models adjusted for age, gender, residential mobility, and history of previous influenza vaccination |
|                    |                                                                          | Q4: n = 54/97           | Q4 vs. Q1: GMT rise = 52.83 (38.92, 71.71) | Results stratified by demographic characteristics, comorbidity, or medication use are not shown here |

(continued)
Table 4. Continued

| Reference            | Outcome                                | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|----------------------|----------------------------------------|-------------------------|--------------------------------------------------|-------------------|
| Looker et al. 2014   | Serum anti-influenza type A/H1N1 antibody: GMT increase, log$_{10}$ titer increase, log$_{10}$ titer ratio, seroconversion, or seroprotection | Q1: 0.25–13.7 ng/mL: n = 103 | Q4: seroconversion OR = 0.87 (0.43, 1.74) | Log$_{10}$ models adjusted for age, gender, residential mobility, and history of previous influenza vaccination |
|                      |                                        | Q2: 13.8–31.5 ng/mL: n = 103 | Q2 (vs. Q1): seroprotection OR = 0.67 (0.35, 1.25) | Results stratified by demographic characteristics, comorbidity, or medication use are not shown here |
|                      |                                        | Q3: 31.6–90 ng/mL: n = 103  | Q3: seroprotection OR = 0.82 (0.42, 1.59) | |
|                      |                                        | Q4: 90.4–2140 ng/mL: n = 102 | Q4: seroprotection OR = 0.73 (0.36, 1.47) | |
|                      | Seroprotection:                        | Q1: n = 83/100           | Per unit increase in log$_{10}$ PFOA: | |
|                      |                                        | Q2: n = 82/102           | Log$_{10}$ titer rise: coefficient = 0.05 (−0.11, 0.21) | |
|                      |                                        | Q3: n = 83/100           | Log$_{10}$ titer ratio: coefficient = 0.05 (−0.09, 0.18) | |
|                      |                                        | Q4: n = 91/101           | Seroprotection OR = 1.17 (0.63, 2.17) | |
|                      | Seroconversion:                        | Q1: n = 97/100           | Seroprotection OR = 0.85 (0.44, 1.64) | |
|                      |                                        | Q2: n = 95/102           | Log$_{10}$ titer ratio: coefficient = 0.04 (−0.27, 0.08) | |
|                      |                                        | Q3: n = 94/100           | Log$_{10}$ titer ratio: coefficient = 0.04 (−0.29, 0.12) | |
|                      |                                        | Q4: n = 99/101           | Log$_{10}$ titer ratio: coefficient = 0.04 (−0.28, 0.09) | |
| Looker et al. 2014   | Serum anti-influenza type A/H1N1 antibody: GMT increase, log$_{10}$ titer increase, log$_{10}$ titer ratio, seroconversion, or seroprotection | Q1: 0.1–5.8 ng/mL: n = 106 | Log$_{10}$ titer rise: coefficient = 0.07 (−0.06, 0.21) | |
|                      |                                        | Q2: 5.9–9.2 ng/mL: n = 101 | Seroprotection OR = 1.59 (0.33, 7.70) | |
|                      |                                        | Q3: 9.3–14.5 ng/mL: n = 102 | Seroprotection OR = 6.47 (0.91, 45.85) | |
|                      |                                        | Q4: 14.7–42.3 ng/mL: n = 102 | Per unit increase in log$_{10}$ PFOA: | |
|                      | Seroconversion:                        | Q1: n = 88/104           | Log$_{10}$ titer rise: coefficient = −0.03 (−0.14, 0.09) | |
|                      |                                        | Q2: n = 81/97            | Log$_{10}$ titer ratio: coefficient = 0.07 (−0.06, 0.21) | |
|                      |                                        | Q3: n = 82/100           | Seroprotection OR = 1.51 (0.89, 2.56) | |
|                      |                                        | Q4: n = 88/102           | Seroprotection OR = 2.34 (0.91, 6.07) | |
|                      | Seroconversion:                        | Q1: n = 101/104          | Log$_{10}$ models adjusted for age, gender, residential mobility, and history of previous influenza vaccination | |
|                      |                                        | Q2: n = 90/97            | Results stratified by demographic characteristics, comorbidity, or medication use are not shown here | |
|                      |                                        | Q3: n = 97/100           | |
|                      |                                        | Q4: n = 97/102           | |
| Reference | Outcome | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors |
|-----------|---------|-------------------------|-----------------------------------------------|------------------|
| Looker et al. 2014 | Serum anti-influenza type A/H3N2 antibody: GMT increase, log_{10} titer increase, log_{10} titer ratio, seroconversion, or seroprotection | PFOA quartiles:  
Q1 (0.25–13.7 ng/mL): n = 103  
Q2 (13.8–31.5 ng/mL): n = 103  
Q3 (31.6–90 ng/mL): n = 103  
Q4 (90.4–2140 ng/mL): n = 102  
  Seroconversion:  
Q1: n = 68/100  
Q2: n = 68/102  
Q3: n = 69/100  
Q4: n = 56/101  
  Seroprotection:  
Q1: n = 91/100  
Q2: n = 82/102  
Q3: n = 80/100  
Q4: n = 85/101 | Log_{10} titer ratio: coefficient = 0.10 (−0.11, 0.30)  
Seroconversion OR = 1.10 (0.51, 2.37)  
Seroprotection OR = 0.93 (0.23, 3.71)  
PFOA quartiles:  
Q1: GMT rise = 228.86 (161.53, 324.27)  
Q2: GMT rise = 125.36 (86.01, 182.73)  
Q3: GMT rise = 104.13 (72.47, 149.64)  
Q4: GMT rise = 183.73 (127.28, 265.23); p ≤ 0.05  
Q2 (vs. Q1): log_{10} titer rise = −0.28 (−0.51, −0.06)  
Q3: log_{10} titer rise = −0.37 (−0.60, −0.13)  
Q4: log_{10} titer rise = −0.12 (−0.36, 0.13)  
Q2 (vs. Q1): log_{10} titer ratio = −0.10 (−0.30, 0.10)  
Q3: log_{10} titer ratio = −0.07 (−0.28, 0.14)  
Q4: log_{10} titer ratio = −0.04 (−0.28, 0.21)  
Q2 (vs. Q1): seroconversion OR = 0.90 (0.48, 1.68)  
Q3: seroconversion OR = 1.13 (0.59, 2.17)  
Q4: seroconversion OR = 0.62 (0.33, 1.16)  
Q2 (vs. Q1): seroprotection OR = 0.34 (0.14, 0.83)  
Q3: seroprotection OR = 0.28 (0.11, 0.70)  
Q4: seroprotection OR = 0.39 (0.15, 0.99)  
Per unit increase in log_{10} PFOA:  
Log_{10} titer rise: coefficient = −0.01 (−0.17, 0.14)  
Log_{10} titer ratio: coefficient = −0.12 (−0.25, 0.02)  
Seroconversion OR = 0.76 (0.51, 1.15)  
Seroprotection OR = 0.66 (0.39, 1.12)  
PFOA quartiles:  
Q1: GMT rise = 137.69 (98.70, 192.09)  
Q2: GMT rise = 147.27 (99.27, 218.47)  
Q3: GMT rise = 211.02 (141.24, 315.27)  
Q4: GMT rise = 126.69 (88.85, 180.65)  
Q2 (vs. Q1): log_{10} titer rise = −0.13 (−0.06, 0.41)  
Q3: log_{10} titer rise = −0.04 (−0.28, 0.21)  
Q4: log_{10} titer ratio = −0.03 (−0.24, 0.19)  
Q2 (vs. Q1): seroconversion OR = 1.08 (0.59, 1.97)  
Q3: seroconversion OR = 1.10 (0.59, 2.06)  
Q4: seroconversion OR = 1.41 (0.72, 2.78)  
Q2 (vs. Q1): seroprotection OR = 0.85 (0.38, 1.88)  
Q3: seroprotection OR = 1.09 (0.47, 2.56)  
Q4: seroprotection OR = 0.56 (0.24, 1.28)  
Per unit increase in log_{10} PFOS:  
Log_{10} titer rise: coefficient = −0.09 (−0.13, 0.32)  
Log_{10} titer ratio: coefficient = −0.005 (−0.20, 0.19)  
Seroconversion OR = 1.17 (0.63, 2.15)  
Seroprotection OR = 0.63 (0.26, 1.49)  
Per 2-fold increase in serum PFOA:  
% change = −0.22 (−0.85, 6.44)  

Looker et al. 2014 | Serum anti-influenza type A/H3N2 antibody: GMT increase, log_{10} titer increase, log_{10} titer ratio, seroconversion, or seroprotection | PFOS quartiles:  
Q1 (0.1–5.8 ng/mL): n = 106  
Q2 (5.9–9.2 ng/mL): n = 101  
Q3 (9.3–14.5 ng/mL): n = 102  
Q4 (14.7–42.3 ng/mL): n = 102  
  Seroconversion:  
Q1: n = 65/104  
Q2: n = 60/97  
Q3: n = 64/100  
Q4: n = 72/102  
  Seroprotection:  
Q1: n = 89/104  
Q2: n = 82/97  
Q3: n = 87/100  
Q4: n = 80/102 | Log_{10} models adjusted for age, gender, residential mobility, and history of previous influenza vaccination  
Results stratified by demographic characteristics, comorbidity, or medication use are not shown here

Kielsen et al. 2015 | Serum anti-diphtheria toxoid antibody, log_{10} titer % change | n = 12 | Log_{10} models adjusted for age, gender, residential mobility, and history of previous influenza vaccination  
Results stratified by demographic characteristics, comorbidity, or medication use are not shown here

(continued)
outside the reference range indicate immune abnormalities (Emmett et al. 2006b; Grandjean et al. 2012; Looker et al. 2014). A limitation of this approach is that a reference range is typically determined based on the mean plus or minus two standard deviations calculated from a group of healthy adults or children. By definition, 5% of the normal population falls outside of such a reference range (AACC 2015). The only way to determine whether a given value outside a reference range is truly “abnormal” is to associate it with a clinical abnormality, yet this has not been done in most epidemiologic studies of immune biomarkers. Studies also have not demonstrated whether immune parameters measured in clinically normal individuals can accurately predict the risk of future immunological diseases. Given the immune system’s capacity for repair and regeneration, apparent immune abnormalities that are detected at one point in time may even resolve before producing any adverse clinical health effect. Thus, biomarkers that do not accurately diagnose or predict the presence or absence of a clinical health condition are not clinically useful.

Outcome assessment of clinical immune conditions

Studies of PFOA and PFOS in relation to clinically recognizable immune conditions discussed in this review, including atopic, infectious, autoimmune, and inflammatory disorders, mostly used self- or parent-reported information as a basis for outcome assessment (Anderson-Mahoney et al. 2008; Granum et al. 2013; Humblet et al. 2014; Innes et al. 2011; Looker et al. 2014; Okada et al. 2012, 2014; Smit et al. 2015; Steenland et al. 2013, 2015; Uhl et al. 2013; Wang et al. 2011). The majority did not validate self-reported data based on medical records or other official sources, such that outcome misclassification was probable but unquantifiable. Study validity would be particularly threatened in studies where participants were probably aware of whether they were relatively highly exposed to PFOA from a contaminated public water supply and/or from their workplace (Anderson-Mahoney et al. 2008; Emmett et al. 2006b; Innes et al. 2011; Looker et al. 2014; Steenland et al. 2013, 2015). In these studies, self-reported outcomes might be influenced by the perception that higher exposure could increase risk of adverse health conditions, thereby biasing associations in a positive direction.

Even in the studies that attempted to validate self-reported outcomes (Steenland et al. 2013, 2015), only positive self-reports were compared with medical records in a subset of subjects, excluding those who did not consent to medical records retrieval or whose records could not be retrieved, and also excluding...
Table 5. Results of epidemiologic studies of the association between exposure to perfluorooctanoic acid (PFOA) and/or perfluorooctanesulfonate (PFOS) and autoimmune or chronic inflammatory conditions.

| Reference            | Outcome                      | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors                                                                                                                                                                                                 |
|----------------------|------------------------------|-------------------------|--------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Innes et al. 2011    | Osteoarthritis               |                         |                                                  | Age, body mass index, race/ethnicity, gender, socioeconomic status, marital status, menopausal status, hormone replacement therapy, smoking status, current alcohol consumption, vegetarian diet, regular exercise program, years of education, annual household income, employment status/disability, comorbidity Overall (non-stratified) ORs also adjusted for military service, harmful chemical exposure, sleep impairment, frequent mood disturbance, frequent recent memory loss, and serum estradiol, cholesterol, uric acid, and C-reactive protein levels Further adjustment for other PFAS did not substantially change estimates |
|                      |                              | PFOA quartiles:         |                                                  |                                                                                                                                                                                                                  |
|                      |                              | Q1 (0.25–13.5 ng/mL): n = 737 | PFOA quartiles:                                 | Age, body mass index, race/ethnicity, gender, socioeconomic status, marital status, menopausal status, hormone replacement therapy, smoking status, current alcohol consumption, vegetarian diet, regular exercise program, years of education, annual household income, employment status/disability, comorbidity Overall (non-stratified) ORs also adjusted for military service, harmful chemical exposure, sleep impairment, frequent mood disturbance, frequent recent memory loss, and serum estradiol, cholesterol, uric acid, and C-reactive protein levels Further adjustment for other PFAS did not substantially change estimates |
|                      |                              | Q2 (13.6–28.0 ng/mL): n = 838 | Q1: referent                                    |                                                                                                                                                                                                                  |
|                      |                              | Q3 (28.1–71.9 ng/mL): n = 994 | Q2: OR = 1.16 (1.03, 1.31)                      |                                                                                                                                                                                                                  |
|                      |                              | Q4 (≥72.0 ng/mL): n = 1162 | Q3: OR = 1.21 (1.07, 1.36)                      |                                                                                                                                                                                                                  |
|                      |                              |                          | Q4: OR = 1.42 (1.26, 1.59)                      |                                                                                                                                                                                                                  |
|                      |                              |                          | P-trend = 0.00001                               |                                                                                                                                                                                                                  |
|                      |                              |                          | Per unit increase in log PFOA: OR = 1.07 (1.04, 1.11) |                                                                                                                                                                                                                  |
|                      |                              |                          | P-interaction with age <0.00001                  |                                                                                                                                                                                                                  |
|                      |                              |                          | P-trend for ages <55 years = 0.000001           |                                                                                                                                                                                                                  |
|                      |                              |                          | P-trend for ages ≥55 years = 0.11               |                                                                                                                                                                                                                  |
|                      |                              |                          | P-interaction with obesity = 0.0005             |                                                                                                                                                                                                                  |
|                      |                              |                          | P-trend for non–obese <0.00001                  |                                                                                                                                                                                                                  |
|                      |                              |                          | P-trend for obese = 0.25                        |                                                                                                                                                                                                                  |
|                      |                              |                          | Results not appreciably changed after restriction to PFOA levels <90th percentile (<193 ng/mL) Association "largely eliminated" after restriction to PFOA levels ≤20 ng/mL (n = 17,885, including 1167 cases) |                                                                                                                                                                                                                  |
|                      |                              |                          | Per unit increase in log PFOS: OR = 0.88 (0.84, 0.93) |                                                                                                                                                                                                                  |
|                      |                              |                          | Results not appreciably changed after stratification by age group or obesity status or restriction to relatively low PFOS levels |                                                                                                                                                                                                                  |
| Innes et al. 2011    | Osteoarthritis               |                         |                                                  |                                                                                                                                                                                                                  |
|                      |                              | PFOS quartiles:          |                                                  |                                                                                                                                                                                                                  |
|                      |                              | Q1 (0.25–13.6 ng/mL): n = 861 | PFOS quartiles:                                 | Age, body mass index, race/ethnicity, gender, socioeconomic status, marital status, menopausal status, hormone replacement therapy, smoking status, current alcohol consumption, vegetarian diet, regular exercise program, years of education, annual household income, employment status/disability, comorbidity Overall (non-stratified) ORs also adjusted for military service, harmful chemical exposure, sleep impairment, frequent mood disturbance, frequent recent memory loss, and serum estradiol, cholesterol, uric acid, and C-reactive protein levels Further adjustment for other PFAS did not substantially change estimates |
|                      |                              | Q2 (13.7–20.2 ng/mL): n = 868 | Q1: referent                                    |                                                                                                                                                                                                                  |
|                      |                              | Q3 (20.3–29.3 ng/mL): n = 964 | Q2: OR = 0.91 (0.81, 1.01)                      |                                                                                                                                                                                                                  |
|                      |                              | Q4 (≥29.4 ng/mL): n = 1038 | Q3: OR = 0.94 (0.84, 1.06)                      |                                                                                                                                                                                                                  |
|                      |                              |                          | Q4: OR = 0.76 (0.68, 0.85)                      |                                                                                                                                                                                                                  |
|                      |                              |                          | P-trend = 0.00001                               |                                                                                                                                                                                                                  |
|                      |                              |                          | Per unit increase in log PFOS: OR = 0.88 (0.84, 0.93) |                                                                                                                                                                                                                  |
|                      |                              |                          | Results not appreciably changed after restriction to PFOA levels <90th percentile (<193 ng/mL) Association "largely eliminated" after restriction to PFOA levels ≤20 ng/mL (n = 17,885, including 1167 cases) |                                                                                                                                                                                                                  |
| Steenland et al. 2013| Ulcerative colitis, lifetime | n = 151 validated        |                                                  | Age as time scale, sex, race/ethnicity, time-dependent smoking, time-dependent body mass index, and time-dependent alcohol consumption                                                                                                                                 |
|                      |                              | (n = 596 self-reported)  |                                                  |                                                                                                                                                                                                                  |
| Reference     | Outcome                                | Number (%) with outcome | Estimate of association (95% confidence interval)                                                                 | Adjustment factors                                                                 |
|---------------|----------------------------------------|-------------------------|----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
|               |                                        |                         | **Q1: referent**                                                                                               | **Age as time scale, sex, race/ethnicity, time-dependent smoking, time-dependent body mass index, and time-dependent alcohol consumption** |
|               |                                        |                         | **Q2: OR** = 1.59 (0.95, 2.65)                                                                               |                                                                                   |
|               |                                        |                         | **Q3: OR** = 2.40 (1.44, 3.99)                                                                               |                                                                                   |
|               |                                        |                         | **Q4: OR** = 2.33 (1.37, 3.97)                                                                               |                                                                                   |
|               |                                        |                         | **P-trend** < 0.0001                                                                                           |                                                                                   |
|               |                                        |                         | **PFOA quartiles, excluding background exposures:**                                                             |                                                                                   |
|               |                                        |                         | **Q1: referent**                                                                                               |                                                                                   |
|               |                                        |                         | **Q2: OR** = 1.27 (0.78, 2.08)                                                                               |                                                                                   |
|               |                                        |                         | **Q3: OR** = 2.08 (1.26, 3.44)                                                                               |                                                                                   |
|               |                                        |                         | **Q4: OR** = 2.30 (1.36, 3.91)                                                                               |                                                                                   |
|               |                                        |                         | **P-trend** < 0.0001                                                                                           |                                                                                   |
| Steenland et al. 2013 | Ulcerative colitis, incident        | \textit{n} = 30 validated | **PFOA quartiles, unlagged:**                                                                                   |                                                                                   |
|               |                                        |                         | **Q1 (<159 ng/mL/year): referent**                                                                            |                                                                                   |
|               |                                        |                         | **Q2 (18–586 ng/mL/year): OR = 1.68 (0.62, 4.66)**                                                            |                                                                                   |
|               |                                        |                         | **Q3 (587–3500 ng/mL/year): OR = 2.10 (0.75, 5.85)**                                                           |                                                                                   |
|               |                                        |                         | **Q4 (>3501 ng/mL/year): OR = 1.62 (0.57, 4.61)**                                                             |                                                                                   |
|               |                                        |                         | **P-trend** = 0.21                                                                                             |                                                                                   |
|               |                                        |                         | **PFOA quartiles, 10-year lag:**                                                                              |                                                                                   |
|               |                                        |                         | **Q1: referent**                                                                                               |                                                                                   |
|               |                                        |                         | **Q2: OR** = 1.21 (0.43, 3.34)                                                                               |                                                                                   |
|               |                                        |                         | **Q3: OR** = 2.16 (0.80, 5.81)                                                                               |                                                                                   |
|               |                                        |                         | **Q4: OR** = 1.51 (0.43, 4.30)                                                                               |                                                                                   |
|               |                                        |                         | **P-trend** = 0.12                                                                                             |                                                                                   |
| Steenland et al. 2013 | Crohn's disease, lifetime           | \textit{n} = 95 validated \textit{(n} = 178 self-reported) | **PFOA quartiles, unlagged:**                                                                                   |                                                                                   |
|               |                                        |                         | **Q1: referent**                                                                                               |                                                                                   |
|               |                                        |                         | **Q2: OR** = 1.25 (0.61, 2.58)                                                                               |                                                                                   |
|               |                                        |                         | **Q3: OR** = 1.15 (0.55, 2.41)                                                                               |                                                                                   |
|               |                                        |                         | **Q4: OR** = 1.00 (0.48, 2.09)                                                                               |                                                                                   |
|               |                                        |                         | **P-trend** = 0.73                                                                                             |                                                                                   |
|               |                                        |                         | **PFOA quartiles, 10-year lag:**                                                                              |                                                                                   |
|               |                                        |                         | **Q1: referent**                                                                                               |                                                                                   |
|               |                                        |                         | **Q2: OR** = 0.80 (0.32, 1.99)                                                                               |                                                                                   |
|               |                                        |                         | **Q3: OR** = 0.97 (0.36, 2.60)                                                                               |                                                                                   |
|               |                                        |                         | **Q4: OR** = 0.69 (0.26, 1.82)                                                                               |                                                                                   |
|               |                                        |                         | **P-trend** = 0.79                                                                                             |                                                                                   |
|               |                                        |                         | **Results were similar when follow-up started at the time of first residence in the study area or first employment at the plant, or when only cumulative exposures above background levels were considered (results not published)** |                                                                                   |
| Steenland et al. 2013 | Rheumatoid arthritis, lifetime     | \textit{n} = 346 validated \textit{(n} = 1292 self-reported) | **PFOA quartiles, unlagged:**                                                                                   |                                                                                   |
|               |                                        |                         | **Q1: referent**                                                                                               |                                                                                   |
|               |                                        |                         | **Q2: OR** = 1.24 (0.85, 1.79)                                                                               |                                                                                   |
|               |                                        |                         | **Q3: OR** = 1.40 (0.96, 2.03)                                                                               |                                                                                   |
|               |                                        |                         | **Q4: OR** = 0.99 (0.68, 1.43)                                                                               |                                                                                   |
|               |                                        |                         | **P-trend** = 0.84                                                                                             |                                                                                   |
|               |                                        |                         | **PFOA quartiles, 10-year lag:**                                                                              |                                                                                   |
|               |                                        |                         | **Q1: referent**                                                                                               |                                                                                   |
|               |                                        |                         | **Q2: OR** = 1.53 (0.61, 2.58)                                                                               |                                                                                   |
|               |                                        |                         | **Q3: OR** = 1.73 (1.10, 2.71)                                                                               |                                                                                   |
| Reference  | Outcome                                      | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors                                                                 |
|------------|----------------------------------------------|-------------------------|---------------------------------------------------|-------------------------------------------------------------------------------------|
| Steenland et al. 2013 | Rheumatoid arthritis, incident | $n = 56$ validated      | Q4: OR = 1.35 (0.87, 2.11) P-trend = 0.73       | Results were similar when follow-up started at the time of first residence in the study area or first employment at the plant, or when only cumulative exposures above background levels were considered (results not published) |
|            |                                              |                         | PFOA quartiles, unlagged:                         | Age as time scale, sex, race/ethnicity, time-dependent smoking, time-dependent body mass index, and time-dependent alcohol consumption |
|            |                                              |                         | Q1: referent                                      |                                                                                     |
|            |                                              |                         | Q2: OR = 0.81 (0.39, 1.68)                        |                                                                                     |
|            |                                              |                         | Q3: OR = 2.07 (1.00, 4.27)                        |                                                                                     |
|            |                                              |                         | Q4: OR = 0.52 (0.25, 1.09)                        |                                                                                     |
| Steenland et al. 2013 | Insulin-dependent diabetes, broad definition, lifetime | $n = 160$ validated (n = 342 self-reported) | Q1: referent                                      |                                                                                     |
|            |                                              |                         | Q4: OR = 0.54 (0.22, 1.33)                        |                                                                                     |
|            |                                              |                         | P-trend = 0.84                                    |                                                                                     |
|            |                                              |                         | PFOA quartiles, 10-year lag:                      |                                                                                     |
|            |                                              |                         | Q1: referent                                      |                                                                                     |
|            |                                              |                         | Q2: OR = 0.31 (0.14, 0.71)                        |                                                                                     |
|            |                                              |                         | Q3: OR = 0.90 (0.41, 2.00)                        |                                                                                     |
|            |                                              |                         | Q4: OR = 0.32 (0.14, 0.72)                        |                                                                                     |
| Steenland et al. 2013 | Type I diabetes, narrow definition, lifetime | $n = 85$ validated      | Q1: referent                                      |                                                                                     |
|            |                                              |                         | Q4: OR = 0.54 (0.22, 1.33)                        |                                                                                     |
|            |                                              |                         | P-trend = 0.84                                    |                                                                                     |
|            |                                              |                         | PFOA quartiles, unlagged:                         | Age as time scale, sex, race/ethnicity, time-dependent smoking, time-dependent body mass index, and time-dependent alcohol consumption |
|            |                                              |                         | Q1: referent                                      |                                                                                     |
|            |                                              |                         | Q2: OR = 0.83 (0.25, 2.78)                        |                                                                                     |
|            |                                              |                         | Q3: OR = 1.41 (0.40, 4.95)                        |                                                                                     |
|            |                                              |                         | Q4: OR = 0.88 (0.25, 3.06)                        |                                                                                     |
|            |                                              |                         | P-trend = 0.68                                    |                                                                                     |
|            |                                              |                         | PFOA quartiles, 10-year lag:                      |                                                                                     |
|            |                                              |                         | Q1: referent                                      |                                                                                     |
|            |                                              |                         | Q2: OR = 0.50 (0.05, 4.90)                        |                                                                                     |
|            |                                              |                         | Q3: OR = 1.32 (0.14, 12.40)                       |                                                                                     |
|            |                                              |                         | Q4: OR = 0.71 (0.07, 7.14)                        |                                                                                     |
|            |                                              |                         | P-trend = 0.65                                    |                                                                                     |
|            |                                              |                         | Results were similar when follow-up started at the |                                                                                     |
|            |                                              |                         | time of first residence in the study area or first employment at the plant, or when only cumulative exposures above background levels were considered (results not published) |                                                                                     |

(continued)
| Reference          | Outcome                         | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors                                                                 |
|-------------------|---------------------------------|-------------------------|---------------------------------------------------|-------------------------------------------------------------------------------------|
| Steenland et al. 2013 | Lupus, lifetime                 | $n = 72$ validated      | PFOA quartiles, unlagged:                         | Age as time scale, sex, race/ethnicity, time-dependent smoking, time-dependent body mass index, and time-dependent alcohol consumption |
|                   |                                 | ($n = 187$ self-reported) | Q1: referent                                       |                                                                                     |
|                   |                                 |                         | Q2: OR = 1.49 (0.68, 3.34)                         |                                                                                     |
|                   |                                 |                         | Q3: OR = 1.01 (0.44, 2.30)                         |                                                                                     |
|                   |                                 |                         | Q4: OR = 0.71 (0.31, 1.65)                         |                                                                                     |
|                   |                                 |                         | P-trend = 0.94                                     |                                                                                     |
|                   |                                 |                         | Results were similar when follow-up started at the time of first residence in the study area or first employment at the plant, or when only cumulative exposures above background levels were considered (results not published) |                                                                                     |
| Steenland et al. 2013 | Multiple sclerosis, lifetime   | $n = 99$ validated      | PFOA quartiles, unlagged:                         | Age as time scale, sex, race/ethnicity, time-dependent smoking, time-dependent body mass index, and time-dependent alcohol consumption |
|                   |                                 | ($n = 150$ self-reported) | Q1: referent                                       |                                                                                     |
|                   |                                 |                         | Q2: OR = 0.85 (0.44, 1.63)                         |                                                                                     |
|                   |                                 |                         | Q3: OR = 1.56 (0.81, 3.00)                         |                                                                                     |
|                   |                                 |                         | Q4: OR = 1.26 (0.65, 2.42)                         |                                                                                     |
|                   |                                 |                         | P-trend = 0.22                                      |                                                                                     |
|                   |                                 |                         | Results were similar when follow-up started at the time of first residence in the study area or first employment at the plant, or when only cumulative exposures above background levels were considered (results not published) |                                                                                     |
| Steenland et al. 2015 | Ulcerative colitis            | $n = 28$ validated      | PFOA quartiles, unlagged:                         | Age as time scale, sex, race, education, body mass index, time-dependent smoking, and time-dependent alcohol consumption |
|                   |                                 |                         | Q1: referent                                       |                                                                                     |
|                   |                                 |                         | Q2: OR = 1.57 (0.52, 4.76)                         |                                                                                     |
|                   |                                 |                         | Q3: OR = 0.57 (0.10, 3.11)                         |                                                                                     |
|                   |                                 |                         | Q4: OR = 2.74 (0.78, 9.65)                         |                                                                                     |
|                   |                                 |                         | P-trend, log cumulative PFOA = 0.05                |                                                                                     |
|                   |                                 |                         | P-trend, categorical PFOA = 0.26                   |                                                                                     |
|                   |                                 |                         | PFOA quartiles, 10-year lag:                       |                                                                                     |
|                   |                                 |                         | Q1: referent                                       |                                                                                     |
|                   |                                 |                         | Q2: OR = 3.00 (0.82, 11.0)                         |                                                                                     |
|                   |                                 |                         | Q3: OR = 3.26 (0.70, 15.10)                        |                                                                                     |
|                   |                                 |                         | Q4: OR = 6.57 (1.47, 29.40)                        |                                                                                     |
| Reference          | Outcome            | Number (%) with outcome | Estimate of association (95% confidence interval) | Adjustment factors                                                                 |
|--------------------|--------------------|-------------------------|--------------------------------------------------|-----------------------------------------------------------------------------------|
| Steenland et al. 2015 | Rheumatoid arthritis | n = 23 validated        | P-trend, log cumulative PFOA = 0.05              | P-trend, categorical PFOA = 0.05                                                  |
|                    |                    |                         | PFOA quartiles, unlagged:                        | PFOA quartiles, 10-year lag:                                                      |
|                    |                    |                         | Q1: referent                                     | Q1: referent                                                                      |
|                    |                    |                         | Q2: OR = 2.11 (0.49, 8.98)                       | Q2: OR = 1.74 (0.45, 6.77)                                                        |
|                    |                    |                         | Q3: OR = 4.08 (0.96, 17.2)                       | Q3: OR = 2.12 (0.4, 11.1)                                                         |
|                    |                    |                         | Q4: OR = 4.45 (0.99, 19.9)                       | Q4: OR = 2.62 (0.47, 14.7)                                                        |
|                    |                    |                         | P-trend, log cumulative PFOA = 0.54             | P-trend, log cumulative PFOA = 0.75                                              |
|                    |                    |                         | P-trend, categorical PFOA = 0.04                 | P-trend, categorical PFOA = 0.06                                                  |
|                    |                    |                         | PFOA quartiles, 10-year lag:                      | Age as time scale, sex, race, education, body mass index, time-dependent smoking, and time-dependent alcohol consumption |
| Steenland et al. 2015 | Osteoarthritis     | n = 196 self-reported  | Age as time scale, sex, race, education, body mass index, time-dependent smoking, and time-dependent alcohol consumption |
|                    |                    |                         | OR: odds ratio; PFAS: perfluoroalkyl and polyfluoroalkyl substances; PFOA: perfluorooctanoic acid; PFOS: perfluorooctanesulfonate; Q: quartile. |
negative self-reports. A substantial proportion of subjects with self-reported conditions were excluded due to the absence of confirmation. Thus, bias due to differential outcome misclassification remained possible in these studies if factors related to validation differed by exposure level—although information was not provided to assess this possibility. Additionally, even if physician’s diagnoses of certain immune conditions were accurately self-reported by study participants, the probability of observing such endpoints could be influenced by physicians’ diagnostic criteria or acuity, or by individuals’ access to health care. Therefore, associations of PFOA or PFOS exposure with health care quality or usage (e.g. mediated through socioeconomic status (Nelson et al. 2012; Tyrrell et al. 2013) or occupation) could conceivably be responsible for observed associations with certain physician-diagnosed immune conditions.

A few studies of clinical immunological disorders ascertained outcomes based on death certificates (Leonard et al. 2008), hospital discharge diagnoses (Fei et al. 2010), or a confirmed physician’s diagnosis (Dong et al. 2013). Such objectively collected outcome data are generally less prone to bias than self-reported information, although differential misclassification can be induced if physicians are aware of subjects’ exposure status. Hospital discharge and mortality data are not sensitive for capturing certain immune conditions, such as asthma and common infections, which usually do not result in hospitalization or death. Therefore, results that rely on these data may not be generalizable to less severe conditions. Moreover, some associations may be observed due to an influence of the exposure (or related conditions) on the severity of disease (which would still be important to identify) or the probability of seeking medical attention or being hospitalized, rather than an effect on disease development itself.

**Exposure assessment**

Exposure assessment is as vital to the validity and interpretation of a study as is outcome assessment. Of the 24 epidemiologic studies included in this review, 19 measured PFOA and/or PFOS in the serum or plasma of individual subjects using liquid chromatography tandem mass spectrometry, the standard method for quantitative analysis of these chemicals (Ashley-Martin et al. 2015; Dong et al. 2013; Emmett et al. 2006b; Fei et al. 2010; Grandjean et al. 2012; Granum et al. 2013; Humblet et al. 2014; Innes et al. 2011; Kielsen et al. 2015; Lin et al. 2011; Looker et al. 2014; Olsen et al. 2003; Uhl et al. 2013). Among the 19 studies with serum or plasma PFOA and/or PFOS measurements, eight were cross-sectional (Emmett et al. 2006b; Humblet et al. 2014; Innes et al. 2011; Kielsen et al. 2015; Lin et al. 2011; Looker et al. 2014; Olsen et al. 2003; Uhl et al. 2013), one was retrospective (Dong et al. 2013), and the remainder were prospective in design (Ashley-Martin et al. 2015; Fei et al. 2010; Grandjean et al. 2012; Granum et al. 2013; Okada et al. 2012, 2014; Osuna et al. 2014; Pennings et al. 2015; Smit et al. 2015; Wang et al. 2011). Prospective cohort studies benefit from measuring exposures prior to the diagnosis of health conditions, thereby ensuring that the temporal sequence of exposure and outcome is logically consistent with a potential causal effect. By contrast, when the exposure of interest is measured concurrently with the outcome (as in cross-sectional studies) or after the outcome has been ascertained (as in retrospective case-control studies), it may be difficult to determine which preceded the other in time, thereby prohibiting conclusions about causation. In the case of circulating PFOA and PFOS levels, reverse causation is a possible concern, given that disease processes or corresponding treatments could conceivably affect physiological clearance of these chemicals, and possibly also changes in behavioral patterns related to exposure (e.g. tap water consumption). However, such mechanisms are not well studied, and the potential direction and magnitude of bias are unknown.

Another issue related to exposure assessment is whether a single measurement of circulating PFOA or PFOS is etiologically relevant, even if measured prior to onset of the health condition of interest. Among all epidemiologic studies included in this review, only two (Grandjean et al. 2012; Osuna et al. 2014) analyzed PFOA and PFOS concentrations at more than one time point. One study found pairwise Pearson correlations of 0.19 for PFOA and 0.27 for PFOS measured in serum from
mothers during pregnancy and in children at age 5 years (Grandjean et al. 2012), while the other found correlations of 0.33 for PFOA and 0.28 for PFOS measured in maternal prenatal serum and in child serum at age 7 years (Osuna et al. 2011). These results suggest limited correlation between maternal and childhood exposure, perhaps due to changes in exposure levels over the course of early childhood, the effects of rapid growth and a high renal clearance rate in early childhood, or individual variability in uptake (during pregnancy) and clearance (during and after pregnancy). PFOA and PFOS have clearance half-lives of approximately 2.5 years and 4.8 years, respectively, in humans (Bartell 2012; Chang et al. 2012; Olsen et al. 2007), indicating that there is little fluctuation within individuals in the presence of constant exposure sources. However, whether those sources are indeed constant is unknown and perhaps unlikely, given the widespread use and release of these chemicals (Buck et al. 2011). In the absence of adequate evidence, unanswered questions are the degree to which circulating PFOA and PFOS levels change within individuals over time, and whether specific time windows exist during which exposure to PFOA or PFOS might have an effect on the development of immune disorders in humans. To the extent that a single exposure measurement does not capture individual variation in circulating PFOA and PFOS levels and is not taken during an etiologically important time window, the pertinent exposure will be misclassified.

**Confounding**

Control for confounding varied substantially among epidemiologic studies in this review, ranging from no or minimal adjustment (Anderson-Mahoney et al. 2008; Ashley-Martin et al. 2015; Emmett et al. 2006b; Granum et al. 2013; Kielsen et al. 2015; Leonard et al. 2008; Looker et al. 2014; Osuna et al. 2014; Pennings et al. 2015) to adjustment for at least 10 covariates potentially related to the exposure and outcome (Fei et al. 2010; Innes et al. 2011; Lin et al. 2011; Okada et al. 2012). In virtually any observational study, but especially those that adjust for no or few potential confounders, confounding cannot be eliminated; that is, an exposure and an outcome can appear to be associated due to independent associations with a third, unmeasured or incompletely adjusted variable. Indeed, several authors acknowledged that uncontrolled confounding, including residual confounding due to imprecise adjustment, remained a potential explanation for observed results (e.g. (Anderson-Mahoney et al. 2008; Ashley-Martin et al. 2015; Dong et al. 2013; Humblet et al. 2014; Innes et al. 2011; Leonard et al. 2008; Steenland et al. 2013; Uhl et al. 2013; Wang et al. 2011)).

Although some sociodemographic and behavioral determinants of PFAS levels have been identified (Calafat et al. 2007b; Emmett et al. 2006a; Eriksen et al. 2011; Jain 2013, 2014; Nelson et al. 2012; Tyrrell et al. 2013), the list is almost certainly incomplete, and influential factors – as well as the direction and magnitude of their associations – may vary across populations. In addition, risk factors for immune conditions are incompletely recognized. Thus, the potential effect of confounding on observed estimates is complex and difficult to quantify. Nevertheless, especially in studies with minimal adjustment for covariates, the potential influence of confounding should be taken into account when interpreting positive, negative, and null reported results. Sensitivity analysis comparing results with different covariate adjustment strategies could help to clarify the impact of specific confounders.

**Selection bias**

Other than bias due to confounding or systematic differences in the reporting of outcomes, discussed above, selection bias is another potential concern in some of the studies discussed in this review. Particularly in cross-sectional and case-control studies, in which subjects may be aware of their health status and exposure status at the time of enrollment, selection bias may arise if the decision to participate is influenced by this awareness. Even if the exposure and outcome themselves do not directly affect participation rates, selection bias can occur if participation is influenced by other factors, such as sociodemographic characteristics, that are associated with the exposure and outcome. In some cross-sectional and case-control studies, fewer than half of eligible subjects elected to participate (Anderson-Mahoney et al. 2008; Emmett et al. 2006b; Lin et al. 2011), and none had participation rates over 75% after accounting for exclusions due to missing data (Innes et al. 2011; Olsen et al. 2003) (omitting those that did not report participation rates (Costa et al. 2009; Dong et al. 2013; Humblet et al. 2014; Kielsen et al. 2015; Uhl et al. 2013)). In the presence of substantial non-participation, the potential magnitude of selection bias is greater.

In prospective cohort studies, the likelihood of selection bias due to unequal participation rates is lower because subjects are recruited prior to the onset of health conditions. Selection bias may occur at the time of enrollment if the decision to participate is affected by one’s awareness of their future disease risk
(e.g. due to having a positive family history) and exposure level, or factors associated with both, but this is a less likely scenario. Additionally, selection bias can occur during follow-up if the decision to drop out of the study is related to exposure and outcome. Therefore, reported study follow-up rates of 12–89% among subjects originally enrolled in prospective cohorts (Ashley-Martin et al. 2015; Grandjean et al. 2012; Granum et al. 2013; Okada et al. 2012, 2014; Pennings et al. 2015; Smit et al. 2015; Steenland et al. 2013, 2015; Wang et al. 2011) raise varying degrees of concern about potential selection bias.

Selection bias can also arise if the source populations for exposed and unexposed subjects differ systematically by outcome status, or if the source populations for cases and controls differ systematically by exposure status, independently of any true association between the exposure and outcome. Although most studies used internal comparison groups, thereby avoiding bias due to non-comparable source populations, studies susceptible to this bias were a case-control study of children with asthma diagnosed at one of two hospitals, compared with children without asthma selected from seven public schools in the same geographic region of Taiwan (Dong et al. 2013); and especially a cross-sectional study of volunteers included in a class action lawsuit due to their residence near a PFOA-contaminated river in Ohio and West Virginia, compared with nationally representative survey data (Anderson-Mahoney et al. 2008).

**Statistical considerations**

Any given statistical association may be due to chance. In studies that test a large number of hypotheses, the expected number of false-positive results (typically set at 5%) increases correspondingly. Selective reporting of statistically significant results and omission of non-significant results, a common practice in epidemiologic studies (Kavoura et al. 2007), can lead to undercounting of the total number of tests conducted and the corresponding expected number of false-positive findings. Especially when *a posteriori* analyses are conducted with exposures and outcomes classified in several ways or focusing on various subgroups of subjects in an effort to detect significant results, chance should be mentioned as a plausible explanation for any statistically significant result. Replication of findings in multiple independent study settings is critical to determining whether an association is unlikely to be explained by chance.

Conversely, low statistical power should be taken into account as an explanation for statistically non-significant findings in studies with a small number of subjects. However, because sampling and measurement error cannot be assumed to be completely at random, one cannot assume that a larger study would necessarily yield the same relative risk point estimates with greater statistical precision. Moreover, the lower the power of a study, the lower the probability that an observed, nominally statistically significant association is due to a true effect; that is, significant associations in smaller studies, on average, are more likely to be false (Button et al. 2013).

In summary, key characteristics for evaluating the quality of an epidemiologic study are outcome assessment methods, exposure assessment methods, control for confounding, potential for selection bias, and appropriateness of the statistical approach and its presentation. In the studies of associations between PFOA and/or PFOS and immune conditions, issues such as the clinical relevance, intra-individual variability, temporal sequence, and validity of outcome and exposure measures; the potential direction and magnitude of bias due to uncontrolled confounding and selection bias; and the roles of selective reporting, insufficient power, and chance should be taken into account when interpreting the results of each study. Those results are summarized in the next sections, where studies are grouped by type of immunological health condition.

**Results**

**Immune biomarkers**

Nine studies reported associations between serum or plasma levels of PFOA and/or PFOS and various circulating immune biomarkers measured using standard assays (Table 1) (Ashley-Martin et al. 2015; Costa et al. 2009; Dong et al. 2013; Emmett et al. 2006b; Granum et al. 2013; Lin et al. 2011; Okada et al. 2012; Olsen et al. 2003; Wang et al. 2011). One other study reported associations of PFAS (including PFOA and PFOS) levels with gene expression patterns, which were in turn related to immune-related outcomes (Pennings et al. 2015); this study is discussed in this section because, like biomarkers, gene expression patterns are nonspecific indicators that do not necessarily correspond to a clinically recognizable condition. Only four specific biomarkers were evaluated in more than one study: white blood cell count, total IgE, eosinophil count, and C-reactive protein. Five studies (Ashley-Martin et al. 2015; Granum et al. 2013; Okada et al. 2012; Pennings et al. 2015; Wang et al. 2011) involved prospective follow-up of birth cohorts in which prenatal or perinatal PFOA and PFOS levels were analyzed in relation to outcomes measured subsequently at birth or in early childhood (although Okada
et al. (2012) collected an unspecified number of maternal serum samples after delivery). The remaining five studies were cross-sectional or case-control in design and analyzed PFOA, PFOS, and biomarker levels measured at the same time, even if repeated specimens were collected (Costa et al. 2009; Dong et al. 2013; Emmett et al. 2006b; Lin et al. 2011; Olsen et al. 2003). Thus, the latter studies were unable to establish the temporal sequence between PFOA or PFOS concentrations and immune biomarkers.

White blood cell count

No significant association between serum PFOA or PFOS and white blood cell count was detected in a cross-sectional analysis of medical surveillance examination data from 518 workers at two fluorochemical manufacturing facilities in Belgium and the United States (quantitative results not reported) (Olsen et al. 2003). Likewise, no significant difference in white blood cell count was detected in a cross-sectional analysis of medical surveillance examination data comparing 34 workers currently employed in a PFOA production department at a chemical plant in Italy and up to 107 workers never occupationally exposed to PFOA (regression coefficient for exposed versus unexposed $= 0.58 \times 10^9$/L, 95% confidence interval [CI] = −0.19, 1.35), nor was a significant association detected between concurrently measured serum PFOA level and white blood cell count (coefficient $= 0.029 \times 10^9$/L per 1000 ng/mL PFOA, 95% CI = −0.011, 0.071) (Costa et al. 2009). In a cross-sectional study of 371 residents in a Mid-Ohio Valley water district contaminated with PFOA from a nearby polymer manufacturing plant, serum PFOA was not significantly associated with higher white blood cell count assessed as a continuous outcome (coefficient $= 0.0004$ [units not reported], $p = 0.09$, $p = 0.08$), and no significant difference in serum PFOA was observed between those with abnormal and normal white blood cell counts ($p = 0.64$) (Emmett et al. 2006b).

All three of these studies were limited by their cross-sectional design and use of one-time exposure and outcome measurements. Participation rates of 75% and 52% in the study by Olsen et al. (2003) and 36–49% in the study by Emmett et al. (2006b) (participation rates were not reported by Costa et al. 2009)) could have produced selection bias, and Emmett et al. (2006b) did not control for any potential confounders. Finally, by not assessing individual- or group-level exposures, the study by Costa et al. (2009) implicitly assumed that all PFOA production workers were similarly exposed. Nevertheless, the generally consistent null results suggest no substantial, detectable effect of PFOA or PFOS on total white blood cell count.

Childhood IgE

The relationship between PFOA or PFOS levels and newborn or childhood total IgE levels was reported in four studies (Table 1) (Ashley-Martin et al. 2015; Dong et al. 2013; Okada et al. 2012; Wang et al. 2011). In a prospective birth cohort of 244 children in Taiwan, log-transformed cord serum PFOA and PFOS levels measured at delivery were not significantly associated with serum total IgE levels at age 2 years (regression coefficient $= 0.037$ kU/L per 1 log ng/mL PFOA, $p = 0.870$; coefficient $= 0.251$ kU/L per 1 log ng/mL PFOS, $p = 0.147$) (Wang et al. 2011). However, in a cross-sectional analysis of this cohort, significant positive associations of log-transformed cord serum PFOA and PFOS with cord serum total IgE at birth were detected among boys (PFOA coefficient $= 0.206$, 95% CI $= 0.047$, 0.702; PFOS coefficient $= 0.175$, 95% CI $= 0.004$, 0.704), but not girls (PFOA coefficient $= 0.067$, $p = 0.823$; PFOS coefficient $= 0.151$, $p = 0.616$).

Discordant heterogeneity by sex was reported in a prospective birth cohort study of 343 Japanese mother–child pairs, among whom log$_{10}$-transformed third-trimester maternal prenatal serum PFOA was inversely associated with cord serum total IgE at birth in quadratic and cubic (but not linear) models for girls, suggesting a curvilinear relationship (linear coefficient $= 0.766$ IU/mL per 1 log$_{10}$ ng/mL PFOA, 95% CI $= 0.104$, 1.428; quadratic coefficient $= −1.429$, 95% CI $= −2.416$, −0.422; cubic coefficient $= −3.078$, 95% CI $= −5.431$, −0.726), whereas no significant association was observed in boys (Okada et al. 2012). In both boys and girls in this study, no significant association was found between maternal prenatal serum PFOS levels and cord serum IgE levels.

In a cross-sectional analysis within a case-control study of 231 children with physician-diagnosed asthma and 225 controls in Taiwan, higher quartiles of child serum PFOA and PFOS were not significantly associated with serum total IgE among controls ($P_{trend} = 0.123$ and 0.404, respectively), but statistically significant positive exposure-response trends with both PFOA and PFOS were seen in asthmatic children ($P_{trend} = 0.005$ and 0.008, respectively) (Dong et al. 2013).

A third prospective birth cohort study, in which maternal first-trimester plasma PFOA and PFOS levels were studied in relation to cord plasma IgE levels in 1242 mother–infant pairs in Canada, found no significant associations (odds ratio (OR) for elevated IgE $\geq 0.5$ kU/L per unit increase in log$_{10}$-transformed maternal PFOA $= 1.1$, 95% credible interval (CrI) $= 0.6$, 1.9; OR for
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maternal PFOS = 1.1, 95% CrI = 0.6, 1.9) (Ashley-Martin et al. 2015). Unlike the results of Okada et al. (2012), these null associations did not vary appreciably in analyses stratified by infant sex.

Three of these studies are strengthened by their prospective design (Ashley-Martin et al. 2015; Okada et al. 2012; Wang et al. 2011), but remain constrained by the reliance on a single measurement of exposure and outcome per subject. The case-control study (Dong et al. 2013), besides being limited by its reliance on serum PFOA, PFOS, and total IgE levels measured simultaneously, was susceptible to selection bias due to the differently defined case and control source populations, as well as nonparticipation. Given the contradictory evidence of subgroup heterogeneity and inconsistency in the direction and magnitude of the reported associations, if any, it remains uncertain whether PFOA or PFOS affects total IgE levels in all children or in certain susceptible subgroups.

**Eosinophil count**

The study of Ohio residents of a PFOA-contaminated water district examined both absolute eosinophil count and percentage of eosinophils among white blood cells, and found no significant association of serum PFOA levels with either outcome in unadjusted analyses (regression coefficient for absolute eosinophils = 0.00000252 [units not reported], $\rho = 0.00$, $p = 0.90$; coefficient for percent eosinophils = −0.0000652, $\rho = 0.01$, $p = 0.82$) (Emmett et al. 2006b) (Table 1). Similarly, the Taiwan case-control study of asthma reported no significant cross-sectional trend between serum PFOA or PFOS levels and absolute eosinophil count among children without asthma ($P_{\text{trend}} = 0.224$ and 0.445, respectively) (Dong et al. 2013). However, significant positive trends with both PFOA and PFOS concentrations ($P_{\text{trend}} = <0.001$ and 0.009, respectively) were found among asthmatic children. Similar patterns of association, with positive trends in asthmatic but not in non-asthmatic children, were also observed with serum eosinophil cationic protein levels in the latter study. Taken together, these two studies suggest no apparent effect of PFOA or PFOS on eosinophil count in non-asthmatic individuals at a single time point. Given the cross-sectional nature of the Taiwan study, the temporal directionality of the observed associations in children with asthma is unclear.

**C-reactive protein**

In the two cross-sectional studies that examined C-reactive protein levels as an outcome (Table 1), no significant association with occupational PFOA exposure was detected among Italian chemical plant workers (regression coefficient = −0.020 mg/L per 1000 ng/mL PFOA, 95% CI = −0.268, 0.228) (Costa et al. 2009). No significant association with either PFOA or PFOS was found in a cross-sectional study of 287 adolescents and young adults with or without hypertension in Taiwan ($P_{\text{trend}} = 0.932$ and 0.957, respectively) (Lin et al. 2011). Limitations include the cross-sectional study design, the single exposure and outcome measures for a biomarker that fluctuates within individuals, the lack of quantitative exposure assessment in the former study (Costa et al. 2009), and participation rates of 10% and 49% for normotensive and hypertensive subjects, respectively, in the latter study (Lin et al. 2011). Even so, these statistically null results do not suggest any substantial impact of PFOA or PFOS on C-reactive protein levels.

**Gene expression**

In a prospective birth cohort study set in Norway, perinatal maternal plasma levels of PFOA, PFOS, and two other PFASs (perfluorononanoate and perfluorohexane sulfonate) from 66 women were analyzed in relation to expression levels of 19,595 unique genes based on microarrays (Pennings et al. 2015). Gene expression levels were also analyzed with respect to mother-reported episodes of common cold up to age 3 years ($n = 73$ children) and post-vaccination anti-rubella antibody titers at 3 years ($n = 58$ children) – two immune-related outcomes that previously were found to be associated with PFAS levels in the same cohort (Granum et al. 2013). Expression levels of 453 genes were significantly ($P < 0.05$) positively correlated with PFOA levels and 490 genes were inversely correlated; 636 genes were positively correlated with PFOS levels and 671 were inversely correlated; 294 genes were positively correlated with levels of at least two PFASs and 284 were inversely correlated; 330 genes were positively correlated with common cold episodes and 250 were inversely correlated; and 522 genes were positively correlated with anti-rubella antibody titers and 709 were inversely correlated. Expression levels of 27 genes – including 3 related to immunological and/or hematopoietic functions and 6 involved in development and/or morphogenesis – were associated with both PFAS levels and common cold episodes, while 26 genes – including 2 involved in regulation of T-cell activation, 1 other associated with immunological functionality, and 7 involved in development and/or morphogenesis – were associated with both PFAS levels and anti-rubella antibody titers. The authors interpreted these results as providing a mechanistic link between prenatal PFAS
exposure and impaired immune function in early childhood. However, the interpretation is not clear-cut, especially given that expression levels of hundreds of immune-related genes were not correlated with the exposure or outcomes. Moreover, the small number of subjects and the large number of comparisons raise concerns about a large number of false-positive findings; thus, independent confirmation and targeted mechanistic studies are needed to substantiate these results.

**Other biomarkers**

Other studies of immune biomarkers, which included counts and percentages of lymphocytes, basophils, neutrophils, and monocytes (Emmett et al. 2006b), IgA, IgG, IgM, α1 globulins, α2 globulins, β globulins, and γ globulins (Costa et al. 2009), early-childhood specific IgE levels against a variety of common food and inhalant allergens (Granum et al. 2013), and cord plasma levels of thymic stromal lymphopoietin and interleukin-33 (Ashley-Martin et al. 2015), found few statistically significant associations. These were a “very weak” positive correlation ($\rho = 0.13$, $p = 0.01$) between PFOA and absolute monocyte count (but no significant difference in PFOA levels between subjects with normal and abnormal monocyte counts), significantly lower serum PFOA levels in those with abnormal percentages of lymphocytes ($n = 18$; $p = 0.01$) or neutrophils ($n = 35$; $p = 0.02$) (Emmett et al. 2006b), and a positive association between serum PFOA and α2 globulin levels (regression coefficient $= -0.026$, 95% CI $= 0.007$, 0.045, but no significant difference in α2 globulin levels between PFOA-exposed and non-exposed workers) (Costa et al. 2009). Otherwise, reported associations were statistically non-significant. Given many of the methodological limitations identified above [e.g. cross-sectional design, probable confounding, and selection bias in Emmett et al. (2006b), lack of quantitative exposure assessment in Costa et al. (2009)], these isolated, as-yet unreplicated results do not establish any association of PFOA or PFOS with biomarkers of adverse immune function.

**Atopic conditions**

Ten studies investigated associations between PFOA and/or PFOS exposure and the occurrence of specific or overall atopic or allergic disorders, including seven studies of asthma (Anderson-Mahoney et al. 2008; Dong et al. 2013; Granum et al. 2013; Humblet et al. 2014; Leonard et al. 2008; Smit et al. 2015; Steenland et al. 2015), five studies of wheezing (Granum et al. 2013; Humblet et al. 2014; Okada et al. 2012, 2014; Smit et al. 2015; Wang et al. 2011). Two cohort studies were conducted at a West Virginia polymer manufacturing plant; one was a retrospective cohort study that compared mortality between plant workers and general populations or other regional workers (Leonard et al. 2008), and the other was a cohort study that analyzed estimated cumulative serum PFOA levels in relation to validated self-reported asthma that occurred before or after study entry (Steenland et al. 2015). Another study used a case-control design to compare serum PFOA and PFOS levels between children with physician-diagnosed asthma and children from the same region without a personal or family history of asthma (Dong et al. 2013). Finally, two were cross-sectional studies, including one that used serum PFOA and PFOS measurements and self-reported data on asthma and wheezing in a representative group of U.S. adolescents (Humblet et al. 2014), and another that collected self-reported health information during in-person group sessions or telephone interviews with members of a class action lawsuit involving PFOA contamination of drinking water in the Mid-Ohio Valley community of Ohio and West Virginia (Anderson-Mahoney et al. 2008).

**Asthma**

This last study (Anderson-Mahoney et al. 2008) was severely methodologically limited by the low participation rate (2.5%), the lack of an appropriate comparison population, the failure to control for confounders other than age and sex, the absence of quantitative exposure data, the lack of validation of self-reported health conditions, and the absence of blinding to PFOA exposure status. Therefore, its finding of an 82% higher (95% CI = 47%, 125%) prevalence of asthma among 566 participants compared with representative adults in the 2001–2002 U.S. National Health and Nutrition Examination Survey (NHANES) (Table 2) cannot be interpreted in terms of causality.

The other cross-sectional analysis focused on NHANES data for adolescents from 1999–2008 (Humblet et al. 2014), and found in some statistical models that a two-fold increase in serum PFOA concentration was
associated with a significantly higher odds of ever
having been diagnosed with asthma, based on self-
report (OR = 1.18, 95% CI = 1.01, 1.39). However, this
association was attenuated and statistically non-signifi-
cant in other models (e.g. OR for doubling in serum
PFOA = 1.11, 95% CI = 0.87, 1.42 after using sample
weights to account for the probability of selection,
nonresponse adjustment, and adjustment to indepen-
dent population controls; OR for highest versus lowest
tertile of serum PFOA = 1.11, 95% CI = 0.94, 1.31). Serum
PFOA was not significantly associated with current
asthma, and serum PFOS was not significantly associated
with ever or current asthma. Inference based on these
findings is constrained by the single cross-sectional
assessment of exposures and outcomes and the lack of
validated outcome data.

The cohort mortality study of 6027 workers ever
employed at the polymer manufacturing plant between
1948 and 2002 reported no deaths from asthma, compared
with 2.1 deaths expected in the U.S. population, 1.8 deaths expected in the West Virginia population,
and 0.7 deaths expected in other regional workers
for the same company (Leonard et al. 2008). However,
mortality data are not sensitive for studying risk factors
for the onset or exacerbation of asthma, which is seldom
fatal, and the study was based on small numbers of
expected deaths.

Based on post-diagnosis serum PFOA and PFOS levels
in 231 asthma cases and 225 controls, the Taiwan case-
control study detected a significant positive association
and exposure-response trend between both PFOA and
PFOS and recently diagnosed asthma (OR for top versus
bottom quartile of serum PFOA = 4.05, 95% CI = 2.21,
7.42, \( P_{\text{trend}} < 0.001 \); OR for top versus bottom quartile of
serum PFOS = 2.63, 95% CI = 1.48, 4.69, \( P_{\text{trend}} = 0.003 \))
(Dong et al. 2013). In case-only analyses, neither
exposure was significantly related to asthma control
(based on a five-item questionnaire about asthma
symptoms, use of rescue medication, and limitation of
daily activities) in the four weeks prior to the study.
PFOA was not significantly related to asthma severity,
but a significant positive association was observed
between PFOS levels and asthma severity (\( P_{\text{trend}} = 0.045 \)). Causal inference in this study is limited by
the retrospective exposure assessment after asthma
onset in cases. Moreover, selection bias may have
influenced the findings to an unknown extent because
hospital-based cases and school-based controls were
drawn from different source populations (the authors
did not state whether the cases attended the same
schools as the controls) with potentially different serum
PFOA and PFOS concentrations. Selection bias could also
have occurred if exposure levels differed between study
participants and nonparticipants; the response rate
among potential controls contacted by phone was
72%, not accounting for those who could not be
contacted (response rate among cases not stated). On
the other hand, this study, unlike any others reviewed, is
strengthened by the ascertainment of cases with
validated, physician-diagnosed asthma.

In a prospective birth cohort study of asthma onset,
no significant association was detected between mater-
nal perinatal plasma PFOA or PFOS levels and asthma
incidence, based on 11 cases in the first three years of
childhood for 76 Norwegian mother–child pairs with
available questionnaire data (OR per 1 ng/mL
PFOA = 3.56, 95% CI = 0.84, 15.02; OR per 1 ng/mL
PFOS = 1.22, 95% CI = 0.89, 1.66) (Granum et al. 2013).
The small size of this study gave it limited power to
detect any association.

However, a substantially larger prospective birth
cohort study, based on 1024 mother–child pairs from
Greenland and Ukraine, also found no significant
association between maternal prenatal serum PFOA or
PFOS levels and the incidence of asthma in children
aged 5–9 years (Smit et al. 2015). In analyses including
70 children who had ever been diagnosed with asthma,
the OR per 1-standard-deviation increase in PFOA was
0.80 (95% CI = 0.62, 1.04) in subjects from both countries
combined, and the OR per 1-standard-deviation increase
in PFOS was 0.86 (95% CI = 0.67, 1.10). The authors
conducted a principal component analysis to identify
distinct patterns of exposure to 16 environmental
contaminants, and found no significant associations
with asthma for either of the two principal components
with high loadings for PFOA or PFOS (data not shown in
Table 2). Limitations of this study include its one-time
perinatal measurement of PFOA and PFOS levels, the
reliance on self-reported information on asthma, and the
lack of control for confounders in the analyses of PFOA
and PFOS as single exposures. However, the study is
strengthened by its prospective exposure assessment
and relatively large size.

At the same polymer manufacturing plant as that
studied by Leonard et al. (2008), 3713 workers (or their
next of kin, for 6%) were interviewed regarding their
health history and provided sufficient data for computa-
tion of retrospective estimates of PFOA exposure,
making them eligible for an analysis of several diseases
including asthma (Steenland et al. 2015). Historical
annual serum PFOA levels from occupational exposure
since 1951 were estimated using a job-exposure matrix,
and historical annual serum PFOA levels from environ-
mental exposure since 1951 were estimated based on a
multistage modeling procedure that incorporated an
environmental fate and transport model, information
about residential history and drinking water sources and consumption, and a pharmacokinetic model. Subjects who reported having asthma currently treated with medication were asked to give consent for medical records review; of 138 self-reported cases, 108 (78%) had a medical record reviewed and 84 of those (78%) were confirmed as asthma, with 82 included in the analysis. A borderline significant inverse trend was detected between increasing quartiles of cumulative serum PFOA and risk of currently treated asthma in unlagged analyses (OR for top versus bottom quartile = 0.53, 95% CI = 0.16, 1.69, \( P_{\text{trend}} = 0.27 \) for continuous log PFOA, \( P_{\text{trend}} = 0.05 \) for categorical PFOA), and no significant association was detected in analyses with a 10-year lag (OR for top versus bottom quartile = 0.52, 95% CI = 0.09, 2.84, \( P_{\text{trend}} = 0.53 \) for continuous log PFOA, \( P_{\text{trend}} = 0.17 \) for categorical PFOA). Although PFOA exposure in this study was modeled rather than measured, with an unknown degree of misclassification, it has the advantage of accounting for changes in exposure over time. Self-reported outcomes were validated, but only for positive (not negative) self-reports and not for subjects whose medical records were not retrieved.

Overall, given the conflicting findings, the temporal ambiguity of exposure and outcome assessment in most studies, potential misclassification of self-reported asthma in several studies, and the greater weight accorded to the Norway and Greenland/Ukraine studies due to their prospective design and direct measurement of prenatal exposures, these studies collectively do not indicate a causal relationship between PFOA or PFOS exposure and asthma risk.

**Eczema and wheezing**

Associations with eczema and wheezing were investigated in four prospective birth cohort studies (Table 2) (Granum et al. 2013; Okada et al. 2012, 2014; Smit et al. 2015), and wheezing was also examined in the cross-sectional analysis of 1999–2008 NHANES data (Humblet et al. 2014). In the latter study, whose limitations were discussed earlier, no significant associations were detected between serum PFOA or PFOS concentrations and self-reported wheezing in the last 12 months (OR per two-fold increase in PFOA = 1.00, 95% CI = 0.80, 1.23; OR per two-fold increase in PFOS = 0.83, 95% CI = 0.67, 1.02).

In the Norwegian birth cohort (Granum et al. 2013), no significant association was detected between maternal perinatal plasma PFOA or PFOS levels and the onset of parent-reported doctor-diagnosed atopic eczema (\( n = 14 \) cases) or eczema and itchiness in the face or at joints (\( n = 32 \) cases) during the first three years of childhood (OR for atopic eczema per 1 ng/mL PFOA = 1.31, 95% CI = 0.37, 4.68; OR per 1 ng/mL PFOS = 0.96, 95% CI = 0.73, 1.27). The Norwegian study also did not detect a significant association of maternal PFOA or PFOS levels with eczema and itchiness in the third year of life (\( n = 21 \) cases, 16 with blood specimens), nor did it find a significant association with the onset of wheezing during the first three years (\( n = 29 \) cases) or in the third year of childhood (\( n = 18 \) cases, 11 with blood).

No significant association between maternal prenatal serum PFOA or PFOS and childhood eczema (\( n = 37 \) cases) was reported with follow-up through age 18 months in the prospective cohort of 343 mother–child pairs in the city of Sapporo, Japan (OR per 10-fold increase in PFOA = 0.96, 95% CI = 0.23, 4.02; OR per 10-fold increase in PFOS = 0.87, 95% CI = 0.15, 5.08) (Okada et al. 2012). In a substantially larger cohort including 2062 mother–child pairs throughout Hokkaido Prefecture, Japan (which includes Sapporo; however, the cohorts appear not to overlap (Kishi et al. 2013)), no significant positive association was again observed between maternal prenatal plasma PFOS and the onset of childhood eczema through 12 months or 24 months of age (\( n = 367 \) cases) (Okada et al. 2014). Instead, a significant inverse exposure-response trend was detected with PFOA (\( P_{\text{trend}} = 0.025 \) ), as eczema risk at 24 months was 38% lower (95% CI = 0%, 49%) in the top quartile than the bottom quartile of maternal prenatal plasma PFOA. Neither of the Japanese cohorts detected a significant association between maternal prenatal PFOA or PFOS levels and the onset of wheezing in the first 18 months (\( n = 33 \) cases) (Okada et al. 2012) or 12 or 24 months of life (\( n = 397 \) cases) (Okada et al. 2014).

The Greenland/Ukraine birth cohort study found no significant association between maternal prenatal serum PFOA or PFOS and current eczema (\( n = 74 \) cases, ever eczema (\( n = 132 \) cases), current wheeze (\( n = 130 \) cases), or ever wheeze (\( n = 43 \) cases) at ages 5–9 years in the majority of comparisons, which included separate analyses of subjects from Greenland and Ukraine and pooled analyses of all subjects (i.e. 24 odds ratios for two exposures, four outcomes, and three country groups) (Smit et al. 2015). For example, the ORs in association with a 1-standard-deviation increase in exposure in the combined Greenland/Ukraine subjects were 0.97 (95% CI = 0.81, 1.17) for PFOA and ever eczema, 0.98 (95% CI = 0.82, 1.18) for PFOS and ever eczema, 0.91 (95% CI = 0.76, 1.10) for PFOA and ever wheeze, and 0.83 (95% CI = 0.69, 1.00) for PFOS and ever wheeze. The only statistically significant association was an inverse (i.e. protective) association between a 1-standard-deviation increase in maternal prenatal PFOS and risk of current
wheeze in Ukrainian subjects (OR = 0.60, 95% CI = 0.38, 0.92). Associations between principal components heavily loaded with PFOS or PFOA were also generally statistically null, with only a single statistically significant inverse association between the principal component with high factor loadings for PFOA, PFOS, and perfluorohexanoic acid and risk of current wheeze (data not shown in Table 2).

Overall, despite being constrained by the lack of repeated exposure assessment and modest case numbers, the four birth cohort studies suggest no significant adverse impact of prenatal PFOA or PFOS exposure on the onset of eczema or wheezing in early childhood, and the cross-sectional study indicates no apparent association between PFOA or PFOS exposure and the prevalence of wheezing in adolescence.

### Food allergy

In the Sapporo birth cohort, no significant association was observed between maternal prenatal serum PFOA or PFOS and the development of food allergy during the first 18 months of life (OR per 10-fold increase in PFOA = 1.67, 95% CI = 0.52, 5.37; OR per 10-fold increase in PFOS = 3.72, 95% CI = 0.81, 17.10) (Table 2) (Okada et al. 2012). Likewise, as mentioned earlier in the section on immune biomarkers, the Norwegian prospective birth cohort study found no significant associations of maternal PFOA and PFOS levels at delivery with child sensitization to a variety of food and inhalant allergens at age 3 years, based on allergen-specific IgE levels (Table 1) (Granum et al. 2013). Taken together, these studies provide no evidence for a causal relationship between early-life PFOA or PFOS exposure and the development of food allergy in childhood.

### Other atopic conditions

Other atopic health conditions were assessed by only one study each, and thus lack sufficient evidence to assess consistency across studies. In the Hokkaido birth cohort study, no association with total allergic diseases (including eczema, wheezing, and allergic rhinoconjunctivitis symptoms as of age 24 months) was observed for maternal prenatal plasma PFOS levels (Okada et al. 2014). For maternal PFOA levels, no association was observed among boys, but a significant inverse trend was detected among girls ($P_{\text{trend}} = 0.017$; OR for top versus bottom quartile = 0.64, 95% CI = 0.42, 0.97) and in all children combined ($P_{\text{trend}} = 0.030$; OR = 0.79, 95% CI = 0.59, 1.04). The single study of atopic dermatitis, a prospective birth cohort study of 244 mother–child pairs in Taiwan, found no significant association in adjusted models relating cord serum PFOA or PFOS levels to the onset of atopic dermatitis by age 2 years, based on 43 cases classified using questionnaire data (Table 2) (Wang et al. 2011). Overall, despite their reliance on unvalidated parent-reported outcomes and use of a single exposure measurement per subject, these studies suggest no apparent relationship between pre- or perinatal PFOA or PFOS levels and risk of various atopic disorders in early life.

### Infectious diseases

Five studies (Fei et al. 2010; Granum et al. 2013; Leonard et al. 2008; Looker et al. 2014; Okada et al. 2012) reported on PFOA or PFOS levels in relation to infectious diseases (Table 3). Two of these studies evaluated the occurrence of common colds (Granum et al. 2013; Looker et al. 2014), two evaluated the occurrence of otitis media (Granum et al. 2013; Okada et al. 2012), and two examined all infectious and parasitic diseases combined (Fei et al. 2010; Leonard et al. 2008), while other infectious diseases were evaluated by only one study each. Two studies used registry-based hospitalization or mortality data on infectious diseases (Fei et al. 2010; Leonard et al. 2008), whereas the rest used parent- or self-reported health outcomes. Three studies prospectively followed mother–child pairs from birth to age 8.2 years (on average) (Fei et al. 2010), 3 years (Granum et al. 2013), and 18 months (Okada et al. 2012), respectively; one was a retrospective cohort mortality study of workers at a West Virginia polymer production plant that used PFOA (Leonard et al. 2008); and the remaining study was a cross-sectional analysis of adults exposed to a PFOA-contaminated public water supply in the Mid-Ohio Valley of Ohio and West Virginia (Looker et al. 2014).

### Common cold

The prospective birth cohort study of mother–child pairs in Norway, described earlier, found significant positive associations between a 1-ng/mL increase in maternal perinatal plasma PFOA and number of episodes of the common cold in the first three years of life as well as during the third year, with regression coefficients of 0.42 in both multivariate models (95% CI = 0.21, 0.62 and 0.16, 0.72, respectively) (Table 3) (Granum et al. 2013). No significant association was detected between maternal perinatal plasma PFOA and the incidence of any common cold (yes versus no) in the third year ($n = 70$ positive reports among 85 children). For maternal PFOS levels, no significant association was detected with number of cold episodes in the first three years or the
third year of life, or with the incidence of any common cold in the third year.

In the cross-sectional study of 755 adults in the Mid-Ohio Valley, neither PFOA nor PFOS levels in serum were significantly associated with the presence of any cold (reported by 538 subjects) or the number of cold episodes in the last 12 months (Looker et al. 2014). The OR for any cold per 1 log_{10} ng/mL PFOA was 0.83 (95% CI = 0.61, 1.13) and that for PFOS was 0.83 (0.51, 1.34) (Table 3). These study subjects were a selected subgroup of 69,030 participants in the C8 Health Project, a study of individuals who had lived, worked, or attended school for at least 12 months in one of six public water districts contaminated with PFOA from a nearby polymer manufacturing plant (Frisbee et al. 2009). Methodological limitations include the cross-sectional exposure and outcome assessment, the potential for selection bias, and possible confounding by risk factors other than age and sex (the only adjusted covariates). Overall, these findings provide inconsistent evidence regarding a potential effect of PFOA exposure on the frequency of common cold episodes, and they suggest no significant association with PFOS exposure.

**Otitis media**

Childhood otitis media (middle ear infection) was investigated with respect to maternal prenatal and perinatal PFOA and PFOS levels in two prospective birth cohort studies based on 343 mother–child pairs in Sapporo, Japan, followed to age 18 months (n = 61 cases) (Okada et al. 2012) and up to 85 mother–child pairs in Norway followed to age 3 years (n = 27 cases in all three years) (Granum et al. 2013), respectively (Table 3). Neither study reported a significant association with PFOA or PFOS exposure. The OR per 10-fold increase in PFOA was 1.51 (95% CI = 0.45, 5.12) and that for PFOS was 1.40 (95% CI = 0.33, 6.00) in the Japanese study, while the OR per 1 ng/mL PFOA for all three years was 0.76 (95% CI = 0.81, 1.29) and that for PFOS was 1.02 (95% CI = 0.81, 1.29) in the Norwegian study. Despite their limitations, discussed earlier, these studies provide no solid evidence of an effect of PFOA or PFOS on otitis media in young children.

**Other infections**

One of the two registry-linkage studies that evaluated all infectious and parasitic diseases combined was the occupational cohort mortality study, in which PFOA-exposed workers experienced significantly fewer deaths from infectious and parasitic diseases (one death observed) than expected in the general U.S. population (15.0 deaths expected), but no significant difference compared with the general West Virginia population (3.4 deaths expected) or the regional worker population (1.1 deaths expected) (Leonard et al. 2008).

The other study involved the linkage of a cohort of 1400 mother–child pairs with the national hospital discharge registry in Denmark, yielding 577 hospitalizations for infectious diseases (including all infectious and parasitic diseases plus additional organ-specific infections) among 363 children followed prospectively from birth to 5.8–10.7 years (mean = 8.2 years) (Fei et al. 2010). In this cohort, maternal first-trimester plasma PFOA and PFOS levels were not significantly associated with increased risk of infectious disease hospitalization for boys and girls combined, with consistently statistically null findings across strata of age group and birth order. The only statistically significant association overall was an inverse association between the second versus first quartile of maternal PFOA and risk of infectious disease hospitalization. Significant positive associations of both exposures with infectious disease hospitalization were observed among girls (per-quartile RR for PFOA = 1.21, 95% CI = 1.04, 1.42; per-quartile RR for PFOS = 1.18, 95% CI = 1.03, 1.36). Among boys, however, the opposite was true, with a significant inverse association between maternal PFOA and hospitalization for infectious disease (per-quartile RR = 0.83, 95% CI = 0.73, 0.95) but no significant association for maternal PFOS (per-quartile RR = 0.90, 95% CI = 0.80, 1.02).

This study is strengthened by the use of validated, objectively recorded outcome data and prospectively collected exposure data, although infectious diseases requiring hospitalization represent only a subset of all infections. The analysis of infectious and parasitic disease deaths in the occupational cohort study included an even smaller subset of all infections, and was further limited by the implicit assumption that all workers were equally exposed to PFOA (Leonard et al. 2008). In the absence of a biologically plausible explanation for opposite effects by sex, the results of these two studies collectively do not suggest a consistent or convincing association between PFOA or PFOS and serious infectious diseases leading to death or hospitalization.

Infectious diseases examined in single studies were mortality from influenza and pneumonia, “flu” infection, and gastroenteritis (Table 3). In the West Virginia worker cohort, significantly fewer deaths from influenza and pneumonia were observed (14 deaths) than expected in the general U.S. population (26.5 deaths expected) and the general West Virginia population (28.2 deaths expected), with no significant difference.
from expectation in the regional worker population (15.7 deaths expected) (Leonard et al. 2008). In the cross-sectional study of Mid-Ohio Valley adults, the occurrence of any “flu” infection in the last 12 months (self-reported by 163 of 755 subjects) was not significantly associated with serum PFOA or PFOS levels, nor was the occurrence of any cold or “flu” infection significantly associated with either exposure (OR per 1 log_{10} ng/mL PFOA = 0.85, 95% CI = 0.62, 1.16; OR per 1 log_{10} ng/mL PFOS = 0.90, 95% CI = 0.55, 1.48) (Looker et al. 2014). The Norwegian cohort study reported a marginally significant positive association between a 1-ng/mL increase in maternal perinatal plasma PFOA concentration and parent-reported number of episodes of gastroenteritis in the third year (coefficient = 0.31, 95% CI = 0.002, 0.61), but no significant association with number of gastroenteritis episodes in the first three years or the presence versus absence of gastroenteritis in years 1–3 or 3 (Granum et al. 2013). Maternal PFOS levels also were not significantly associated with any measure of gastroenteritis. Collectively, in light of the equivocal findings and the methodological limitations discussed earlier, these studies do not offer consistent evidence to support any effect of PFOA or PFOS on the occurrence of infectious diseases, and the few significant results (in either direction) could have occurred by chance.

**Vaccine response**

Four studies, including two prospective birth cohort studies (Grandjean et al. 2012; Granum et al. 2013) and two cross-sectional studies of adults (Kielsen et al. 2015; Looker et al. 2014), investigated whether PFOA and PFOS levels are related to antibody response to common vaccines (Table 4). The first of these was a study of 587 Faroe Islands children from birth through age 5 years (before and after immunization with the diphtheria and tetanus booster vaccine) up to age 7 years, with exposure information on serum PFOA and PFOS levels in mothers during the third trimester of pregnancy and in children at age 5 years prior to receiving the booster (Grandjean et al. 2012). Of the 587 children, 460 (78%) participated in both the 5-year and 7-year examinations, and 431 (73%) had complete data with serum analyses. Vaccine antibody titers prior to the five-year booster, four weeks after the five-year booster, and at age 7 years (with or without adjustment for antibody titer at age 5 years) were analyzed in terms of percentage difference in concentration, as well as in terms of the percentage with insufficient antibody concentration for seroprotection ($\leq 0.1$ IU/mL).

Maternal prenatal serum PFOA and PFOS levels generally were not associated with a significant difference in the tetanus vaccine response, other than a significant positive association between higher maternal PFOS and higher anti-tetanus toxoid antibody titer at age 7 years, adjusted for titer at age 5 years (Table 4). Maternal PFOA and PFOS levels were generally associated with a poorer childhood diphtheria vaccine response, as measured based on antibody titers and the presence of a non-protective antibody level, although most differences were statistically non-significant. Several significant associations were observed between child serum PFOA level at age 5 years and poorer antibody response to both tetanus and diphtheria vaccines at age 7 years, but not at age 5 years. For example, the OR for inadequate anti-tetanus toxoid antibody concentration at age 7 per two-fold increase in PFOA at age 5 was 4.20 (95% CI = 1.54, 11.44), and that for anti-diphtheria toxoid antibody was 3.27 (95% CI = 1.43, 7.51). For PFOS, child serum levels at age 5 years were consistently, although mostly non-significantly, associated with indicators of poorer antibody response to the tetanus and diphtheria vaccines at ages 5 and 7 years. For example, the OR for inadequate anti-tetanus toxoid antibody concentration at age 7 per two-fold increase in PFOA at age 5 was 2.61 (95% CI = 0.77, 8.92), and that for anti-diphtheria toxoid antibody was 2.38 (95% CI = 0.89, 6.35). When PFOS isomers were separately classified as linear or branched, estimated associations with child PFOS levels did not differ substantially by isomer, whereas associations with maternal PFOS levels were similar between linear and total PFOS but attenuated for branched PFOS.

The second study of vaccine response was the Norwegian birth cohort study, which collected outcome information on antibody titers against four vaccine antigens at age 3 years among children who followed the standard national childhood vaccination program ($n = 49–51$ subjects with outcome data, depending on the vaccine type) (Table 4) (Granum et al. 2013). Although 1-ng/mL increases in maternal perinatal plasma PFOA and PFOS levels were associated with significantly lower anti-rubella antibody levels at age 3 years (PFOA coefficient = -0.49 optical density units, 95% CI = -0.64, -0.17; PFOS coefficient = -0.08, 95% CI = -0.14, -0.02), no significant association was detected with antibody levels against measles virus, *Haemophilus influenza* type b, or tetanus toxoid.

The third study of vaccine response was a cross-sectional analysis of 403 Mid-Ohio Valley adults who participated in the C8 Health Project and subsequently completed a second interview and blood study. Information was collected on influenza-specific antibody
levels before and three weeks after immunization with a trivalent influenza vaccine (Table 4) (Looker et al. 2014). Vaccine responses against influenza type A/H1N1, type A/H3N2, and type B were evaluated in several ways: increase in titer after vaccination, \( \log_{10} \)-transformed increase in titer after vaccination, \( \log_{10} \)-transformed ratio of postvaccine to prevaccine antibody titer, seroconversion (defined as a four-fold or greater rise in postvaccination titer), and seroprotection (defined as postvaccination hemagglutination inhibition titer \( \geq 1:40 \)). Serum PFOA and PFOS levels were analyzed as quartiles and as \( \log_{10} \)-transformed continuous variables. In addition, results were reported for all subjects combined and after stratification by patient demographic characteristics, comorbidities, or medication use – at least 18 stratification factors in total. Therefore, the interpretation of results must be considered in light of the fact that hundreds of hypotheses were tested and numerous statistically significant findings would be expected to arise by chance.

In general, no clear pattern emerged between serum PFOA or PFOS level and postvaccination rise in geometric mean titer of antibodies against any of the three influenza strains, other than a significantly lower anti-influenza type B antibody GMT in the top quartile of PFOA concentration (Table 4) (Looker et al. 2014). When antibody titer increases and ratios were \( \log_{10} \)-transformed, nearly all multivariate adjusted associations were also statistically non-significant, except for scattered inverse associations, with no clear negative exposure-response trend, between serum PFOA level and \( \log_{10} \)-transformed antibody titer rise and ratio for anti-influenza A/H3N2. No significant associations were detected between PFOA or PFOS and seroconversion against any of the three influenza strains. When seroprotection was examined as the outcome, serum PFOA level was inversely associated with the prevalence of seroprotection against influenza A/H3N2, with a marginally significant exposure-response trend (\( P_{\text{trend}} = 0.07; \) OR for top versus bottom quartile of PFOA = 0.39, 95% CI = 0.15, 0.99), but an opposite trend toward a higher prevalence of seroprotection was observed for influenza A/H1N1 (\( P_{\text{trend}} = 0.02; \) OR for top versus bottom quartile of PFOA = 6.47, 95% CI = 0.91, 45.85).

Most recently, a small study of 12 healthy, previously immunized adult volunteers in Denmark measured anti-diphtheria and anti-tetanus toxoid antibody titers at the time of diphtheria and tetanus booster vaccination and at 2, 4, 7, 10, 14, and 30 days after vaccination to document the pattern of antibody response (Table 4) (Kielsen et al. 2015). The key time interval of antibody titer change was 4 to 10 days after vaccination, and serum levels of PFOA, PFOS, and six other PFASs were measured on post-vaccination day 10; thus, this study was cross-sectional in design. Associations were estimated as the \( \log_{10} \)-linear relationship between serum PFAS levels and percentage change in anti-diphtheria and anti-tetanus toxoid antibody titers from days 4 to 10, with PFAS and antibody concentrations modeled on the \( \log_{10} \) scale. A significant inverse association was detected between a doubling in serum PFOS concentration and percentage change in anti-tetanus toxoid antibody titer (\(-11.90\%, \) 95% CI = \(-21.92\%, -0.33\%\)). Otherwise, no significant association was detected between a doubling in serum PFOS concentration and percentage change in anti-tetanus toxoid antibody titer (\(-3.59\%, \) 95% CI = \(-11.91\%, 5.51\%\)) or between a doubling in serum PFOA concentration and either outcome (diphtheria: \(-8.22\%, \) 95% CI = \(-20.85\%, 6.44\%; \) tetanus: \(0.23\%, \) 95% CI = \(-10.40\%, 12.1\%\)). These results were not adjusted for any potential confounders, but the authors stated that models adjusted for age and sex “showed similar results.”

As a whole, these four studies (Grandjean et al. 2012; Granum et al. 2013; Kielsen et al. 2015; Looker et al. 2014) do not provide consistent evidence of a significant association between PFOA or PFOS exposure and serological vaccine responses in general. Within each study, most estimated associations were statistically non-significant, and results were inconsistent by vaccine type and by outcome classification. Authors provided no a priori biological hypothesis to explain why PFOA or PFOS exposure would impair the antibody response to one vaccine type but not another. Some authors suggested that their results could be explained by different immunostimulatory effects of different vaccines, but they did not elaborate on this hypothesis or provide supporting mechanistic evidence.

Although Grandjean et al. (2012) found fairly consistent – albeit mostly statistically non-significant – intra-study associations between childhood serum PFOA and PFOS levels and poorer antibody responses against tetanus and diphtheria toxoids, associations with maternal prenatal serum PFOA and PFOS levels were inconsistent between vaccine types. Two studies were strengthened by their measurement of PFOA and PFOS levels prior to ascertaining vaccine response (Grandjean et al. 2012; Granum et al. 2013) and one had the additional advantage of collecting exposure and outcome information at two time points each (Grandjean et al. 2012). However, the variability in findings by timing of exposure and outcome measurement in the latter study (e.g. mostly non-significant associations with prenatal PFOA and PFOS concentrations, but several
significant associations between higher PFOA and PFOS concentrations at age 5 years and poorer vaccine response at age 7 years) makes the results difficult to interpret. This pattern of results could reflect a window of susceptibility in early childhood, but such an explanation remains conjectural. None of the studies demonstrated a clinically recognizable increased risk of infectious diseases as a consequence of a diminished vaccine response. Overall, although these results are not sufficient to establish a causal effect of PFOA or PFOS exposure on an impaired serological vaccine response, some of the positive associations are striking in magnitude and require replication in independent studies.

**Autoimmune and inflammatory conditions**

Two cross-sectional studies and one mixed retrospective/prospective cohort study evaluated whether PFOA and PFOS levels were associated with the prevalence of self-reported osteoarthritis (Innes et al. 2011; Steenland et al. 2015; Uhl et al. 2013), two cohort studies with both retrospective and prospective follow-up evaluated whether PFOA levels were related to risk of validated self-reported autoimmune disorders (Steenland et al. 2013, 2015), and one prospective birth cohort study assessed associations of prenatal or childhood PFOS levels with autoantibody levels in children (Osuna et al. 2014).

The earlier cross-sectional study of osteoarthritis was based on 3731 adults with osteoarthritis and 45 701 without osteoarthritis who lived, worked, or attended school in one of six PFOA-contaminated water districts in the Mid-Ohio Valley and were enrolled in the C8 Health Project. Significant positive associations were found between increasing serum PFOA levels and osteoarthritis prevalence ($P_{\text{trend}} = 0.00001; \text{OR per log-transformed 1-ng/mL increase in PFOA} = 1.07, 95\% \text{CI} = 1.04, 1.11$) (Table 5) (Innes et al. 2011). The observed association was limited to adults under age 55 years ($P_{\text{interaction}}$ by age $<0.00001$) and those who were not obese ($P_{\text{interaction}}$ by obesity status $= 0.0005$), and was not detected when the analysis was restricted to serum PFOA levels typical of the general U.S. population ($\leq 20 \text{ng/mL}$). By contrast, this study found a significant inverse association between serum PFOS levels and osteoarthritis prevalence ($P_{\text{trend}} = 0.00001; \text{OR per log-transformed 1-ng/mL increase in PFOS} = 0.88, 95\% \text{CI} = 0.84, 0.93$) that was not appreciably altered after stratification by age group or obesity status, or after restriction to relatively low PFOS levels.

To follow up on these findings, a cross-sectional study was conducted using 2003–2008 NHANES data from 3809 adults sampled to be representative of the general U.S. population, including 365 who reported ever having been diagnosed with osteoarthritis (Table 5) (Uhl et al. 2013). No significant association was detected between serum PFOA levels and osteoarthritis prevalence among men, but among women a positive exposure-response pattern was detected (OR per log-transformed 1-ng/mL increase in PFOA $= 1.35, 95\% \text{CI} = 1.02, 1.79$) and risk was significantly higher in the top quartile of PFOA than the bottom (OR $= 1.98, 95\% \text{CI} = 1.24, 3.19$). In contrast to the earlier study, this association was detected only among obese adults. Findings were also more pronounced among younger (ages 20–49 years) than older women. Also in contrast to the significant inverse association with PFOS reported by Innes et al. (2011), Uhl et al. (2013) reported a positive association (OR per log-transformed 1-ng/mL increase in PFOS $= 1.15, 95\% \text{CI} = 0.94, 1.40$; OR for top versus bottom quartile of PFOS $= 1.77, 95\% \text{CI} = 1.05, 2.96$), again with stronger associations in women than men and in younger (ages 20–49 years) than older women.

The occupational cohort study of osteoarthritis included 3713 workers at a West Virginia polymer plant who self-reported whether they were currently taking prescription medication for osteoarthritis (Steenland et al. 2015). No significant association was observed between estimated cumulative serum PFOA levels and medicated osteoarthritis ($n = 196$ cases) in either unlagged analyses (OR for highest versus lowest quartile $= 0.97, 95\% \text{CI} = 0.59, 1.59$, $P_{\text{trend}} = 0.92$ for continuous log PFOA, $P_{\text{trend}} = 0.48$ for categorical cumulative PFOA) or 10-year lagged analyses (OR for highest versus lowest quartile $= 0.67, 95\% \text{CI} = 0.39, 1.14$, $P_{\text{trend}} = 0.13$ for continuous log PFOA, $P_{\text{trend}} = 0.15$ for categorical cumulative PFOA).

The two cross-sectional studies of osteoarthritis cited an Australian study in which 81% of “definite” osteoarthritis (67/83), 57% of “possible” osteoarthritis (8/14), and 89% of “negative” osteoarthritis (8/9) self-reported by adults aged 45–64 years was validated by physical examination (March et al. 1998). However, the results of this small study of highly selected adult volunteers may not be generalizable to other populations; thus, the validity of self-reported osteoarthritis in these studies remains unclear. The occupational cohort study restricted the outcome to osteoarthritis currently treated with prescription medication with the goal of minimizing misclassification, but this restriction also limited osteoarthritis cases to a relatively severe subset of disease. Taken together, the results of these three studies (Innes et al. 2011; Steenland et al. 2015; Uhl et al. 2013) do not demonstrate a consistent association between serum PFOA or PFOS levels and osteoarthritis.
Key methodological limitations of the two cross-sectional studies, including the temporal ambiguity of exposure and outcome, reliance on PFOA and PFOS measured at a single point in time, absence of information on timing of osteoarthritis onset, and probably misclassified outcome data, preclude a causal interpretation of the observed positive associations and accord greater weight to the occupational cohort study, despite its use of estimated exposure levels and a relatively restrictive case definition.

One study of PFOA exposure and autoimmune diseases was based on 32,254 adults who were enrolled in the C8 Health Project or a separate study of workers at the nearby polymer manufacturing plant, and who completed a follow-up demographic and medical history survey that assessed whether respondents had ever been diagnosed with certain autoimmune diseases (Table 5) (Steenland et al. 2013). Cumulative PFOA exposure since 1952 (the year of first PFOA emissions from the polymer plant) was estimated for each subject based on the estimated intake of PFOA-contaminated drinking water, as well as occupational exposure for plant workers. In retrospective analyses, no significant associations were detected between estimated PFOA exposure and lifetime history of Crohn's disease (95 cases), rheumatoid arthritis (346 cases), insulin-dependent diabetes (160 cases), type I diabetes (85 cases), lupus (72 cases), or multiple sclerosis (99 cases). A significant positive association was detected between estimated PFOA exposure and lifetime history of ulcerative colitis (151 cases; OR for top versus bottom quartile with no exposure lag period = 2.86, 95% CI = 1.65, 4.96, $P_{trend} < 0.0001$; OR with 10-year lag = 3.05, 95% CI = 1.56, 5.96, $P_{trend} < 0.0001$). Estimates were comparable when person-time was counted from the first year of residence in the study area or the first year of employment at the plant, or when estimated background-level exposure to PFOA was excluded. In prospective analyses, positive but statistically non-significant ORs were detected between PFOA levels and incident ulcerative colitis newly diagnosed between C8 Health Project baseline in 2005–2006 and follow-up in 2008–2011, with no significant trend (30 cases; unlagged $P_{trend} = 0.21$, lagged $P_{trend} = 0.12$), and no significant association was detected with incident rheumatoid arthritis (56 cases). No a priori explanation was provided for why PFOA exposure might be associated with ulcerative colitis but not other autoimmune disorders.

Validated self-reported autoimmune diseases were also analyzed separately among the 3,713 polymer plant workers in this cohort study, with results reported for ulcerative colitis and rheumatoid arthritis (Steenland et al. 2015). Some evidence of a positive trend was observed between estimated cumulative serum PFOA and risk of rheumatoid arthritis with no lag (23 cases; OR for highest versus lowest quartile = 4.45, 95% CI = 0.99, 19.9, $P_{trend}$ for continuous log PFOA = 0.54, $P_{trend}$ for categorical PFOA = 0.04), although the association was attenuated with a 10-year lag (OR = 2.62, 95% CI = 0.47, 14.7, $P_{trend}$ for continuous log PFOA = 0.75, $P_{trend}$ for categorical PFOA = 0.06). For ulcerative colitis, no significant association was observed in the unlagged analysis (28 cases; OR for highest vs. lowest quartile of estimated cumulative PFOA = 2.74, 95% CI = 0.78, 9.65, $P_{trend}$ for continuous log PFOA = 0.05, $P_{trend}$ for categorical PFOA = 0.26), but a significant positive association was observed with a 10-year lag (OR = 6.57, 95% CI = 1.47, 29.40, $P_{trend}$ for continuous log PFOA = 0.05, $P_{trend}$ for categorical PFOA = 0.05).

Outcome validation in these studies (Steenland et al. 2013, 2015) was attempted only for positive self-reports. Approximately one-quarter of combined community members and workers did not grant consent for validation, and medical records were not obtained for approximately 8% of those who consented; among workers only, 17% of subjects who self-reported disease did not have medical records available for validation. Overall, only 34% of self-reported autoimmune disorders in the combined study and 44% in the occupational study were validated, and the rest were excluded from the analysis. Exposure misclassification is also a concern, given that cumulative PFOA exposure was estimated based on a model that was validated against measured serum PFOA levels in 2005–2006 (Spearman’s $r = 0.67$) (Shin et al. 2011), but serum measurements were not available in other years. However, exposure and outcome misclassification, and possibly selection and/or reporting bias, might be expected to lead to false associations with multiple autoimmune diseases. Therefore, the positive association with ulcerative colitis but not with other autoimmune disorders may not be due to bias alone. The sizeable, statistically significant ORs and exposure-response trends for ulcerative colitis in this large cohort study are noteworthy and require replication in independent study settings. Given that all of the subjects in the occupational study (Steenland et al. 2015) were also included in the combined study of community members and workers (Steenland et al. 2013), and the authors did not report whether the association was detected separately among community members, these two studies cannot be considered as mutually independent. Overall, the results from these studies do not establish an association between PFOA exposure and risk of any autoimmune disease, and the results for ulcerative colitis require independent confirmation.
A pilot prospective birth cohort study of 38 children in the Faroe Islands evaluated whether PFOA and PFOS levels measured in cord blood at birth and in serum at age 7 years were associated with serum concentrations of IgG and IgM autoantibodies against six neural proteins and three non-neural proteins in children at age 7 (Osuna et al. 2014). This study was based on the premise that increased autoantibodies might indicate tissue damage (and the subsequent release of self-antigens) following chemical exposure. Prenatal and age-7 PFOA levels were not significantly associated with any of the 18 autoantibodies measured (2 isotypes each of 9 autoantibodies). For PFOS most associations were also non-significant, except for a single significant inverse association between prenatal PFOS levels and anti-actin IgG levels at age 7 (−22% change in autoantibody concentration per 2-fold increase in cord blood PFOS, \( p \leq 0.05 \)). Given the numerous associations tested, the lack of adjustment for confounders, and the selection of subjects with available data, this single association—which would suggest a protective effect against tissue damage, but which the authors interpreted as potentially indicating an immunosuppressive effect—could well be a spurious finding, and its clinical relevance is unclear.

**Weight-of-evidence evaluation**

The ensuing sections provide an evaluation of the weight of epidemiologic evidence addressing the causal question of interest, that is, whether PFOA and PFOS are causally related to adverse immunological health conditions in humans. This evaluation was conducted using the framework of the Bradford Hill “viewpoints” (Hill 1965), which consist of strength of association, consistency, temporality, biological gradient, plausibility, coherence, specificity, experiment, and analogy. As stated by Sir Austin Bradford Hill, none of these nine viewpoints can prove or disprove a causal relationship, and none can be required as essential (except that an exposure must precede an effect temporally). Nevertheless, they provide a rational and convenient framework by which to assess the overall balance of epidemiologic evidence for or against a causal hypothesis.

**Strength of association**

In general, a strong association is less likely than a weak association to be explained by confounding or bias (Hill 1965). The strength of an association is not straightforward to evaluate when the exposure and outcome are analyzed on a continuous scale, for example, as when an association is reported as the per-unit increase in a biomarker per 1-nM/L increase in serum PFOA or PFOS. Nevertheless, most estimated coefficients for the relationship between circulating PFOA or PFOS concentrations and immune biomarkers were close to the null value of zero.

Most relative risk estimates for the association between increasing PFOA or PFOS exposure (whether analyzed as exposed versus unexposed, in tertiles or quartiles, on the \( \log_2 \) or \( \log_{10} \) scale, or per unit increase—scales that are not directly comparable) and atopic, infectious, and autoimmune and inflammatory disorders and anti-vaccine antibody seroconversion or seroprotection were between 0.5 and 2.0. Thus, in general, the strength of the observed associations does not offer compelling evidence in favor of a causal interpretation.

Stronger ORs were reported for 10-fold increases in maternal prenatal serum PFOA and PFOS concentration and development of food allergy or wheezing in young Japanese children (Okada et al. 2012), but these estimates were statistically unstable and non-significant. ORs above 2.0 were also reported for PFOA or PFOS exposure in association with asthma (Anderson-Mahoney et al. 2008; Dong et al. 2013; Granum et al. 2013); with inadequate anti-vaccine antibody concentrations at age 7 years (Grandjean et al. 2012); with seroprotection against influenza type A/H1N1 (Looker et al. 2014); and with ulcerative colitis (Steenland et al. 2013, 2015). These ORs warrant greater attention, although the studies in which they were detected could not convincingly exclude confounding or bias as explanations for these results, which also may be due to chance.

**Consistency**

Positive associations with only two immune conditions were detected in at least two independent studies. The prevalence of self-reported asthma was significantly positively associated with residence in a PFOA-contaminated water district (Anderson-Mahoney et al. 2008) and with concurrently measured serum PFOA in some but not all analyses of representative U.S. adolescents (Humblet et al. 2014). Additionally, risk of incident asthma was significantly positively associated with PFOA and PFOS levels in Taiwanese children (Dong et al. 2013). By contrast, no significant associations between PFOA or PFOS exposure and risk of asthma were detected in Norwegian children up to age 3 years (Granum et al. 2013), children from Greenland and Ukraine up to ages 5–9 years (Smit et al. 2015), or PFOA-exposed polymer production workers (Leonard et al. 2008; Steenland et al. 2015). The methodologically stronger studies in this group are the Taiwan study with physician-confirmed
incident asthma (Dong et al. 2013), the European studies with prospective exposure assessment and follow-up for asthma development (Granum et al. 2013; Smit et al. 2015), and the occupational cohort study with long-term cumulative exposure estimates (Steenland et al. 2015), although these studies still have important methodological limitations. Three of the four higher-quality studies found no significant association between PFOA or PFOS exposure and asthma risk. Thus, taken together, the results of these studies do not establish a consistent positive association between PFOA exposure and risk of asthma.

Two cross-sectional studies found a significant positive association between serum PFOA concentration and the prevalence of self-reported osteoarthritis (Innes et al. 2011; Uhl et al. 2013), whereas one occupational cohort study found no significant association (Steenland et al. 2015). The positive association was restricted to adults under 55 years and non-obese subjects in the study of Mid-Ohio Valley residents (Innes et al. 2011), whereas it was restricted to women and obese adults in the study of NHANES participants (Uhl et al. 2013). Thus, the subgroup-specific results were inconsistent between studies, as were the reported associations with PFOS, and no clearly consistent positive association between PFOA exposure and osteoarthritis risk is demonstrated by these studies.

Otherwise, no significant positive associations with any specific outcome (considering antibody responses to different vaccines as distinct outcomes) were reported in multiple studies. Instead, consistency across studies was observed mainly for null results, as detected for white blood cell count (Costa et al. 2009; Emmett et al. 2006b; Olsen et al. 2003), eosinophil count among non-asthmatic subjects (Dong et al. 2013; Emmett et al. 2006b), C-reactive protein (Costa et al. 2009; Lin et al. 2011), wheezing (Granum et al. 2013; Humblet et al. 2014; Okada et al. 2012, 2014; Smit et al. 2015), eczema (Granum et al. 2013; Okada et al. 2012; Smit et al. 2015) (although a significant inverse association with PFOA was found by Okada et al. (2014)), food allergy (Granum et al. 2013; Okada et al. 2012), and otitis media (Granum et al. 2013; Okada et al. 2012).

**Specificity**

No specific relationship was apparent between PFOA or PFOS and any particular immune condition. However, the overall lack of specificity does not provide evidence for or against a causal conclusion (Hill, 1965). In the study by Steenland et al. (2013), the specific relationship of estimated PFOA exposure with prevalent ulcerative colitis but not five other autoimmune disorders is unlikely to be explained by bias particular only to ulcerative colitis. However, the lack of a significant association with prospectively ascertained incident ulcerative colitis in that study may argue against a causal interpretation of the association detected in the retrospective analysis.

**Temporality**

The issue of temporality was discussed earlier in the section on exposure assessment. Eleven of the reviewed studies were cross-sectional or retrospective in design, and were therefore unable to establish whether the exposure preceded the outcome (Anderson-Mahoney et al. 2008; Costa et al. 2009; Dong et al. 2013; Emmett et al. 2006b; Humblet et al. 2014; Innes et al. 2011; Kielsen et al. 2015; Lin et al. 2011; Looker et al. 2014; Olsen et al. 2003; Uhl et al. 2013). The remaining studies measured or estimated circulating PFOA and/or PFOS levels before ascertaining immunological endpoints (Ashley-Martin et al. 2015; Fei et al. 2010; Grandjean et al. 2012; Granum et al. 2013; Okada et al. 2012, 2014; Osuna et al. 2014; Pennings et al. 2015; Smit et al. 2015; Steenland et al. 2015; Steenland et al. 2013; Wang et al. 2011) or, in the case of the retrospective cohort study, evaluated occupational PFOA exposure prior to death (Leonard et al. 2008). Thus, associations detected in the second set of studies are temporally sequenced in a manner consistent with a potential causal effect. However, given the substantial uncertainty about the magnitude of changes in circulating PFOA and PFOS levels and some immune conditions over time, and whether there exist certain time windows of exposure susceptibility (and, if so, whether study exposures were measured during those intervals), the prospective measurement of PFOA or PFOS concentrations does not substantially augment the quality of a study’s evidence for or against a causal interpretation.

**Biological gradient**

Regression models in which circulating PFOA and PFOS concentrations are analyzed on a continuous scale are based on the assumption of a linear or log-linear exposure-response gradient. Similarly, Pearson’s correlation coefficients measure the strength of the assumed linear relationship between continuous variables. Studies that used these approaches generally did not explicitly test the shape of the exposure-response curve. Only Ashley-Martin et al. (2015) reported that they explicitly tested the linearity of associations using restricted cubic spline models, and they found no significant non-linear associations between PFOA or
PFOS and immune biomarkers. In addition, several other studies classified PFOA or PFOS levels into ordinal categories and tested for the presence of a monotonic exposure-response trend across those categories. These studies are described below.

Okada et al. (2012) found a curvilinear, overall inverse relationship between maternal prenatal serum PFOA and total IgE in cord serum among girls, but not boys. By contrast, Dong et al. (2013) reported significant positive trends between serum PFOA and PFOS quartiles and serum total IgE, absolute eosinophil count, and eosinophil cationic protein among children with asthma, but not those without asthma. In addition, the same authors observed significant positive exposure-response trends between serum PFOA and PFOS quartiles and risk of asthma and, for PFOS, asthma severity. However, no significant trend between tertiles of serum PFOA or PFOS and ever or current asthma was reported by Humblet et al. (2014). Okada et al. (2014) detected significant inverse exposure-response trends between maternal prenatal plasma PFOA (but not PFOS) quartiles and risk of combined eczema, wheezing, and allergic rhinoconjunctivitis symptoms (especially among girls) and eczema alone in early childhood.

Fei et al. (2010) reported significant positive trends between maternal prenatal plasma PFOA and PFOS quartiles and hospitalization for infectious diseases in early childhood among girls, but the opposite trends were found among boys, resulting in a canceling out of trends when both sexes were combined. Looker et al. (2014) detected inverse trends between quartiles of serum PFOA and post-vaccination titer increase and the odds of seroconversion for anti-influenza type B antibody, but not other classifications of this outcome. However, an opposite trend in the positive direction was seen for serum PFOA quartiles and the odds of seroconversion and seroprotection for anti-influenza type A/H1N1 antibody, and no apparent monotonic trends were seen between serum PFOA quartiles and various classifications of the antibody response to influenza type A/H3N2 vaccination. Serum PFOS quartiles did not appear to exhibit any exposure-response gradients with respect to the antibody response to these three vaccine types.

Innes et al. (2011) observed a significant positive trend between quartiles of serum PFOA and the prevalence of osteoarthritis, especially among adults who were younger than 55 years and not obese, countered by a significant inverse trend with serum PFOS that was not modified by age or obesity status. By contrast, increasing quartiles of serum PFOA and PFOS were not associated with monotonic change in the prevalence of osteoarthritis in the study by Uhl et al. (2013). Steenland et al. (2013) found that the risk of ever having been diagnosed with ulcerative colitis rose significantly in parallel with increasing quartiles of estimated cumulative PFOA exposure, but the risk of newly diagnosed ulcerative colitis did not, nor did lifetime history of Crohn’s disease, rheumatoid arthritis, insulin-dependent or type 1 diabetes, lupus, or multiple sclerosis, or incident rheumatoid arthritis. Positive exposure-response trends between 10-year lagged cumulative PFOA exposure and ulcerative colitis, as well as positive trends with rheumatoid arthritis, were also reported by Steenland et al. (2015) in a subset of workers also included in the 2013 study. Other outcomes, including C-reactive protein (Lin et al. 2011), atopic dermatitis (Wang et al. 2011), wheezing (Humblet et al. 2014; Okada et al. 2014), and flu infections and colds (Looker et al. 2014) were found not to increase or decrease significantly in association with rising categories PFOA or PFOS exposure.

Thus, although some significant increasing and decreasing exposure-response gradients were found for PFOA and PFOS with respect to various immune conditions, thereby offering evidence consistent with a causal mechanism, none were replicated across studies or within studies that evaluated multiple comparable outcomes. If sufficient data were available, it might also be informative to compare results from occupationally exposed workers, who have the highest PFOA and PFOS exposure levels, with results from the Mid-Ohio Valley, where PFOA exposure levels are comparable to background levels in PFOA plants, and results from general populations, in which PFOA exposure levels are another order of magnitude lower (Chang et al. 2014). However, the lack of comparability in outcomes evaluated across studies, as well as the small number of studies of any given outcome, precludes such an analysis.

**Plausibility and coherence**

Experimental evidence of immunotoxic effects of PFOA and PFOS in laboratory animals was briefly summarized earlier in this review. In the absence of an established mode of action/adverse outcome pathway, it is unclear whether any of these findings can be translated to humans and especially whether they are relevant to clinically measurable human immunological disorders. Synthesis of IgM, the main immunologic outcome suppressed by PFOA in experimental animals (Corsini et al. 2014; DeWitt et al. 2012), does not play a major role in vaccine-related immunity, which is mediated mainly by IgG. Thus, although animal evidence suggests that it is biologically plausible that PFOA and PFOS could adversely affect some aspects of immune function in humans, it is also plausible that effects observed in
Experiment

The 3M Company, a major international producer of PFOA and PFOS, phased out the manufacture and use of PFOA by 2002 and PFOA by 2008 (U.S. EPA 2014), and the world’s eight major fluoropolymer and telomere manufacturers agreed to reduce PFOA emissions and product content by 95% no later than 2010 and completely by 2015 (U.S. EPA 2006). Consequently, PFOS serum levels in the U.S. population have declined steadily since 1999–2000, around the time that the 3M phase-out began, and PFOA serum levels have also declined since that time (except in 2007–2008) (CDC 2015). These circumstances create a natural experiment by which one might examine whether the incidence of certain immune conditions decreased in parallel with documented declines in serum PFOS levels in the U.S. population (Kato et al. 2011; Olsen et al. 2012), and whether future anticipated declines in serum PFOA levels might also be reflected in lower rates of immune disorders. However, due to the lack of population-based surveillance for the immune conditions of interest in this review, as well as the high potential for confounding by other time-dependent risk factors that may have changed since the early twentieth century, such ecologic data are unavailable to offer any evidence concerning a causal hypothesis.

Analogy

Numerous analogies can be drawn to other chemicals that cause immune disorders in humans, but just as many can be drawn to other chemicals that do not. Such analogies are tenuous and are not helpful for clarifying the relationship of PFOA and PFOS to these conditions.

Conclusions

Based on a maximum of only seven epidemiologic studies of any particular condition (asthma) and a body of literature with major methodological limitations, an evaluation of the weight of epidemiologic evidence according to the Bradford Hill viewpoints reveals generally weak associations, no specific endpoints with consistent findings across all relevant studies, uncertainty about any critical duration of exposure and window(s) of susceptibility, mixed exposure-response trends, and a dearth of supportive animal and mechanistic data. Thus, the available evidence is insufficient to conclude that a causal relationship has been established between PFOA or PFOS exposure and any immune condition in humans. Most existing studies were cross-sectional or retrospective in design, evaluated PFOA and/or PFOS exposure at a single point in time, and relied upon self-reported health outcomes. Going forward, rigorously designed epidemiologic studies are needed to shed new light on whether or not PFOA and PFOS cause human immune disorders, and priority should be given to conditions with suggestive positive results in the available studies (e.g. ulcerative colitis). When a clear link with clinical disease is lacking, as in the case of subtle changes in immune biomarkers, immune system impairment cannot be presumed. However, such evidence might inform areas for additional research on whether a mechanistic tie exists with clinically recognizable health effects. In some study settings, linkages to high-quality disease registries or administrative health databases may enable connections to be drawn with clinical outcomes.

As indicated throughout this review, greater knowledge is needed on the magnitude and timing of intra-individual variation in circulating PFOA and PFOS levels. If serum or plasma levels of these chemicals are shown to fluctuate over time, then studies should incorporate repeated sampling to accurately capture variation in the intensity, duration, timing, and trajectory of individual-level exposure. For example, in prospective birth cohort studies, multiple samples could be drawn during gestation and, if immune conditions are assessed in children rather than infants, during childhood.

In addition, to enable adequate control of confounders, more research is needed to identify the determinants of personal PFOA and PFOS exposure, and studies should collect sufficiently detailed data on these determinants to permit statistical adjustment. For example, factors that have been shown to affect serum PFAS concentrations in the general population, and that could confound associations with certain health outcomes, include age, sex, race/ethnicity, education, income, body size, tap water consumption and filtering, diet and total energy intake, cooking methods, smoking status, alcohol intake, caffeine consumption, and pregnancy and breastfeeding status (Calafat et al. 2007b; Emmett et al. 2006a; Eriksen et al. 2011; Jain 2013, 2014; Nelson et al. 2012; Tyrrell et al. 2013).

Other design features that will help to produce more informative data include prospective follow-up to clarify the temporal relationship between exposure and outcome and reduce information bias; collection of
complete, accurate, reliable, and clinically relevant health outcome data using medical records or direct assessment with validated and standardized tools; and enrollment of large numbers of subjects with sufficient variation in exposure levels to enable detection of any corresponding health differences. In addition, a priori hypotheses and post hoc exploratory analyses should be explicitly described, null results should be fully reported, and the potential influence of bias and chance should be thoughtfully considered, along with quantitative sensitivity analyses to the extent possible. These improvements will help to clarify whether the lack of persuasive evidence is due to a shortage of well-designed epidemiologic studies or the absence of a true demonstrable immunotoxic effect of these chemicals. At present, however, the epidemiologic evidence does not establish a causal effect of PFOA or PFOS on immune conditions in humans.

Acknowledgements

We thank Ms. Laura Elkayam (Exponent, Inc.) for her review of the text and tables for accuracy.

Declaration of interest

This manuscript was supported by the 3M Company. 3M was not involved in the preparation of the manuscript. The sponsors were provided the opportunity to review draft versions and to offer suggestions, whose inclusion in the manuscript was left to the discretion of the authors. The authors retained sole control of the manuscript content and the findings, and statements in this paper are those of the authors and not those of the author’s employer or the sponsors.

No authors were directly compensated by 3M. This project was funded through a contract between 3M and Exponent, an international science and engineering company that provided salary compensation to J.S.M. and E.T.C. H.O.A. and P.B. were compensated with consulting fees from Exponent and had no direct correspondence with 3M related to this manuscript. H.J.W. was not financially compensated for his work on the manuscript. The team of authors was assembled based on existing professional connections (between E.T.C., H.O.A., P.B., and J.M.) and a colleague’s referral to an expert in clinical immunology (H.J.W.).

H.J.W. declares no conflict of interest related to the subject of this manuscript. J.S.M. was a consultant to 3M and previously testified as an expert in legal proceedings related to PFOA and PFOS; he is now retired. E.T.C. is a consultant to 3M and other industry clients on issues related to perfluoroalkyl and polyfluoroalkyl substances, including PFOA and PFOS. P.B. was a consultant to other industry clients on issues related to PFOA. J.S.M., E.T.C., P.B., and H.O.A. co-authored a peer-reviewed, published manuscript on PFOA, PFOS, and cancer that was financially supported by 3M. None of the authors are currently engaged to testify as experts on behalf of the sponsors in litigation related to the compounds discussed in this manuscript.

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