NTP | National Toxicology Program
U.S. Department of Health and Human Services

NTP Monograph

Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid or Perfluorooctane Sulfonate

September 2016

National Toxicology Program
U.S. Department of Health and Human Services

NTP MONOGRAPH ON IMMUNOTOXICITY ASSOCIATED WITH EXPOSURE TO PERFLUOROOCTANOIC ACID (PFOA) OR PERFLUOROOCTANE SULFONATE (PFOS)

September, 2016

Office of Health Assessment and Translation

Division of the National Toxicology Program

National Institute of Environmental Health Sciences

National Institutes of Health

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

TABLE OF CONTENTS

LIST OF TABLE AND FIGURES

TABLES:

FIGURES:

DATA FIGURES:

ABSTRACT

Although emissions of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been dramatically reduced, the persistence and bioaccumulation of these chemicals result in detectable levels in the U.S. population. Despite declining emissions, there is continued widespread exposure to both chemicals and a number of studies have reported potential PFOA- and PFOS-associated immunotoxicity in both humans and non-human animals. The NTP conducted a systematic review to evaluate the evidence on exposure to PFOS or PFOA and immune-related health effects to determine whether exposure to either chemical is associated with immunotoxicity for humans. The literature search and screening process identified 33 human studies, 93 animal studies, and 27 *in vitro*/mechanistic studies relevant for addressing the objective. The health effects data for PFOA and PFOS were considered separately in developing hazard identification conclusions. Conclusions for each chemical were reached by integrating evidence from human and animal studies with consideration of any mechanistic data.

The NTP concludes that PFOA is *presumed to be an immune hazard to humans* based on a high level of evidence that PFOA suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans. Although the strongest evidence for an effect of PFOA on the immune system is for suppression of the antibody response, there is additional, although weaker, evidence that is primarily from epidemiological studies that PFOA reduced infectious disease resistance, increased hypersensitivity-related outcomes, and increased autoimmune disease incidence. The evidence indicating that PFOA affects multiple aspects of the immune system supports the overall conclusion that PFOA alters immune function in humans. However, the mechanism(s) of PFOA-associated immunotoxicity is not clearly understood and effects on diverse endpoints such as suppression of the antibody response and increased hypersensitivity may be unrelated.

The NTP concludes that PFOS is *presumed to be an immune hazard to humans* based on a high level of evidence that PFOS suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans. Although the strongest evidence for an effect of PFOS on the immune system is for suppression of the antibody response, there is additional, although weaker, evidence that is primarily from studies in experimental animals that PFOS suppresses disease resistance and natural killer (NK) cell activity. The evidence indicating that PFOS suppresses multiple aspects of the immune system supports the overall conclusion that PFOS alters immune function in humans. Although the mechanism(s) of PFOS-associated immunotoxicity is not clearly understood, suppression of the antibody response and NK cell function are both potential mechanisms by which PFOS may reduce disease resistance.

PEER REVIEW OF THE DRAFT NTP MONOGRAPH

Peer review of the draft NTP Monograph was conducted by an *ad hoc* expert panel in a public meeting held July 19, 2016, at the National Institute of Environmental Health Sciences, Research triangle park and via WebEx (see http://ntp.niehs.nih.gov/go/37090 for meeting materials and peer review report). The selection of panel members and conduct of the peer review were performed in accordance with the Federal Advisory Committee Act and Federal policies and regulations. The panel members served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members had two major responsibilities in reviewing the draft NTP Monograph: (1) to determine whether the scientific information cited in the draft monograph was technically correct and clearly stated, and whether NTP has objectively presented and assessed the scientific evidence; (2) to determine whether the scientific evidence presented in the draft monograph supported the NTP's conclusions regarding whether immunotoxicity is associated with exposure to PFOA or PFOS.

The panel agreed with the draft conclusions that PFOA and PFOS are both *presumed to be immune hazards to humans*; however, they recommended changing the level of evidence conclusion for hypersensitivity-related outcomes for the animal body of evidence from "high" to "moderate". NTP concurred with this recommendation. Comments from the peer reviewers and written public comments were considered during finalization of the document.

Peer-Review Panel

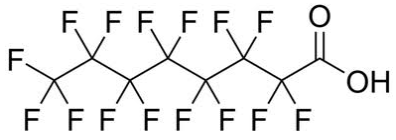
Name	Affiliation
Weihsueh Chiu, PhD (chair)	College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX
Joseph Braun, PhD	Department of Epidemiology, School of Public Health, Brown University, Providence, RI
Emanuela Corsini, PhD	School of Pharmacy, Università degli Studi di Milano, Milano, Italy
Berit Granum, PhD	Department of Food, Water, and Cosmetics Norwegian Institute of Public Health, Oslo, Norway
Deborah Keil, PhD, DABT	Department of Microbiology and Immunology, Montana State University, Bozeman, MT
Michael Woolhiser, PhD	Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI

INTRODUCTION

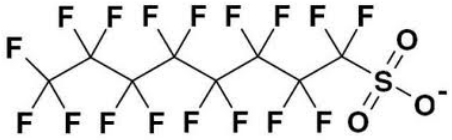
The NTP's Office of Health Assessment and Translation (OHAT) conducted a systematic review to evaluate the evidence that exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS) is associated with immune-related health effects. This review was initiated because there are studies reporting immune-related health effects of PFOA and PFOS in both humans and animals [e.g., epidemiological studies of the antibody response to vaccines (Grandjean *et al.* 2012, Granum *et al.* 2013, Looker *et al.* 2014), experimental animal studies of both innate and adaptive immunity (reviewed in DeWitt *et al.* 2012) and wildlife studies of infectious disease (Kannan *et al.* 2006, Kannan *et al.* 2010)], and the observation that the general U.S. population has detectable blood levels of these chemicals (CDC 2015) despite actions that have substantially reduced emissions.

PFOA and PFOS are extremely persistent chemicals (**Figure 1**) that are widely distributed in the environment as a result of extensive use over the last 50 years in commercial and industrial applications including fluoropolymer manufacturing, food packaging, lubricants, water-resistant coatings, and aqueous fire-fighting foams. They have high chemical stability and are not expected to degrade under typical environmental conditions (Lau *et al.* 2007, EFSA 2008, ATSDR 2009, US EPA 2014b, ATSDR 2015, US EPA 2016b, a). Once in surface water, apparent half-lives of PFOS and PFOA are 41 and 92 years respectively. Estimated half-lives in the human body are also long, ranging from 2 to 8 years (ATSDR 2009, Steenland *et al.* 2010, US EPA 2014b).

Figure 1. Structure of PFOA and PFOS



perfluorooctanoic acid (PFOA; CAS# 335-67-1)



perfluorooctane sulfonate (PFOS; CAS# 1763-23-1)

Through voluntary agreements, the primary manufacturer of PFOS phased out production in 2002 and PFOS is no longer manufactured in the United States (US EPA 2006, ATSDR 2009, US EPA 2009, 2014b, ATSDR 2015, US EPA 2015). Similar arrangements were made for PFOA and eight companies that manufactured PFOA in the United States eliminated emissions and product content by the end of 2015 (US EPA 2006, ATSDR 2009, US EPA 2013, 2014b, ATSDR 2015, US EPA 2015).

Although emissions have been dramatically reduced in the United States and Western Europe, it is not clear if global production has changed as there has been a shift in production and use of long-chain perfluoroalkyl acids (PFAAs) including PFOA and PFOS to emerging economies in continental Asia (Wang *et al.* 2014, Li *et al.* 2015, OECD 2015). The persistence and presence of both PFOA and PFOS as drinking water contaminants (e.g., reviewed in Lindstrom *et al.* 2011, Post *et al.* 2012) along with bioaccumulation result in detectable levels in the U.S. population and therefore they remain of public health concern (US EPA 2014b). PFOA and PFOS were present in all serum samples tested for perfluorinated compounds from the general U.S. population in the 1999 National Health and Nutrition Examination Survey (NHANES 1999-2000) (Calafat *et al.* 2007). While blood levels have declined from 1999 to 2012, PFOA (from 5.2 to 2.08 ng/ml or µg/L geometric mean) and PFOS (from 30.4 to 6.3 ng/ml geometric mean) remain the two highest concentrations among perfluorinated compounds (PFCs) or more specifically the PFAAs) measured in the general U.S. population in the most recent National Report on Human Exposure to Environmental Chemicals for 2009-2012 (CDC 2015). There are also regions in the United States such as Washington County Minnesota where higher levels of PFOA and PFOS have

been detected in drinking water and serum samples from community members (Landsteiner *et al.* 2014), and the Ohio Valley where residents and workers have higher serum PFOA concentrations (33.74 ng/ml geometric mean or ~4- to 10-times the general US population) due to high levels of PFOA in drinking source water contaminated by fluorochemical production facilities (e.g., C8 studies Steenland *et al.* 2010).

Given the widespread exposure to PFOA and PFOS and the availability of data to evaluate immunotoxicity in both humans and non-human animals, this systematic review was developed to evaluate the evidence of PFOA- and PFOS-associated immunotoxicity.

OBJECTIVE AND SPECIFIC AIMS

Objective

The overall objective of this evaluation is to undertake a systematic review to develop NTP hazard identification conclusions on the association between exposure to PFOA or PFOS (or their salts) and immunotoxicity based on integrating levels of evidence from human and non-human animal studies with consideration of the degree of support from mechanistic data.

Specific Aims

- Identify literature reporting the effects of PFOA or PFOS exposure on immune endpoints in humans, animals (experimental and wildlife), or *in vitro* model systems.
- Extract data on immune health effects from relevant studies.
- Assess the internal validity (risk of bias) of individual studies.
- Summarize the extent of evidence available.
- Synthesize the evidence using a narrative approach or meta-analysis (if appropriate) considering limitations on data integration such as study design heterogeneity.
- Rate the confidence in the body of evidence for human and animal studies separately according to one of four statements: (1) High, (2) Moderate, (3) Low, or (4) Very Low/No Evidence Available.
- Translate confidence ratings into level of evidence of health effects for human and animal studies separately according to one of four statements: (1) High, (2) Moderate, (3) Low, or (4) Inadequate.
- Combine the level of evidence ratings for human and animal data and consider the degree of support from mechanistic data to reach one of five possible hazard identification conclusions: (1) Known, (2) Presumed, (3) Suspected, (4) Not classifiable, or (5) Not identified to be an immune hazard to humans.
- Describe limitations of the systematic review, limitations of the evidence base, identify data gaps and key research needs, and describe findings in the context of human exposure levels.

METHODS

Problem Formulation and Protocol Development

The research question and specific aims stated above were developed and refined through a series of problem formulation steps including: (1) review of the topic by the evaluation design team and other technical experts with backgrounds in immunotoxicology, PFOA and PFOS, and systematic review; (2) deliberation with NTP staff and consultation with scientists at other Federal agencies represented on the NTP Executive Committee[1]; (3) comments received on the draft case-study protocol posted for public comment April 9, 2013 (http://ntp.niehs.nih.gov/go/36501); (4) public review of the concept for "Evaluation of Immunotoxicity Associated with Exposure to PFOA or PFOS" at the December 10, 2014 meeting of the NTP Board of Scientific Counselors (http://ntp.niehs.nih.gov/go/9741); (5) guidance outlined in the OHAT Handbook for Conducting a Literature-Based Health Assessment (NTP 2015b); and (6) external peer-review of the draft protocol. The protocol was posted in June 2015 (http://ntp.niehs.nih.gov/go/749926) and used to conduct this review. A brief summary of the methods is presented below.

PECO Statements

PECO (**P**opulation, **E**xposure, **C**omparators and **O**utcomes) statements were developed as an aid to identify search terms and inclusion/exclusion criteria as appropriate for addressing the research question for the systematic review (Higgins and Green 2011). The PECO statements are listed below for the human and animal studies (**Table 1**) and the *in vitro*/mechanistic studies (**Table 2**).

For the evaluation of immunotoxicity associated with PFOA or PFOS exposure, the evaluation focused on primary immune outcomes (i.e., immune function and immune disease data) from studies in humans, animals, or *in vitro* exposures because primary outcomes are more predictive of an immune-related health effect. The evaluation also collected information on secondary immune outcomes (i.e., observational immune data or data on upstream indicators that are less predictive of immune-related health effects) that were used to provide supportive evidence.

Many of the studies on secondary immune outcomes provide data that are relevant for potential mechanisms of immune-related health effects. Mechanistic data can come from a wide variety of studies that are not intended to identify a disease phenotype. This source of experimental data includes *in vitro* and *in vivo* laboratory studies directed at cellular, biochemical, and molecular mechanisms that explain how a chemical produces particular adverse health effects.

[1] Consumer Product Safety Commission (CPSC), Department of Defense (DoD), Environmental Protection Agency (EPA), Food and Drug Administration (FDA), National Cancer Institute (NCI), National Center for Environmental Health/Agency for Toxic Substances and Disease Registry (NCEH/ATSDR), National Institute of Environmental Health Sciences (NIEHS), National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration (OSHA) http://ntp.niehs.nih.gov/go/163

Table 1. Human and Animal PECO (Population, Exposure, Comparator and Outcome) Statement	
Element	**Evidence**
Population	Humans or animals (experimental and wildlife) without restriction based on age or sex
Exposure	Exposure to PFOA (CAS# 335-67-1) and PFOS (CAS# 1763-23-1) based on administered dose or concentration, biomonitoring data (e.g., urine, blood, or other specimens), environmental measures (e.g., air, water levels), or (humans only) indirect measures such as job title
Comparators	*Humans:* Comparable populations exposed to lower levels of PFOA or PFOS *Animals:* Comparable animal populations exposed to vehicle-only treatment in experimental animal studies or lower levels of PFOA or PFOS in wildlife studies
Outcomes	**Primary outcomes:** *Humans:* Immune-related diseases and measures of immune function: immunosuppression (e.g., otitis, infections, or decreased vaccine antibody response); hypersensitivity-related outcomes (e.g., atopic dermatitis or asthma); autoimmunity (e.g., thyroiditis) *Animals:* Disease resistance assays (e.g., host resistance to influenza A) or Immune function assays following *in vivo* exposure to PFOA or PFOS (e.g., antibody response [T-cell dependent IgM antibody response], natural killer cell [NK] activity, delayed-type hyper-sensitivity [DTH] response, phagocytosis by monocytes, local lymph-node assay [LLNA]) **Secondary outcomes:** *Humans or animals:* Observational immune endpoints (e.g., lymphocyte counts, lymphocyte proliferation, cytokine levels, serum antibody levels, serum autoantibody levels); or immunostimulation (e.g., unintended stimulation of humoral immune function)

Table 2. *In Vitro*/mechanistic PECO (Population, Exposure, Comparator and Outcome) Statement	
Element	**Evidence**
Population	Human or animal cells, tissues or model systems with *in vitro* exposure regimens
Exposure	Exposure to PFOA (CAS# 335-67-1) and PFOS (CAS# 1763-23-1) based on administered dose or concentration
Comparators	Comparable cells or tissues exposed to vehicle-only treatment or untreated controls
Outcomes	**Primary outcomes:** Immune function assays following *in vitro* exposure to PFOA or PFOS (e.g., natural killer cell [NK] activity, phagocytosis or bacterial killing by monocytes, proliferation following anti-CD3 antibody stimulation of lymphocytes) **Secondary outcomes:** Observational immune endpoints *in vitro* exposure to PFOA or PFOS (e.g., general mitogen-stimulated lymphocyte proliferation, cytokine production)

Literature Search

Search terms were developed to identify all relevant published evidence on immunotoxicity or immune-related health effects potentially associated with exposure to PFOA or PFOS by (1) reviewing Medical Subject Headings for relevant and appropriate immune terms, (2) extracting key immune health effects and immunotoxicity terminology from reviews and a sample of relevant primary data studies, (3) use of the chemical-specific search terms for PFOA from a draft systematic review of developmental PFOA exposure and fetal growth (Johnson *et al.* 2013, Koustas *et al.* 2013), and adaptation of the chemical-

specific PFOA search strategy to generate search terms for PFOS. A combination of relevant subject headings and keywords were subsequently identified. A test set of relevant studies was used to ensure the search terms retrieve 100% of the test set. The following 9 electronic databases were searched using a search strategy tailored for each database (specific search terms used for the PubMed search are presented in **Appendix 1**; the search strategy for other databases are available in the protocol http://ntp.niehs.nih.gov/go/749926). No language restrictions or publication year limits were imposed, and databases were searched October of 2014 and 2015, with a final updated search on May 18, 2016.

Databases Searched

- Cochrane Library
- EMBASE
- PubChem
- PubMed
- Scopus
- Toxline
- Web of Science

Searching Other Resources

The reference lists of all included studies, relevant reviews, finalized or recent draft federal hazard assessments (US EPA 2005, ATSDR 2009, US EPA 2014c, a, ATSDR 2015, US EPA 2016a, b), commentaries, and other non-research articles were manually searched for additional relevant publications. NTP published a Request for Information about ongoing studies or upcoming publications on immune-related health effects of PFOA or PFOS in the Federal Register [80 FR 48886 (August 14, 2015)]. Studies identified by the public in response to the 2015 request for information or after posting of the protocol were also included.

Unpublished Data

Unpublished data were eligible for inclusion provided the owner of the data was willing to have the data made public and peer reviewed (see protocol for more details http://ntp.niehs.nih.gov/go/749926).

Study Selection

Evidence Selection Criteria

In order to be eligible for inclusion, studies had to satisfy eligibility criteria that reflect the PECO statements in **Table 1** and **Table 2**. The following additional exclusion criteria were applied: (1) studies only reporting concentrations of PFOA or PFOS in immune tissues are not considered immune outcomes; (2) articles without original data (e.g., editorials or reviews); (3) studies published in abstract form only (grant awards and conference abstracts); and (4) retracted articles.

Screening Process

References retrieved from the literature search were screened for relevance and eligibility against the evidence selection criteria using DistillerSR® (Evidence Partners; http://www.systematic-review.net). Screeners from the evaluation team were trained with an initial pilot phase on 25 studies undertaken to improve clarity of the evidence selection criteria and to improve accuracy and consistency among screeners. All references were independently screened by two trained screeners (one of which was the project lead) at the title and abstract level to determine whether a reference met the evidence selection criteria. Studies that were not excluded by reviewing the title and abstract were screened with a full-text

review. Screening conflicts were resolved through discussion. Following full-text review, the remaining studies were "included" and used for the evaluation.

Data Extraction

Extraction Process and Data Warehousing

Data were collected (i.e., extracted) from included studies by one member of the evaluation team and checked by a second member for completeness and accuracy. Any discrepancies in data extraction were resolved by discussion. Information that was inferred, converted, or estimated during data extraction is annotated, e.g., using brackets [n = 10]. The study extraction files note whether an attempt was made to contact study authors by email for missing data considered important for evaluating key study findings (and whether or not a response was received).

Data extraction was completed using ICF International's proprietary Dose Response Analytical Generator and Organizational Network (DRAGON) software[2] and exported to the Health Assessment Workspace Collaborative (HAWC) software, an open source and freely available web-based interface application, for visualization and warehousing.[3] Data extraction elements are listed separately for human, animal, and *in vitro* studies in the protocol (http://ntp.niehs.nih.gov/go/749926). The data extraction results for included studies are publicly available (https://hawcproject.org/assessment/57/) and can be downloaded in Excel format through HAWC.

Quality Assessment of Individual Studies

Risk of bias was assessed for individual studies using a tool developed by OHAT that outlines a parallel approach to evaluating risk of bias from human, animal, and *in vitro* studies to facilitate consideration of risk of bias across evidence streams with common terms and categories (NTP 2015a). The risk-of-bias tool is comprised of a common set of 11 questions that are answered based on the specific details of individual studies to develop risk-of-bias ratings for each question. Study design determines the subset of questions used to assess risk of bias for an individual study (**Table 3**).

Assessors were trained with an initial pilot phase undertaken to improve clarity of rating criteria and to improve consistency among assessors. Studies were independently evaluated by two trained assessors who answered all applicable risk-of-bias questions with one of four options in **Table 4** following pre-specified criteria detailed in the protocol (http://ntp.niehs.nih.gov/go/749926). The criteria describe aspects of study design, conduct, and reporting required to reach risk-of-bias ratings for each question and specify factors that can distinguish among ratings (e.g., what separates "definitely low" from "probably low" risk of bias).

Several risk-of-bias questions were considered Key Questions or key elements in evaluating the studies because the questions may have greater impact on the overall bias. There were three Key Questions for observational human studies: confounding, exposure characterization, and outcome assessment. There were also three Key Questions for experimental animal studies: randomization, exposure characterization, and outcome assessment.

---

[2] DRAGON (Dose Response Analytical Generator and Organizational Network) developed by ICF International (http://www.icfi.com/insights/products-and-tools/dragon-online-tool-systematic-review).

[3] HAWC (Health Assessment Workspace Collaborative): A Modular Web-based Interface to Facilitate Development of Human Health Assessments of Chemicals (https://hawcproject.org/portal/).

Table 3. OHAT Risk-of-Bias Questions and Applicability by Study Design

Risk-of-Bias Questions	Experimental Animal*	*In Vitro* Experimental Studies	Human Controlled Trials**	Cohort	Case-Control	Cross-Sectional***	Case Series
1. Was administered dose or exposure level adequately randomized?	X	X	X				
2. Was allocation to study groups adequately concealed?	X	X	X				
3. Did selection of study participants result in the appropriate comparison groups?				X	X	X	
4. Did study design or analysis account for important confounding and modifying variables?				X	X	X	X
5. Were experimental conditions identical across study groups?	X	X					
6. Were research personnel blinded to the study group during the study?	X	X	X				
7. Were outcome data complete without attrition or exclusion from analysis?	X	X	X	X	X	X	
8. Can we be confident in the exposure characterization?	X	X	X	X	X	X	X
9. Can we be confident in the outcome assessment (including blinding of outcome assessors)?	X	X	X	X	X	X	X
10. Were all measured outcomes reported?	X	X	X	X	X	X	X
11. Were there no other potential threats to internal validity?	X	X	X	X	X	X	X

*Experimental animal studies are controlled exposure studies. Non-human animal observational studies can be evaluated using the design features of observational human studies such as cross-sectional study design.

**Human Controlled Trials are studies in humans with controlled exposure (e.g., Randomized Controlled Trials, non-randomized experimental studies)

***Cross-sectional studies include population surveys with individual data (e.g., NHANES) and surveys with aggregate data (i.e., ecological studies).

Any discrepancies in ratings between assessors were resolved through discussion to reach the final recorded risk-of-bias rating for each question along with a statement of the basis for that rating. Members of the evaluation team were consulted for assistance if additional expertise was necessary to reach final risk of bias ratings based on specific aspects of study design or performance reported for individual studies. Information or study procedures that were not reported is assumed not to have been conducted, resulting in an assessment of "probably high" risk of bias. Authors were queried by email to obtain missing information and responses received were used to update risk-of-bias ratings.

Table 4. The Four Risk-of-Bias Rating Options	
Answers to the risk-of-bias questions result in one of the following four risk-of-bias ratings	
++	***Definitely Low*** *risk of bias*: There is direct evidence of low risk-of-bias practices
+	***Probably Low*** *risk of bias*: There is indirect evidence of low risk-of-bias practices **OR** it is deemed that deviations from low risk-of-bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias
– NR	***Probably High*** *risk of bias*: There is indirect evidence of high risk–of-bias practices (indicated with "-") **OR** there is insufficient information provided about relevant risk-of-bias practices (indicated with "NR" for not reported). Both symbols indicate probably high risk of bias.
––	***Definitely High*** *risk of bias*: There is direct evidence of high risk-of-bias practices

Organizing and Rating Confidence in Bodies of Evidence

Health Outcome and Endpoint Grouping by Three Main Categories of Immune Response

After data were extracted from all studies, the health effects results were grouped across studies to develop bodies of evidence or collections of studies with data on the same or related outcomes for the 3 main categories of immune response: (1) immunosuppression, (2) hypersensitivity-related outcomes and (3) autoimmunity (see **Table 5**). Within each of the main categories, the focus for evaluating the evidence of immunotoxicity was on primary outcomes – the endpoints generally considered to have greater predictive value for overall immunotoxicity or a health effect (e.g., disease resistance assays and functional immune parameters)(WHO 1999). Outcomes that alone would be considered indirect evidence or less indicative of overall immunotoxicity (e.g., organ weights, cell counts, cytokine levels or other observational parameters) were considered secondary outcomes. Because there was sufficient evidence for health effects based on primary health outcomes, data on secondary outcomes were considered with the corresponding primary health effects to examine support for biological plausibility of those outcomes. **Table 5** lists representative endpoints or assays considered primary outcomes and secondary outcomes for each of these categories.

Table 5. Health Outcome Grouping and Identification of Primary and Secondary Outcomes			
	Humans	**Animals***	***In vitro* Assays**
Primary Outcomes	**Immune-related diseases and measures of immune function:** ***(1) Immunosuppression*** (e.g., otitis, infections, or decreased vaccine antibody response); ***(2) Hypersensitivity-related outcomes*** (e.g., atopic dermatitis asthma, total IgE, rhinitis); ***(3) Autoimmunity*** (e.g., thyroiditis or ulcerative colitis)	**Disease resistance assay or measures of immune function following *in vivo* exposure:** ***(1) Immunosuppression*** disease resistance assays (e.g., host resistance to influenza) or immune function assays (e.g., antibody response [T-cell dependent IgM antibody response (TDAR)], natural killer cell [NK] activity, delayed-type hypersensitivity [DTH] response, monocyte phagocytosis); ***(2) Hypersensitivity*** (e.g., airway resistance, local lymph-node assay); ***(3) Autoimmunity*** changes in incidence or progression in animal models of autoimmune disease	***Immune function assays following <u>in vitro exposure</u>:*** ***(1) Immunosuppression*** immune function assays (e.g., natural killer cell [NK] activity, phago-cytosis or bacterial killing by monocytes, proliferation following anti-CD3 antibody stimulation of spleen cells or lymphocytes)
Secondary	***Observational immune endpoints*** (e.g., lymphocyte counts, proliferation, cytokine levels, or serum antibody levels) ***Immunostimulation**** (e.g., unintended stimulation of humoral immune function)	***Observational immune endpoints*** (e.g., lymphoid organ weight, lymphocyte counts or subpopulations, lymphocyte proliferation, cytokine production, serum antibody levels, serum or tissue autoantibody levels, or histological changes in immune organs)	***Observational immune endpoints*** (e.g., general mitogen-stimulated lymphocyte proliferation, cytokine production)

* Note the evaluation considered experimental animal and observational animal studies (e.g., wildlife studies).
** Note that stimulation of the immune response is not adverse *per se*. It is generally agreed that stimulation of the immune system should not be disregarded (WHO 2012). Unintended immunostimulation was considered for possible hazard if there is consistent evidence for persistent elevated immune response.

This dichotomy separating the more from less predictive measures of immunotoxicity is consistent with testing strategies that rely on more sensitive and predictive immune assays (see Luster *et al.* 1992, US EPA 1996a, b, 1998) and the NTP and WHO methods to categorize the evidence of immune system toxicity. Under these systems, measures of immune function or the ability of the immune system to respond to a challenge are weighed more heavily than observational parameters (Germolec 2009, WHO 2012).

Considerations for Pursuing a Narrative or Quantitative Evidence Synthesis

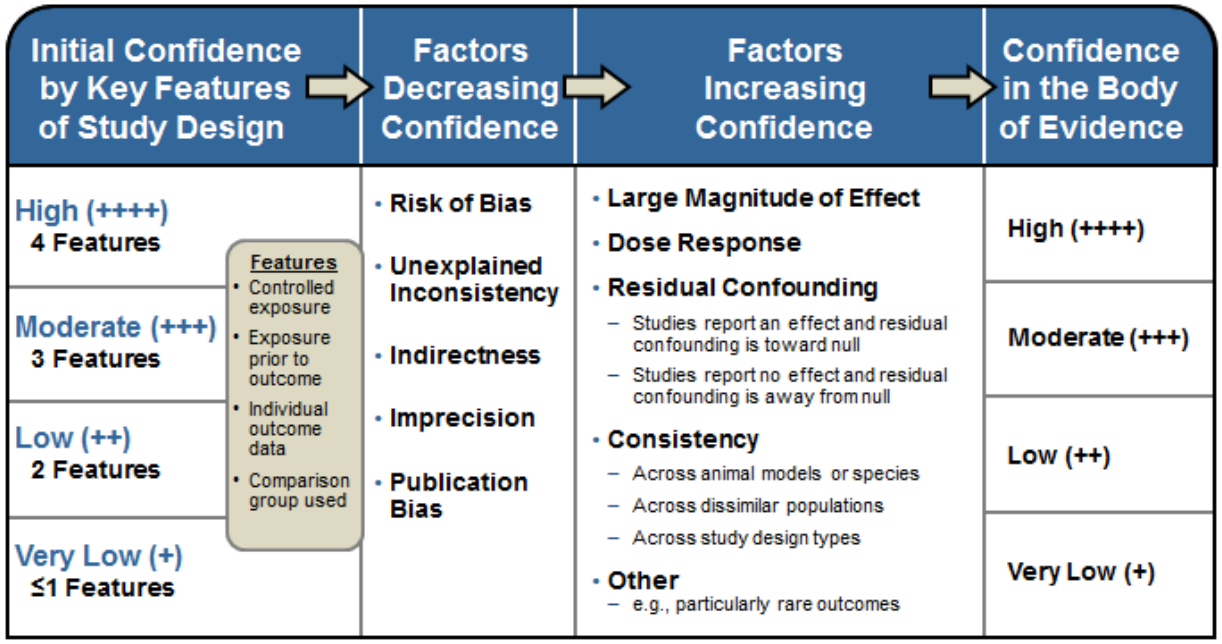
Heterogeneity within the available evidence was used to determine the type of evidence integration that is appropriate: either a quantitative synthesis (meta-analysis) or narrative approach for evidence integration. No meta-analyses were conducted due to heterogeneity of studies and small bodies of evidence across primary health outcomes including the antibody response, disease resistance, hypersensitivity, and autoimmunity-related endpoints. Meta-analysis approaches are considered most suitable if there are at least six to ten studies for a continuous variable and at least four studies for a categorical variable (Fu *et al.* 2011). Although there were larger bodies of evidence for spleen and thymus weight (see **Appendix 5**), both are secondary health outcomes and therefore a meta-analysis for these endpoints was not performed.

Confidence Rating: Assessment of Body of Evidence

The quality of evidence for each immune outcome was evaluated using the GRADE system for rating the confidence in the body of evidence (Guyatt *et al.* 2011, Rooney *et al.* 2014). More detailed guidance on

reaching confidence ratings in the body of evidence as "high," "moderate," "low," or "very low" is provided in the OHAT Handbook for Conducting a Literature-Based Health Assessment (http://ntp.niehs.nih.gov/go/38673, see STEP 5). In brief, available human and animal studies on a particular health outcome in **Table 5** (i.e., immunosuppression, hypersensitivity, and autoimmunity) were initially grouped by key study design features, and each grouping of studies was given an initial confidence rating by those features. Starting at this initial rating (column 1 of **Figure 2**), potential downgrading of the confidence rating was considered for factors that decrease confidence in the results (column 2 of **Figure 2** [risk of bias, unexplained inconsistency, indirectness or lack of applicability, imprecision, and publication bias]); and potential upgrading of the confidence rating was considered for factors that increase confidence in the results (column 3 of **Figure 2** [large magnitude of effect, dose response, consistency across study designs/populations/animal models or species, consideration of residual confounding, and other factors that increase our confidence in the association or effect]). Consideration of consistency across study designs, human populations, or animal species is not included in the GRADE guidance (Guyatt *et al.* 2011); however, it is considered in the modified version of GRADE used by OHAT (Rooney *et al.* 2014, NTP 2015b).

Figure 2. Assessing Confidence in the Body of Evidence



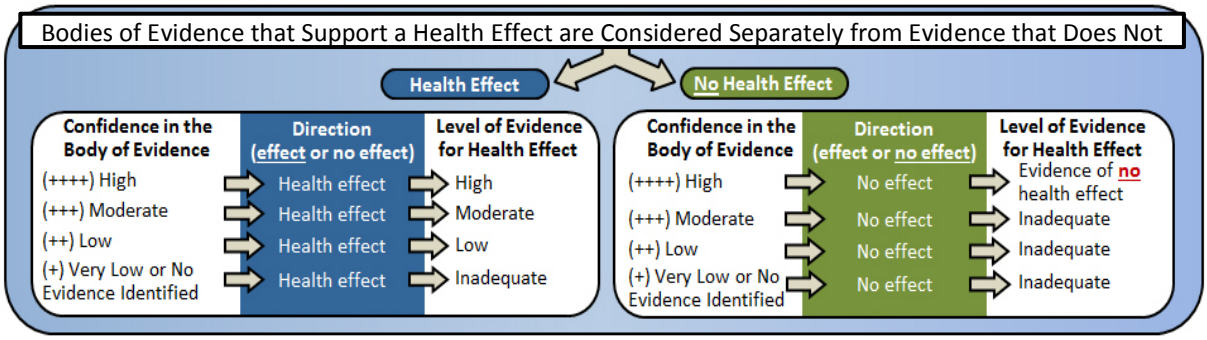
Confidence ratings were assessed by federal staff and reviewed by members of the evaluation review team for accuracy and consistency, and discrepancies were resolved by consensus and consultation with technical advisors as needed. Confidence ratings for the primary outcomes are summarized in evidence profile tables for each outcome.

Preparation of Level of Evidence Conclusions

The confidence ratings were translated into level of evidence of health effects for each type of health outcome separately according to one of four statements: (1) High, (2) Moderate, (3) Low, or (4) Inadequate (**Figure 3**). The descriptor "evidence of no health effect" is used to indicate confidence

that the substance is not associated with a health effect. Because of the inherent difficulty in proving a negative, the conclusion "evidence of no health effect" is only reached when there is high confidence in the body of evidence.

Figure 3. Translate Confidence Ratings into Evidence of Health Effect Conclusions



Evidence Descriptors	Definition
High Level of Evidence	There is high confidence in the body of evidence for an association between exposure to PFOA or PFOS and the health outcome(s).
Moderate Level of Evidence	There is moderate confidence in the body of evidence for an association between exposure to PFOA or PFOS and the health outcome(s).
Low Level of Evidence	There is low confidence in the body of evidence for an association between exposure to PFOA or PFOS and the health outcome(s), or no data are available.
Inadequate Evidence	There is insufficient evidence available to assess if exposure to PFOA or PFOS is associated with the health outcome(s).
Evidence of No Health Effect	There is high confidence in the body of evidence that exposure to PFOA or PFOS is not associated with the health outcome(s).

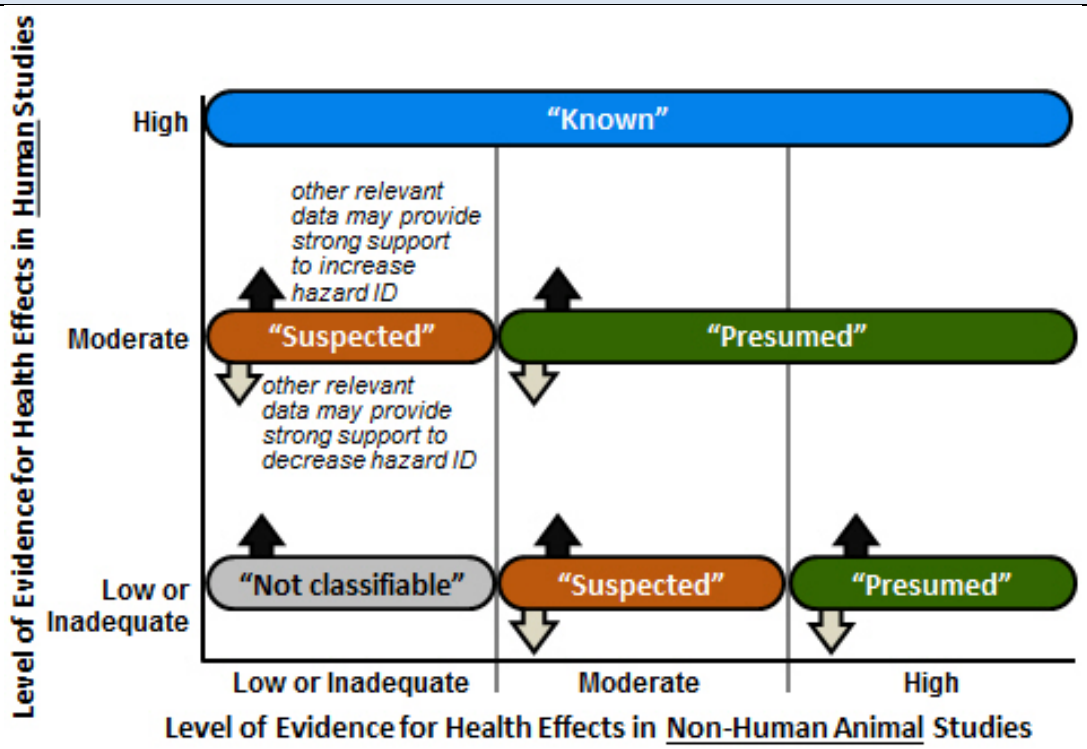
Integrate Evidence to Develop Hazard Identification Conclusions

Finally, the levels of evidence ratings for human and animal data will be integrated with consideration of mechanistic data to reach one of five possible hazard identification categories: (1) Known, (2) Presumed, (3) Suspected, (4) Not classifiable, or (5) Not identified to be an immune hazard to humans (**Figure 4**).

Consideration of Human and Animal Data

Initial hazard identification conclusions were reached by integrating the highest level-of-evidence conclusion for immune effects on an outcome basis for the human and the animal evidence streams. Hazard identification conclusions were reached on the groups of biologically related outcomes using outcome groups identified in **Table 5**) for: (1) immunosuppression, (2) hypersensitivity, and (3) autoimmunity as well as more specific endpoints (e.g., the conclusion on immunosuppression is primarily based on suppression of the antibody response). The level-of-evidence conclusion for human data for each health outcome was considered together with the level of evidence for non-human animal data on that outcome to reach one of four initial hazard identification conclusions: Known, Presumed, Suspected, or Not classifiable. When either the human or animal evidence stream was characterized as "Inadequate Evidence," then conclusions were based on the remaining evidence stream alone (which is equivalent to treating the missing evidence stream as "Low" in **Figure 4**).

Figure 4. Hazard Identification Scheme



Consideration of Mechanistic Data

The NTP does not require mechanistic or mode-of-action data in order to reach hazard identification conclusions, although when available, this and other relevant supporting types of evidence may be used to raise (or lower) the category of the hazard identification conclusion. Mechanistic data can come from a wide variety of studies that are not intended to identify a disease phenotype. This source of experimental data includes *in vitro* and *in vivo* laboratory studies directed at cellular, biochemical, and molecular mechanisms that explain how a chemical produces particular adverse effects.

For the evaluation of immunotoxicity associated with PFOA or PFOS exposure, we were interested in mechanistic data that were relevant for immune outcomes reported from *in vivo* studies in animals or humans. In general, the mechanisms for PFOA- or PFOS- associated immune effects are not well understood at this time (DeWitt *et al.* 2012 for review); however, established mechanisms for the specific immune effects evaluated were used to organize the available data. Mechanistic data from *in vitro* or *in vivo* studies were then used to examine the biological plausibility of the primary health outcomes considered in developing a hazard conclusion (e.g., antibody response). In brief, data on early events in a given immune response (i.e., cell signaling, cell activation, and key cell populations) were examined to inform the biological plausibility of the association between PFOA and PFOS and specific immune effects. PFOA- or PFOS-associated changes in cell populations or cytokines would present a possible mechanism if they occurred at the same or lower concentrations at which the immune effects were reported.

The factors outlined for increasing or decreasing confidence that the mechanistic data support biological plausibility are conceptually similar to those used to rate confidence in bodies of evidence for human or animal *in vivo* studies are listed below and described in depth in the protocol (http://ntp.niehs.nih.gov/go/749926). Four factors were considered that contribute to increased

confidence: potency, dose-response, consistency in terms of cellular events observed at the same or lower doses than *in vivo* health effects, and consistency across cellular targets on the same pathway. And four factors were considered that contribute to decreased confidence: unexplained inconsistency across studies of the same endpoint, risk of bias, indirectness/applicability of the pathway for human health or concentrations for human exposure, and publication bias. Evaluations of the strength of evidence provided by mechanistic data were made on an outcome-specific basis based on discussion by the evaluation team and consultation with technical advisors as needed.

- If mechanistic data provided strong support for biological plausibility of the relationship between exposure and the health effect, the hazard identification conclusion may be upgraded (indicated by black "up" arrows in **Figure 4**) from that initially derived by considering the human and non-human animal evidence together.
- If mechanistic data provided strong opposition for biological plausibility of the relationship between exposure and the health effect, the hazard identification conclusion may be downgraded (indicated by gray "down" arrows in **Figure 4**) from that initially derived by considering the human and non-human animal evidence together.

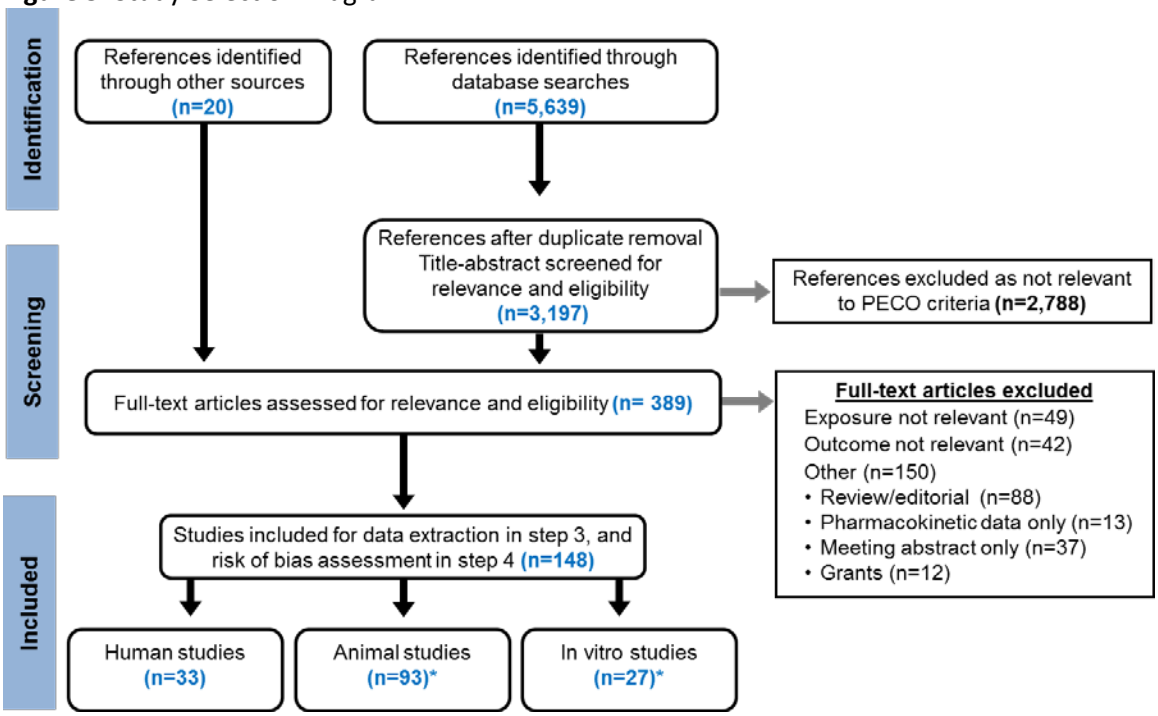
Although it is envisioned that strong evidence for a relevant immune process from mechanistic data alone could indicate a greater potential that the substance is an immune hazard to humans, for this evaluation the mechanistic data were only considered to inform the biological plausibility of observed outcomes from *in vivo* exposure studies in humans or animals. The mechanistic data were collected and then grouped by the immune effects that it would be relevant to and considered when integrating evidence to develop hazard identification conclusions. For example, observational data on total serum immunoglobulin E (IgE) or *in vitro* IgE production would support a functional measure of hypersensitivity-related outcomes, but it would not support suppression of the natural killer (NK) cell response.

RESULTS AND EVIDENCE SYNTHESIS

Literature Search Results

The electronic database searches retrieved 3197 individual references and 20 additional references were identified by technical advisors or from reviewing reference lists in published reviews and included studies. From the total references retrieved, 2788 were excluded during the title and abstract screening and 241 were excluded during the full text review. The screening results are outlined in a study selection diagram with reasons for exclusion documented at the full text review stage (**Figure 5**). The 148 included studies are listed in **Appendix 2**; there are 33 human studies, 93* animal studies, and 27* *in vitro*/ mechanistic studies.

Figure 5. Study Selection Diagram



* Five publications contained data relevant to both experimental animal studies and *in vitro* studies

Health Effects Results

The human and animal immune data across all studies were sorted separately for PFOA and PFOS into the 3 main categories of immune response presented earlier in **Table 5**: (1) immunosuppression, (2) hypersensitivity-related outcomes, and (3) autoimmunity. Within immunosuppression, evidence for effects on the antibody response, natural killer cell (NK) activity, infectious disease resistance and delayed-hyper hypersensitivity (DTH) response were considered separately, as these endpoints may involve different cell populations and mechanisms. For each chemical, the results were organized and presented for primary immune outcomes in these 3 categories. Results for primary outcomes were grouped across studies to develop bodies of evidence or collections of studies with data on the same or related outcomes. More indirect or secondary outcomes such as thymus weight, cell counts and other observational endpoints were summarized in **Appendix 5**.

The majority of data on primary health outcomes were on the antibody response for both PFOA and PFOS, and the collection of studies on this outcome represented the strongest bodies of evidence for both human and experimental animal studies.

For PFOA, there were also epidemiological studies on infectious disease resistance, hypersensitivity-related outcomes and autoimmunity. There were no human studies of PFOA and NK cell activity. Within the animal body of evidence, several PFOA exposure studies were identified that examined airway-hypersensitivity in mouse models of asthma, but there was only a single study on NK cell activity and no experimental studies of disease resistance or autoimmunity.

Similarly for PFOS, there were epidemiological studies on infectious disease resistance and hypersensitivity-related outcomes. There were no human studies of PFOS and NK cell activity and a single study on autoimmunity-related endpoints. Several experimental animal studies were located that tested the effects of PFOS exposure on disease resistance and NK-cell activity, and a single study was located that tested the effect of PFOS on airway-hypersensitivity. There were no experimental studies of autoimmunity in animal models.

The main findings for immune effects of PFOA and PFOS are summarized below. Then, the following sections on **PFOA Immune Evidence** and **PFOS Immune Evidence** present the results and evidence synthesis in detail for all of the primary immune health outcomes considered (antibody response, disease resistance, NK cell activity, hypersensitivity, and autoimmunity). There were no human studies of DTH identified for PFOA or PFOS and few experimental animal studies on the DTH response, the endpoint was not considered for hazard identification and the evidence is discussed in **Appendix 4**.

Main Findings PFOA

The principal findings are outlined below including a brief description of the basis for the confidence ratings and level of evidence conclusions for the human and non-human animal data that support the immune hazard identification conclusions for PFOA. These conclusions are based on evidence that PFOA exposure results in suppression of the antibody response and increased hypersensitivity-related effects, the primary immune outcome with the strongest bodies of evidence from human and experimental animal studies. The human and animal bodies of evidence both indicate that higher serum levels of PFOA are associated with suppression of the antibody response. **Table 6** outlines the evidence profile for PFOA immunotoxicity based on the antibody response data and presents the confidence ratings summaries for the bodies of evidence.

There is moderate confidence that exposure to PFOA is associated with suppression of the antibody response in humans based on the available studies. The results present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFOA were associated with suppression in at least one measure of the anti-vaccine antibody response to common vaccines across multiple studies. There were no changes in the confidence rating for the human body of evidence after considering factors that may increase or decrease confidence. Heterogeneity in the findings may be explained by variation between studies in the different vaccinations tested, time between vaccination and measurement of the antibody response, and analyses or ways to measure the antibody response.

There is high confidence that exposure to PFOA is associated with suppression of the antibody response in animals based on consistent suppression of the primary antibody response from experimental studies in mice. Confidence in the body of evidence was decreased because of serious concern for risk of bias and increased for evidence of dose-response observed across multiple studies to support the final rating of high confidence.

Table 6. Evidence Profile of the Main Findings for PFOA Immunotoxicity										
INITIAL CONFIDENCE for each body of evidence (# of studies)	**Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence**					**Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence**				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
Immunotoxicity Based on Evidence for Suppression of the Antibody Response										
Human										
Initial Moderate (4 prospective studies)[a]	---	---	---	---	---	---	---	---	---	**Moderate**
Initial Low (2 cross-sectional studies)[b]	---	---	---	---	---	---	---	---	---	**Low**
Confidence Across Human Bodies of Evidence	No change for considering across study designs									**Moderate**
Animal										
Initial High (7 mammal studies)	↓	---	---	---	---	---	↑	---	---	**High**
References: Human: Granum (2013)[a], Grandjean (2012)[a], Kielsen (2016)[b], Looker (2014)[a], Mogensen (2015)[a], Stein (2016)[b] Animal: DeWitt (2008, 2009a, 2016), Hu (2010), Loveless (2008), Vetvicka (2013), Yang (2002a)										

The moderate confidence in the human body of evidence for suppression of the antibody response translates into a moderate level of evidence and the high confidence in the experimental animal studies translates into a high level of evidence. Integration of these level-of-evidence conclusions supports an initial hazard identification conclusion of *presumed to be an immune hazard to humans* based on the antibody response data. Relevant mechanistic data (e.g., effects of PFOA on key cell populations, antigen processing and cell activation, or cytokines important for cell signaling during the antibody response) were not considered to provide evidence to support or refute the biological plausibility of PFOA-associated suppression of the antibody response.

- **Human body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Animal body of evidence:** High Confidence = High Level of Evidence
- **Initial hazard conclusion (Moderate x High)** = Presumed to be an Immune Hazard to Humans
- **Final hazard conclusion (after consideration of biological plausibility)** = Presumed to be an Immune Hazard to Humans

Therefore, the antibody data support a final hazard identification conclusion that PFOA is *presumed to be an immune hazard in humans* (see **Table 7**).

Table 7. PFOA Main Immune Effects Summary Table						
Category of Immune Response	**Immune Outcomes**	**Confidence Ratings in the Body of Evidence**		**Level of Evidence in the Body of Evidence**		**Hazard Conclusion**
		Human	**Animal**	**Human**	**Animal**	
Immunosuppression	Antibody response	Moderate	High	Moderate	High	**Presumed to be an Immune Hazard to Humans**

Although the main findings are based on the effects of PFOA on the antibody response, there is additional evidence that PFOA affects the immune system and the following sections (see **PFOA Immune Evidence**) include detailed discussions of the available evidence for effects of PFOA on all of the primary immune health outcomes considered. The additional data are primarily from epidemiological studies reporting that PFOA reduced infectious disease resistance, increased hypersensitivity-related outcomes, and increased autoimmune disease incidence of ulcerative colitis. The evidence indicating that PFOA affects multiple aspects of the immune system supports the overall conclusion that PFOA alters immune function in humans. However, the mechanism(s) of PFOA-associated immunotoxicity is not well understood and effects on diverse endpoints such as suppression of the antibody response and increased hypersensitivity-related endpoints may be unrelated.

Main Findings PFOS

The principal findings are outlined below including a brief description of the basis for the confidence ratings and level of evidence conclusions for the human and non-human animal data that support the immune hazard identification conclusions for PFOS. These conclusions are based on the antibody response, the primary immune outcome with the strongest bodies of evidence for effects of PFOS exposure for both human and experimental animal studies. The human and animal bodies of evidence both indicate that higher serum levels of PFOS are associated with suppression of the antibody response. **Table 8** outlines the evidence profile for PFOS immunotoxicity based on the antibody response data and presents the confidence ratings summaries for the bodies of evidence.

There is moderate confidence that exposure to PFOS is associated with suppression of the antibody response in humans based on the available studies. The results present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFOS were associated with suppression in at least one measure of the anti-vaccine antibody response to common vaccines across multiple studies. There were no changes in the confidence rating for the human body of evidence after considering factors that may increase or decrease confidence. Heterogeneity in the findings may be explained by variation between studies in the different vaccinations tested, time between vaccination and measurement of the antibody response, and analyses or ways to measure the antibody response.

There is high confidence that exposure to PFOS is associated with suppression of the antibody response in animals based on consistent suppression of the primary antibody response from experimental studies in mice. Confidence in the body of evidence was decreased because of serious concern for risk of bias and increased for evidence of dose-response observed across multiple studies to support the final rating of high confidence.

The moderate confidence in the human body of evidence for suppression of the antibody response translates into a moderate level of evidence and the high confidence in the experimental animal studies translates into a high level of evidence. These level-of-evidence conclusions support an initial hazard identification conclusion of *presumed to be an immune hazard to humans* based on the antibody response data. Relevant mechanistic data (e.g., effects of PFOS on key cell populations, antigen processing and cell activation, or cytokines important for cell signaling during the antibody response) were not considered to provide evidence to support or refute the biological plausibility of PFOS-associated suppression of the antibody response.

- **Human body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Animal body of evidence:** High Confidence = High Level of Evidence
- **Initial hazard conclusion (Moderate x High)** = Presumed to be an Immune Hazard to Humans

- **Final hazard conclusion**
  (after consideration of biological plausibility) = Presumed to be an Immune Hazard to Humans

Therefore, the antibody data support a final hazard identification conclusion that PFOS is *presumed to be an immune hazard in humans* (see **Table 9**).

Table 8. Evidence Profile of the Main Findings for PFOS Immunotoxicity

INITIAL CONFIDENCE for each body of evidence (# of studies)	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
Immunotoxicity Based on Evidence for Suppression of the Antibody Response										
Human										
Initial Moderate (4 prospective studies)[a]	---	---	---	---	---	---	---	---	---	**Moderate**
Initial Low (2 cross-sectional studies)[b]	---	---	---	---	---	---	---	---	---	**Low**
Confidence Across Human Bodies of Evidence	No change for considering across study designs									**Moderate**
Animal										
Initial High (8 mammal studies)	↓	---	---	---	---	---	↑	---	---	**High**

References:
Human: Granum (2013)[a], Grandjean (2012)[a], Kielsen (2016)[b], Looker (2014)[a], Mogensen (2015)[a], Stein (2016)[b]
Animal: Dong (2009b, 2011), Keil (2008), Lefebvre (2008), Peden-Adams (2008), Qazi (2010b), Vetvicka (2013), Zheng (2009)

Although the main findings are based on the effects of PFOS on the antibody response, there is additional evidence that PFOS affects the immune system and the following sections (see **PFOS Immune Evidence**) include detailed discussions of the available evidence for effects of PFOS on all of the primary immune health outcomes considered. There is also evidence based primarily on experimental animal studies that PFOS suppresses disease resistance to influenza A virus challenge and NK cell activity. Disease resistance involves multiple components of the immune system, and successful immune response to viral challenge includes rapid responses from the innate arm of the immune system (e.g., NK cell activity) as well as slower responses from the humoral arm of the immune system (antibody mediated responses). Although the mechanism(s) of PFOS-associated immunotoxicity is unclear, suppression of the antibody response and NK cell function are both potential mechanisms by which PFOS may reduce disease resistance. The evidence indicating that PFOS suppresses multiple aspects of the immune system supports the overall conclusion that PFOS alters immune function in humans.

Table 9. PFOS Main Immune Effects Summary Table

Category of Immune Response	Immune Outcomes	Confidence Ratings in the Body of Evidence		Level of Evidence in the Body of Evidence		Hazard Conclusion
		Human	Animal	Human	Animal	
Immunosuppression	Antibody response	Moderate	High	Moderate	High	**Presumed to be an Immune Hazard to Humans**

Risk of Bias Considerations

Risk-of-bias ratings of all of the individual studies for all questions are available in **Appendix 3**. The risk of bias of individual studies in the body of evidence and for the body of evidence as a whole was considered in developing the confidence ratings for each health effect. The key risk-of-bias questions (e.g., confounding, exposure characterization, and outcome assessment for human studies) are discussed in the consideration of the body of evidence for each health effect. Although no study was excluded based on concerns for risk of bias, confidence conclusions were considered with and without high risk of bias studies (e.g., studies rating probably high or definitively high risk of bias for two key risk-of-bias questions) to assess the impact of the high risk of bias studies.

PFOA Immune Evidence

The sections below on each primary immune health effect begin with a brief description of the health effect, followed by a summary of the human evidence and the confidence rating of the body of evidence from human studies. A similar summary of the animal evidence and confidence rating in the animal body of evidence is then presented. *In vitro* or mechanistic studies are then summarized. Then evidence synthesis was conducted in a three-part process for each outcome. First, the confidence ratings were translated into level-of-evidence of health effects conclusions using the procedure outlined in **Figure 3**. Next, initial hazard identification conclusions were reached by integrating the level-of-evidence conclusions for the human and animal evidence streams using the procedure outlined in **Figure 4**. Finally, the degree of support from mechanistic data was considered and discussed in reaching final hazard identification conclusions for each of the major immune health outcomes.

Immunosuppression: Antibody Response

The development of specific antibodies in response to an immune challenge (e.g., injection with sheep red blood cells or SRBC) is a well-accepted measure of immune function included in many guidelines or testing requirements for immunotoxicity (US EPA 1998, ICH 2005, WHO 2012). Antibodies are proteins found in blood and other body fluids that bind to antigens (generally proteins on the cell surface of infectious agents such as viruses or bacteria) and thereby identify them for destruction or removal. The production, release, and increase in circulating levels of antigen-specific antibodies are important for protection against the infectious agent and preventing or reducing severity of influenza, respiratory infection, colds, and other diseases as part of the humoral immune response. Reduced antibody production is an indication of decreased immune function or immunosuppression that may indicate a greater risk of disease. There are 5 antibody or immunoglobulin (Ig) classes in mammals: IgM, IgG, IgA, IgD, and IgE that differ in structure and function.

Antibody assays for immunosuppression generally measure IgM or IgG. IgM is important for the early or primary response after a single antigen challenge and IgG is a later response that is important in recognizing the antigen following re-exposure. Antigen-specific IgM to a T-cell-dependent antigen (e.g., SRBC) is considered one of the most predictive measures of overall immune function because proper response requires cooperation between T-cells, B-cells, and antigen-presenting cells to develop an antibody response (Luster *et al.* 1992). This antibody response can be examined by measuring antigen-specific antibody levels after vaccination in humans and after challenge with SRBC or other antigens in laboratory animals. Measurement of total immunoglobulin levels (rather than antigen-specific IgM or IgG) is considered observational data that is less predictive for immunotoxicity (WHO 2012).

Human Antibody Response Data for PFOA (and PFOS)

The human body of evidence for PFOA and PFOS on the antibody response is based on the same six epidemiological studies with very similar results and findings for both chemicals. The confidence ratings for the human data are the same for both chemicals. Therefore, to avoid repetition, the following text includes discussion of the human data for both PFOA and PFOS. The reader is referred to the section on **Human Antibody Response Data for PFOS** for the summary and confidence conclusion on the human body of evidence for the PFOS antibody data.

Summary: There is moderate confidence that exposure to PFOA is associated with suppression of the antibody response based on the available human studies. The results show consistent PFOA- associated suppression in at least one measure of the anti-vaccine antibody response across multiple studies with evidence from developmental, childhood, and adult exposures (see **Table 10** for list of studies). There were no changes in confidence rating for the body of evidence after considering factors that may increase or decrease confidence (see **Table 12** for confidence ratings summaries for the body of

evidence). Heterogeneity in the findings may be explained by variation between studies in the different vaccinations tested, time between vaccination and measurement of the antibody response, and analyses or ways to measure the antibody response. The confidence rating for the human antibody data is the same for PFOA and PFOS.

Table 10. Studies on Antibody Response in Humans (4 prospective; 2 cross-sectional)

Study	Study design (Location / Study)	n for Ab data	Exposure measure timing	Antibody measure timing	Analysis	Antigens /vaccines tested
Granum (2013)	Prospective (Norwegian/ MoBa birth cohort)	49 to 51	Maternal 0-3d post delivery	Children (age 3)	Linear regression (bivariate and multivariate) β coefficient	measles, Hib, rubella*,†, tetanus
Grandjean (2012) Mogensen (2015)	Prospective (Faroe Islands birth cohort)	Age 5 (509) Age 7 (419)	Maternal Child (age 5) (log transformed)	Children (age 5, 7) (log transformed)	Multiple analyses for different antibody endpoints including Linear regression for: 1) Linear regression (multivariate) % change in antibody level; and 2) Logistic regression OR of antibodies falling below a clinically protective level. Both analyses per 2-fold increase of individual PFAAs(PFOA, PFOS); also structural equation models to examine joint PFAAs	diphtheria*,†, tetanus*,†
Kielsen (2016)	Cross-sectional (Denmark hospital volunteers)	12	10-days post vaccination (log transformed)	Adult 2-30 days post vaccination (log transformed)	Regression model with different phases (linear day 4 to10 with cubic spline function) % change antibody from day 4 to 10 post vaccination per 2-fold increase individual PFAAs(PFOA, PFOS)	diphtheria†, tetanus
Looker (2014)	Prospective (USA OH/ WV residents with elevated PFOA in drinking water)	403	At vaccination (log transformed and quartiles)	Adult 21 days post vaccination	Multiple analyses for different antibody endpoints including Linear regression for: 1) mean rise following vaccination; 2) log-antibody rise; 3) log-antibody titer ratio (post-vaccine: pre-vaccine). And Logistic regression OR of: 1) seroconversion (4-fold increase post vaccination); and 2) seroprotection (1:40 post vaccination)	Influenza type B, A/H1N1, A/H3N2*
Stein (2016)	Cross-sectional (USA, NHANES)	1101 to 1190	In same sample as antibody (log transformed)	Children (age 12-19) (log transformed)	Linear regression % change in antibody per 2-fold increase individual PFAAs (PFOA, PFOS)	measles, mumps*,†, rubella*,†

Antibody level significantly ($p<0.05$) decreased in at least one measure associated with (*) PFOA or (†) PFOS exposure

The available epidemiological studies in the human body of evidence that evaluated the association between exposure to PFOA or PFOS and the antibody response include: (1) two birth cohort studies conducted in Norway and the Faroe Islands; (2) a longitudinal analysis of adult residents and workers in a region of the Ohio Valley with higher PFOA levels in drinking water (part of the C8 studies); and (3) two cross-sectional studies, one using NHANES data on U.S. children ages 12 to 19, and a small study of adult volunteers from a Denmark hospital (see **Table 10** for details). In addition to study design and age of the population when the antibody response was measured (children age 2-19 or adults), the studies varied greatly in size (from n = 12 to over 1100), age and timing of the exposure measurement (maternal, at the same time as vaccination, same time as antibody response), methods for evaluating the antibody response (circulating antibody level or rise in antibody concentration following vaccination), type of statistical analyses (β coefficient from linear regression, change in antibody response for 2-fold increase

in specific PFAAs or OR), and the specific vaccine tested. The two Faroe Island birth cohort studies evaluated different exposure timing (maternal, 5 and 7 years) and antibody concentrations in the same population. Most studies measured antibody concentrations to common childhood vaccines. The study in adults measured antibody concentration, rise and ratio (from pre to post vaccination), and seroconversion and seroprotection to flu vaccines (Looker *et al.* 2014). Some of the studies also measured other immunological related endpoints such as hypersensitivity and infectious disease (see following sections for discussion of these other health outcomes).

As shown in **Table 11**, the results of multiple studies reported PFOA- and PFOS-associated suppression in at least one measure of the anti-vaccine antibody response with evidence from developmental, childhood, and adult exposures. Increased maternal serum or plasma concentrations of PFOA and PFOS were associated with lower anti-vaccine antibody levels for some vaccines, but not others, in children (ages 3-7) in both available prospective studies that examined antibody response in relation to developmental exposure (Grandjean *et al.* 2012, Granum *et al.* 2013). A 2-fold increase in serum concentrations of PFOA and PFOS measured in children were also associated with lower anti-vaccine antibody levels in children (age 5-19), and again for some vaccines, but not all, in a prospective study (Grandjean *et al.* 2012) measuring exposure in 5-year old children and antibody levels at age 7 and in a cross-sectional study of children (age 12-19) using NHANES data (Stein *et al.* 2016). There are also two studies in adults that reported that either PFOA or PFOS (but not both) were associated with reductions in the initial rise in anti-vaccine antibody levels in the first 10-30 days after vaccination (Looker *et al.* 2014, Kielsen *et al.* 2016). After a discussion of common issues such as different methods for measuring the antibody response, the following sections address the three exposure times separately.

One challenge common to the epidemiological studies in general is the ability to control for other exposures, particularly between PFOA and PFOS or for other PFAAs that may also be immunomodulatory. While the available studies corrected for major immunotoxicants if there was a strong reason to suspect they were present (e.g., the Faroe Island study corrected for PCBs because the known exposure and immunotoxicity in this population) (Grandjean *et al.* 2012), the adjustment for other PFAAs was generally not performed. The correlation between individual PFAA exposures varied widely between compounds and between studies. For example, there was moderate to high correlation between PFOA and PFOS (r = 0.70 to 0.72) in the study of children age 12-19 from NHANES depending on survey year (Stein *et al.* 2016). Higher concentrations of PFOA and PFOS were both associated with reduced antibody levels to mumps and rubella, but the analyses were not performed to correct for potential effects of other PFAAs. The NHANES study also measured perfluorohexane sulfonate (PFHxS) and perfluorononanoate (PFNA) and the correlation between all four PFAAs ranged more widely (r = 0.14 to 0.72). Stein *et al.* (2016) reported that PFHxS was also associated with lower antibody levels to rubella, but not to mumps, and that PFNA was not significantly associated with antibody levels to any vaccine. A wider range of correlations were reported across PFAAs in the Faroe Island birth cohort (r = 0.01 to 0.78) for PFOA, PFOS, PFHxS, PFNA and perfluorodecanoate (PFDA); and all of the different PFAAs were associated with reduced antibody response in at least one vaccine/analysis (e.g., diphtheria or tetanus relative to maternal serum or age 5 serum PFAAs) (Grandjean *et al.* 2012). In further analyses, the authors (Grandjean *et al.* 2012, Mogensen *et al.* 2015) report that the antibody response to diphtheria and tetanus and a combined exposure model to a single variable for joint latent exposure to PFAAs that included PFOA, PFOS and PFHxS. The association for PFOA or PFOS was less apparent when adjusted for the other PFAAs, with anti-tetanus antibody borderline significant decrease by 29% (95% CI -0.4 to 50.6). The analysis suggests that individual PFAAs (including PFOA and PFOS) suppressed the antibody response, and there may be some degree of additive effects or cumulative effects of several PFAAs as indicated by the stronger association reported in the combined exposure model. The authors indicate that using the data from this study alone (Grandjean *et al.* 2012, Mogensen *et al.* 2015), the

effects could not be attributed to individual compounds, and that the combined variable to the three major PFAAs (PFOA, PFOS and PFHxS) showed a stronger association with reduced antibody levels.

Table 11. Summary of PFOA/PFOS and Selected Data on Antibody Response in Humans

Vaccine	Change in antibodies with PFOA[a]	Change in antibodies with PFOS[a]	Possible sources of heterogeneity	Exposure measure timing	Study
Rubella	**-0.4 (-0.64 to -0.17)**	**-0.08 (-0.14 to -0.02)**	• developmental exposure metric	Maternal 0-3d post delivery	Granum (2013)
	-8.9 (-14.6 to -2.9)	**-13.3 (-19.9 to -6.2)**	• childhood exposure metric • booster vaccination	Children: current	Stein (2016)
Diphtheria	**-22.8 (-39.4 to -1.7)**	-19.7 (-41.8 to 10.7)	• developmental exposure metric • antibody levels measured at age 7	Maternal week 32 gestation	Grandjean (2012)
	-25.2 (-42.9 to -2.0)	**-27.6 (-45.8 to -3.3)**	• childhood exposure metric • antibody levels measured at age 7	Children (age 5)	Grandjean (2012)
	OR = 3.27 (1.43 to 7.51)	**OR = 2.38 (0.89 to 6.35)**	• outcome is OR of falling below protective level of 0.1 IU/ml) • childhood exposure metric • antibody levels measured at age 7	Children (age 5)	Grandjean (2012)
	-8.2 (-20.8 to 6.4)	**-11.9 (-21.9 to -0.3)**	• outcome is antibody rise not levels • outcome in adults	Adults 10-days post vaccination	Kielsen (2016)
Mumps	**-6.6 (-11.7 to -1.5)**	**-5.9 (-9.9 to -1.6)**	• childhood exposure metric • booster vaccination	Children: current	Stein (2016)
Measles	-0.13 (-0.35 to 0.09)	-0.05 (-0.1 to 0.01)	• developmental exposure metric	Maternal 0-3d post delivery	Granum (2013)
	-3.4 (-16.7 to 11.9)	-2.9 (-17.3 to 13.9)	• childhood exposure metric • booster vaccination	Children: current	Stein (2016)
Tetanus	-0.01 (-0.09 to 0.1)	-0.002 (-0.03 to 0.02)	• developmental exposure metric	Maternal 0-3d post delivery	Granum (2013)
	7.4 (-17.1 to 39.0)	35.3 (-3.9 to 90.6)	• developmental exposure metric • antibody levels measured at age 7	Maternal week 32 gestation	Grandjean (2012)
	-35.8 (-51.9 to -14.2)	-23.8 (-44.3 to 4.2)	• childhood exposure metric • antibody levels measured at age 7	Children (age 5)	Grandjean (2012)
	OR = 4.20 (1.54 to 11.44)	**OR = 2.61 (0.77 to 8.92)**	• outcome is OR of falling below protective level of 0.1 IU/ml) • childhood exposure metric • antibody levels measured at age 7	Children (age 5)	Grandjean (2012)
	0.23 (-10.4 to 12.1)	-3.6 (-11.9 to 5.5)	• outcome is antibody rise not levels • outcome in adults	Adult 10-days post vaccination	Kielsen (2016)
Influenza A H3N2	**Antibody titer ratio** 2nd -0.1 (-0.3 to 0.1) 3rd -0.07 (-0.28 to -0.14) **4th -0.22 (-0.43 to -0.01)** **Antibody titer rise** **2nd -0.28 (-0.51 to -0.06)** **3rd -0.37 (-0.60 to -0.13)** 4th -0.12 (-0.36 to 0.13)	**Antibody titer ratio** 2nd -0.06 (-0.26 to 0.14) 3rd -0.02 (-0.18 to -0.23) 4th -0.03 (-0.24 to -0.19) **Antibody titer rise** 2nd 0.03 (-0.19 to 0.26) 3rd 0.18 (-0.00 to 0.41) 4th -0.04 (-0.28 to 0.21)	• outcome is antibody rise not levels • outcome in adults	Adult at vaccination	Looker (2014)

[a]linear regression (β or % change in antibody per 2-fold increase of individual PFAAs unless noted as OR).
Bold text indicates statistically significant decreased or increased association.
This table highlights select findings to illustrate reductions in the antibody response across multiple vaccines (rubella, diphtheria, mumps, tetanus, and influenza A), multiple measures of the antibody response (anti-vaccine antibody levels, OR of falling below seroprotective antibody levels, antibody rise, and antibody ratio pre- to post-vaccination) and in different populations relative to PFOA and PFOS measured in pregnant women, children, and adults; for additional data see **Figure D1, Figure D2, Figure D3, Figure D4, Figure D5**.

Risk-of-bias ratings of individual studies of the antibody response for all questions are available in **Figure D10** and **Figure D11** (see protocol for additional details http://ntp.niehs.nih.gov/go/749926). The assessment of the key risk-of-bias questions (confounding, exposure characterization, and outcome assessment) are discussed in the following sections when there are particular issues for studies of

exposure during development, childhood, or as adults and measures of the antibody response. A general discussion of each of the key questions is included below.

Risk-of-bias assessment of potential confounders included age, sex, race/ethnicity, smoking, body mass index (BMI), alcohol consumption, variables that represent socioeconomic status, and exposure to other known or suspected immunotoxicants (e.g., PCBs and other potentially immunomodulatory PFAAs) based on prior reports of associations with PFOA and PFOS exposure levels (Calafat *et al.* 2007, Nelson *et al.* 2010) and immune outcomes (WHO 1996, Dallaire *et al.* 2005) (see protocol http://ntp.niehs.nih.gov/go/749926 for more details). The Grandjean *et al.* (2012) population in the Faroe Islands has known exposure to PCBs that have been shown to suppress the antibody response; however, the analysis accounted for PCBs as potential confounders, and the authors report no effect of adjustment for PCBs on the analyses. As discussed above, there may be limited ability to differentiate effects of PFOA or PFOS from other PFAAs given that there is likely to be co-exposure with other PFAAs and there may be similar immunomodulatory effects of the different PFAAs (e.g., suppression of the antibody response as discussed above). Therefore, unless a study controlled for other PFAAs, studies were rated probably high risk of bias in accounting for potential confounders and modifiers because of the limited ability to differentiate effects of PFOA or PFOS from other PFAAs. The effects of individual PFAAs on the antibody response can be considered effect modifiers, rather than true confounders of the association between exposures to individual PFAAs (e.g., PFOA and PFOS) and lower antibody response. No study was excluded based on concerns for risk of bias.

Risk-of-bias assessment of the exposure characterization assessed the consistency and reliability of the exposure measures such as use of established test methods (e.g., high pressure liquid chromatography with tandem mass spectrometry) and whether exposure was assessed in a relevant time-window for development of the outcome (see protocol for more details http://ntp.niehs.nih.gov/go/749926). Given the slow clearance and long biological half-life of PFOA and PFOS in humans (2 to 8 years)(Olsen *et al.* 2007a, Kudo 2015), exposure measured from a single sample/time point is considered a good measure of PFOA or PFOS exposure. The range of exposure was also considered for these studies to determine if it was sufficiently broad to detect an association between exposure and potential differences in the outcome. In general, serum or plasma concentrations spanned a range that was at least 10-fold, suggesting a sufficient range [e.g., 0.2 to 2.7 ng/ml PFOA minimum to maximum in the Granum *et al.* (2013) study]. All of the human studies of antibody response were rated probably low or definitely low risk of bias for exposure (**Figure D10** and **Figure D11**).

Risk-of-bias evaluation of the outcome assessment considered the use of established methods, whether the outcome had been assessed consistently across all groups and whether or not the outcome assessors had been blinded to the study groups or exposure levels prior to assessing the outcomes. Given the study designs for the epidemiological studies that examined antibody response (as for many of the epidemiological studies in this data set), it was unlikely that the laboratory personnel measuring antibody levels would have been aware of the PFOA or PFOS serum levels of individuals and therefore lack of blinding was not a concern. Across studies there were several methods used to measure the antibody response, and all of the methods are considered both valid and reliable; however, they may differ in their clinical relevance as a measure of the ability of individuals to respond to infectious agents such as viruses, bacteria, or mount an effective antibody response to vaccination. Most of the studies of PFOA and PFOS evaluated specific antibody levels to vaccines at a time point substantially removed from vaccination by months or years. Antibody levels rise dramatically in the first several weeks following vaccination, and then decrease over time with a greater time between vaccination and measurement of antibody response; for example dropping 90% by 3-years after primary vaccination (Swartz *et al.* 2003). Lower concentrations of specific antibodies to vaccines are likely to have biological significance on a

population level with some proportion of the population having a decreased ability to mount a defense against viruses or bacteria (WHO 2012). However, for individuals, it is unknown if small to moderate reductions in circulating antibody levels (e.g., 15 to 35%) would affect the immune response to challenge.

In addition to measuring antibody levels, several studies of PFOA and PFOS included analysis of the antibody response that are considered more clinically relevant to the ability of individuals to respond to viral or bacterial challenge. Three studies included analysis for antibody levels dropping below a clinically protective level or failing to increase sufficiently at vaccination to produce immunity considered protective (Grandjean *et al.* 2012, Looker *et al.* 2014, Kielsen *et al.* 2016). For example, a 2-fold increase in serum concentrations of PFOA and PFOS was associated with a greater OR 4.20 (95% CI, 1.54 to 11.44) for falling below the clinically protective antibody level 0.1 IU/ml for diphtheria antibodies in a study of children from the Faroe Islands (Grandjean *et al.* 2012). Both of the studies in adults reported that exposure (one for PFOA and the other for PFOS) were associated with a decreased rise in antibody concentrations post vaccination which would suggest a lower likelihood of achieving a sufficient antibody level to provide effective immunity (Looker *et al.* 2014, Kielsen *et al.* 2016). The outcome measures used in the Looker *et al.* (2014) study are considered particularly strong, clinically and biologically relevant to the individuals in the study as well as on a population level.

Levels of PFOA/PFOS in pregnant women and antibody response in children
Increased maternal PFOA and PFOS concentrations were associated with lower antibody levels to rubella vaccination, but not measles, *Haemophilus influenzae* type b (Hib), or tetanus in 3-year old children from a sub-cohort of the Norwegian Mother and Child Cohort (MoBa) study (n = 49-51) (Granum *et al.* 2013) (**Figure D1**). In another study, increased maternal PFOS was associated with lower antibody levels to diphtheria vaccination in 5-year old children (pre-booster) and maternal PFOA was associated with lower antibody levels to diphtheria in 7-year old children from a Faroe Islands birth cohort (n = 419-509) (Grandjean *et al.* 2012) with additional data on exposure at 7-years of age and analysis in (Mogensen *et al.* 2015) (**Figure D2**). Across these vaccine antibody studies that measured maternal PFOA or PFOS concentrations, only one vaccine (tetanus) was tested in more than one study to allow an examination of consistency of the association. In both studies, there was no association between maternal PFOA or PFOS concentrations and antibody levels to tetanus in children.

Risk-of-bias assessment of potential confounders for the antibody response was discussed previously and both the Grandjean *et al.* (2012) and Granum *et al.* (2013) study adjusted for most important confounders other than co-exposure to other PFAAs. Duration of breastfeeding was considered as a confounder the Granum *et al.* (2013) and the Grandjean *et al.* (2012) studies because of evidence that breast feeding may support immune function and duration of breastfeeding may affect PFOA exposure as well (Karrman *et al.* 2007). In addition, the Grandjean *et al.* (2012) population in the Faroe Islands has known exposure to PCBs that have been shown to suppress the antibody response; however, the analysis accounted for PCBs as potential confounders, and the authors report no effect of adjustment for PCBs on the analyses. Both studies were rated probably high risk of bias in accounting for potential confounders because of the limited ability to differentiate effects of PFOA or PFOS from other PFAAs (**Figure D10** and **Figure D11**). For example, the Granum *et al.* (2013) study of the MoBa birth cohort reported suppression of the antibody response to rubella with higher serum concentrations of PFOA and PFOS, and also with serum levels of PFHxS and PFNA. Within the Granum *et al.* (2013) study the different PFAAs were not highly correlated (r = 0.26 to 0.60) and analyses of individual PFAAs supported effects all in the same direction (i.e., suppression), but the analyses of PFOA or PFOS were not performed to correct for potential effects of other PFAAs.

Risk-of-bias assessment of the exposure characterization for the developmental studies assessed the consistency and reliability of the exposure measures as discussed previously. However, for the developmental studies, there was further consideration of maternal serum or plasma as an indirect measure of exposure to the offspring. Maternal serum PFOA and PFOS were used as the exposure metric for both studies examining potential effects of prenatal exposure on antibody levels in children. The mean maternal serum PFOA concentrations were 1.1 and 3.2 ng/ml, and mean serum PFOS concentrations were 5.6 and 27.3 ng/ml in the Granum *et al.* (2013) and Grandjean *et al.* (2012) studies respectively. The mean serum levels for PFOA and PFOS in these studies are within the range of US exposures [e.g., latest NHANES data for 1999-2012 reported mean (95% CI) was 2.08 ng/ml (1.95-2.22) for serum PFOA (CDC 2015)]. Strong positive correlations (e.g., Pearson's correlation between 0.79 – 0.93) have been reported between maternal PFOA and PFOS and cord levels as a measure of fetal exposure (Glynn *et al.* 2012). However, Glynn *et al.* (2012) did show that the correlation between maternal and fetal levels was strongest for maternal samples taken closer to delivery (i.e., third trimester or within 3 weeks after delivery), with the weakest correlations during the first trimester (Pearson's correlation 0.78 for PFOA and 0.60 for PFOS). Maternal serum concentrations of PFOA and PFOS were measured close to delivery in the Granum *et al.* (2013) (within 3 days of birth) and Grandjean *et al.* (2012) (at week 32 of pregnancy) studies, and therefore, the studies minimize the likelihood of exposure misclassification for prenatal exposure and were rated definitely low risk of bias for exposure characterization (**Figure D10** and **Figure D11**).

Although Granum *et al.* (2013) and Grandjean *et al.* (2012) reported that maternal concentrations of PFOA and PFOS were associated with decreased antibody levels in children, the studies cannot exclude the potential impact of post-natal exposure directly to children. There is no *a priori* reason to suspect a specific window of susceptibility for PFOA or PFOS exposure to affect the antibody response (i.e., developmental, childhood, or adult). The one study that tested both developmental and childhood exposure (Grandjean *et al.* 2012) reported that both exposure windows were associated with lower antibody levels to diphtheria vaccinations and the most consistent results were for the association with childhood exposure measures (**Figure D2** and **Figure D3**). The authors found that maternal PFOA and PFOS serum concentrations had low correlations with exposure measured in children at age 5 (0.19 to 0.27 for PFOA and PFOS respectively). As discussed below, lower antibody levels to vaccines were also reported to be associated with childhood and adult measurements of PFOA and PFOS.

Levels of PFOA/PFOS in children and antibody response

The Grandjean *et al.* (2012) study of children in the Faroe Islands discussed earlier in the context of maternal exposure also reported that serum PFOA at age 5 was associated with lower antibody levels to both tetanus and diphtheria at age 7, and age 7 adjusted for age 5 results; similarly, serum PFOS at age 5 was associated with decreased antibody levels for diphtheria at age 7 and for tetanus at age 5 (**Figure D3**). Adjustment for age 5 results somewhat attenuated the effect size. In addition to a lower antibody level, the results included a separate analysis for the antibody level dropping below a clinically protective level. A 2-fold increase in PFOA and PFOS concentrations at age 5 was associated with odds ratios between 2.38 (95% CI, 0.89 to 6.35) and 4.20 (95% CI, 1.54 to 11.44) for falling below the clinically protective level of 0.1 IU/mL for tetanus and diphtheria antibodies at age 7. As discussed above, lower antibody levels are likely to have biological significance on a population level, and the lower OR for children having antibody levels below the value considered clinically protective is stronger data that is likely to also be meaningful to the individuals in this study. The researchers added additional data on exposure at 7-years of age and further analysis in a subsequent publication that also supported lower antibody levels with 2-fold increases in serum PFOA and PFOS at age 7 (Mogensen *et al.* 2015). The consistency in direction of the effect (i.e., decreased antibody levels) across multiple measures of the antibody response supports PFOA- and PFOS-associated suppression of the antibody response and an

association with both developmental and post-natal exposure. However, chance cannot be ruled out given the number of different analyses in this study (antibody levels pre- and post-booster age 5, age 7, and age 7 adjusted for age 5 response examined in relation to maternal and age 5 serum levels of PFOA and PFOS).

There is also one cross-sectional study based on NHANES data on children age 12-19 (n = 1101 to 1190) that reported current serum concentrations of PFOA and PFOS were associated with lower antibody levels for mumps and rubella (but not measles) using NHANES (1999-2000 and 2003-2004) data (Stein *et al.* 2016). Although these NHANES exposure data were taken from individuals at the same time as the antibody levels, the long (2-8 year) half-lives of PFOA and PFOS increase the likelihood that serum measurements represent past exposure that would be biologically relevant for the antibody response. The cross-sectional nature of the study design is considered in setting the initial confidence as low confidence for this design (see **Table 12**), and therefore the study was not further down-graded for risk of bias in the exposure characterization given the long half-lives of PFOA and PFOS. Both available studies reported that serum concentrations of PFOA and PFOS in children were associated with lower antibody response to vaccinations and there were no shared vaccines to allow examination of the consistency of the association across studies (**Figure D3**).

Levels of PFOA/PFOS in adults and antibody response

The two adult studies measured the antibody response using a different metric – the change in antibody concentration following vaccination was evaluated, rather than circulating antibody concentrations. The Looker *et al.* (2014) study performed prospective analysis using serum exposure concentrations taken immediately prior to vaccination and then subsequent measurement of the antibody response after 21 days in a study of 403 adults living in the Ohio Valley (as part of the C8 studies), a region with elevated drinking water PFOA levels. Serum levels of PFOA were associated with a reduced rise in antibody levels in response to influenza A/H3N2 vaccination (not influenza B or A/H1N1) as determined in several measures (e.g., titer rise, titer ratio, and seroprotection or antibody titer post vaccination >1:40 defined as protective) (Looker *et al.* 2014) (**Figure D4**). The antibody outcome measures used in this study, particularly seroprotection or that higher PFOA concentrations were associated with decreased likelihood of attaining the antibody level considered to provide long-term protection, were considered meaningful to the individuals in this study as well as biologically relevant on a population level. The area is known to have elevated water levels of PFOA and residents have higher serum PFOA than the general US population. Interestingly, this is the only human study that did not report an effect of PFOS on antibody levels and it is the only study where PFOA concentrations were higher than PFOS (in all other studies mean PFOS concentrations are 5x PFOA levels or more). This study was not downgraded for risk of bias for potential confounding due to the potential impact of other PFAAs given the established high concentrations of PFOA in this population, and that the study results did not find and effect of PFOS on measured endpoints. The Looker *et al.* (2014) study provides increased confidence that the observed antibody suppression in humans can be attributed to PFOA rather than a combined effect of PFOA and other PFAAs. In a small study of 12 volunteer hospital workers in Denmark, serum levels of PFOS (but not PFOA) were associated with reduced rise in antibody levels following booster vaccination for diphtheria (but not tetanus) (Kielsen *et al.* 2016) (**Figure D5**). This study was rated probably high risk of bias for two Key Questions (1) selection, for lack of information on the methods and time frame of participant recruitment and (2) confounding, for failing to account for important confounders including race, smoking, BMI, socioeconomic status, and potential exposure to agents that may affect antibody response as part of working in a hospital setting, and additional threats to internal validity given the small sample size (n = 12). Therefore, the Kielsen *et al.* (2016) study was only used for support, rather than deriving conclusions because of the serious risk of bias concerns (**Figure D10** and **Figure D11**).

Total IgM, IgG, and IgA levels were not associated with serum PFOA as part of a medical surveillance of workers in a PFOA production plant (Costa *et al.* 2009); however, as discussed above, total immunoglobulin (IgM or IgG) levels are not considered reliable measures of immunotoxicity or of the antibody response. The medical surveillance did not include any vaccine-specific or antigen-specific antibody measures so these results cannot be compared to the other studies and were not used in deriving conclusions on the antibody response.

Sources of heterogeneity

Suppression of the antibody response was only associated with PFOA and PFOS exposure for a subset of the vaccines examined within each study. This is not considered to represent inconsistency in the results because different responses to different vaccines are expected and often observed in human and experimental animal data as antigens such as vaccines may stimulate different components of the immune system. The strength of an antibody response in terms of antibody level and length of time that an elevated/effective antibody response is maintained is known to differ across vaccines. For example, the antibody response to rubella and mumps are typically robust and last for decades (Vandermeulen *et al.* 2007, Kakoulidou *et al.* 2010); whereas diphtheria and tetanus require booster vaccinations to maintain higher antibody levels that provide effective immunity.

Although a PFOA- and PFOS- associated decrease in specific antibody level to vaccination is reported in almost all of the available human studies, there is limited ability to compare results across studies because studies generally tested the antibody response to different vaccinations. When the antibody response to the same vaccine was examined in multiple studies for the same exposure lifestage (developmental, childhood, or adult), the consistency and potential sources of heterogeneity were discussed in the sections above. Consistency can also be examined across lifestage for diphtheria, measles, rubella and tetanus. The antibody response to diphtheria showed the most consistent response and was suppressed in relation to maternal, childhood, and adult PFOA and PFOS concentrations (Grandjean *et al.* 2012, Mogensen *et al.* 2015, Kielsen *et al.* 2016). The antibody response to measles may represent a specific antibody response that is less susceptible to suppression by PFOA or PFOS exposure, due to a different mechanism or stronger antibody response to this vaccine; it was not associated with PFOA or PFOS in any study (Granum *et al.* 2013, Stein *et al.* 2016). Higher PFOA and PFOS concentrations in children were associated with lower antibody levels for tetanus; but the antibody response was not associated with maternal PFOA or PFOS (Grandjean *et al.* 2012, Granum *et al.* 2013). It is possible that heterogeneity in the association between PFOA and PFOS exposure and reduced antibody levels for different vaccines is a result of differences in the strength of the response (i.e., higher antibody levels), rates at which antibody titers erode over time, or other factors.

It is unclear from these data if exposure during childhood is more likely to be associated with reduced antibody response or if there is a “best” age group to study the association. However, it is well established that antibody levels decrease substantially in the months and years following vaccination. For example, in a study of vaccination to diphtheria, tetanus and pertussis, antibody levels dropped 90% by 3-years after primary vaccination (Swartz *et al.* 2003). There is a greater decrease in antibody level with more time between vaccination and the measurement of the antibody response. It appears that studies in this data set with a greater time since vaccination were more likely to report decreased antibody levels associated with PFOA and PFOS [e.g., antibody levels to tetanus and diphtheria two years after booster vaccination (age 7) were more likely to be associated with PFOA and PFOS than antibody levels immediately following booster (age 5) (Grandjean *et al.* 2012)(Granum *et al.* 2013, Stein *et al.* 2016)].

Confidence in the body of evidence was not increased for dose-response for several reasons including the difficulty in attributing effects to individual compounds. Multiple studies did report that higher PFOA and PFOS concentrations were associated with lower antibody levels; however, none of the studies clearly demonstrated an increase in the effect size (greater reduction in the antibody level or reduced rise in antibody level following vaccination) with higher exposure levels of PFOA or PFOS after controlling for other PFAAs. For example, Grandjean *et al.* (2012) presented mean antibody concentrations in children from the Faroe Islands relative to exposure tertiles for PFOA and PFOS concentrations from both the maternal and childhood (age 5) metrics. Antibody concentrations to diphtheria and tetanus at age 7 show a negative dose response relative to childhood exposure to PFOA and PFOS at age 5 (Grandjean *et al.* 2012). In further analysis on the same population, Mogensen *et al.* (2015) developed general additive models for combined exposure to PFOA, PFOS and PFHxS that indicated a negative dose response for antibody concentrations relative to exposure at age 5. However, when adjusting the model for the impact of other PFAAs, the results were no longer significant. Multiple studies did demonstrate that higher PFOA and PFOS concentrations were associated with lower antibody levels; however, few studies analyzed the data by quartiles to examine the dose-response relationship. Granum *et al.* (2013) reported significant β coefficients or the slope, which support a dose response relationship; however, the analysis did not include adjustment for other PFAAs measured in the study. The Looker *et al.* (2014) study of adults in the Ohio Valley (part of the C8 studies) examined the antibody response where PFOA was more likely to be the important PFAA because of high concentrations of PFOA in this population and no association between PFOS and antibody response in this study. In addition, only the Looker *et al.* (2014) study analyzed the antibody response data by quartiles. Although PFOA was associated with reduced antibody response to influenza A/H1N1, there was inconsistent evidence of a dose-response relationship. The highest quartile was significantly elevated for antibody titer ratio (see **Figure D4**); in contrast the highest exposure quartile was not significantly elevated when evaluating antibody titer rise, although the second and third quartile were elevated. Confidence in the body of evidence was not increased for dose-response given the uncertainty on the dose-response relationship that could be attributed to PFOA or PFOS.

The NHANES data in children 12-19 reported a 15% reduction in antibody levels to rubella for a doubling of PFOS concentration (Stein *et al.* 2016) and the Faroe Islands birth cohort reported 25 to 38% reduction in antibody levels to diphtheria for doubling of childhood or maternal PFOA or PFOS (Grandjean *et al.* 2012). It is unknown if this level of reduction would affect the immune response to a viral or bacterial challenge for these individuals. Nevertheless, immune suppression resulting in a lower antibody response is not a desirable outcome and any lowering of the antibody response may be considered adverse on a population level such that individuals with lower antibody levels may be less able to mount a defense against viruses or bacteria (WHO 2012). In addition to a lower antibody level, several studies included analysis for antibody levels below a clinically protective level (Grandjean *et al.* 2012, Looker *et al.* 2014). A 2-fold increase in PFOS and PFOA concentrations at age 5 was associated with a greater OR 4.20 (95% CI, 1.54 to 11.44) for falling below the clinically protective antibody level for diphtheria antibodies at age 7 in children from the Faroe Islands (Grandjean *et al.* 2012). Both of the adult studies reported that exposure to PFOA and PFOS were associated with a reduced rise in antibody concentrations post vaccination (Looker *et al.* 2014, Kielsen *et al.* 2016). The outcome measures used in the Looker *et al.* (2014) study are considered particularly strong and biologically relevant to the individuals in the study as well as on a population level. The results indicated that higher PFOA concentrations were associated with decreased likelihood of attaining the antibody level considered to provide long-term protection against influenza A/H2N2. Studies of infectious disease or disease resistance may provide additional insight on the relevance of reduced antibody response for health outcomes; however, there are few studies of PFOA or PFOS and infectious disease and the body of

evidence for human studies provides low confidence for PFOA or PFOS associations with infectious disease as discussed in the following section.

Taken together, these studies provide evidence that higher developmental, childhood, or adult serum concentrations of PFOA and PFOS are associated with lower specific antibody responses to commonly used vaccines. The data are considered a consistent pattern of findings for PFOA- and PFOS-associated antibody suppression. The animal and *in vitro* studies on the antibody response are presented below for PFOA (see **Human Antibody Response Data for PFOS** for further discussion of the PFOS antibody response data).

Animal Antibody Response Data for PFOA

Summary: There is high confidence that exposure to PFOA is associated with suppression of the antibody response based on the available animal studies. The results show consistent suppression of the primary antibody response in mice (see **Figure D6** for data figure and study details and **Table 12** for confidence ratings summary for the body of evidence). Confidence in the body of evidence was decreased because of serious concern for risk of bias and increased for evidence of dose-response observed across multiple studies (**Figure D6**) to support the final rating of high confidence. Half of the studies were rated probably high or definitely high risk of bias for exposure characterization (one of the Key Questions) due to use of PFOA <98% purity without independent confirmation. In addition, all of the studies in mammals were rated probably high risk of bias for lack of allocation concealment and lack of researcher blinding during the study (see **Figure D12** and **Figure D13**). Although a conservative approach was taken to downgrade for risk of bias, the studies with probably high risk of bias for exposure characterization reported suppression of the antibody response at similar doses of PFOA as studies with probably low risk of bias for exposure.

There is consistent evidence that PFOA exposure results in suppression of the primary antibody response as determined by antigen-specific IgM antibody production to single challenge with T-cell specific antigens (SRBC and HRBC) in male and female mice (Yang *et al.* 2002a, Dewitt *et al.* 2008, Loveless *et al.* 2008, DeWitt *et al.* 2009a, Vetvicka and Vetvickova 2013, DeWitt *et al.* 2016) (**Figure D6**) at oral doses from 3.75 to 30 mg/kg/day. PFOA exposure of male C57BL/6 mice via diet for 10-16 days was associated with suppression of the primary IgG response as indicated by lower antibody levels for multiple IgG isotypes (IgG1, IgG2, IgG3) (Yang *et al.* 2002a) (**Figure D7**). Antibody suppression in the lower dose range (3.75 to 15 mg/kg/day PFOA) takes place without changes in body weight, spleen or thymus cellularity, or other signs of overt toxicity (e.g., DeWitt *et al.* 2009a).

The antibody response data from mice demonstrate a consistent pattern of findings to support PFOA-associated suppression. The heterogeneity in the data can be explained by differences in the antibody response by species (mice vs rats) and outcome (primary vs secondary response). Rats appear to be less susceptible than mice to PFOA-associated antibody suppression, and the one rat study reported no effect of PFOA up to 30 mg/kg/day on the antibody response (Loveless *et al.* 2008). This may be a result of rapid clearance of PFOA in rats relative to mice (e.g., estimated half-life for PFOA in female rats is 2-4 hours compared to 20-30 days in mice Rodriguez *et al.* 2009). There is limited data on the secondary IgG response from which to draw a conclusion on the effect of PFOA exposure; it was not suppressed in the one study testing it in mice (Dewitt *et al.* 2008) or in Japanese quail (Smits and Nain 2013). In the one developmental exposure study (Hu *et al.* 2010), doses up to 1 mg/kg/day PFOA on GD 7-16 (just below the lowest observed adverse effect level [LOAEL] of 3.75 in other studies) were not associated with suppression of the antibody response in C57BL/6 mice.

The primary antibody response to T-cell specific antigens as determined by antigen-specific IgM is considered among the most predictive measures of immunotoxicity and part of multiple testing

guidelines. Therefore, the data are considered a consistent pattern of findings for PFOA-associated antibody suppression.

In Vitro /Mechanistic Data on Antibody Response for PFOA

The results of *in vitro* antibody response assays can be highly variable; however, Mishell-Dutton assays that include the key cell types for producing a T-cell dependent antibody response (antigen presenting cells, T-cells and B-cells) have been successful in demonstrating reduced antibody production *in vitro* for known immunotoxic chemicals (Fischer *et al.* 2011, Hartung and Corsini 2013). No *in vitro* studies were located that tested PFOA or PFOS effects on antibody production using a Mishell-Dutton assay with antigen (e.g., SRBC) challenge. Two studies were identified that examined the effects of PFOA on IgM antibody secretion and surface IgM expression in B-cells in the absence of antigen challenge. Levitt *et al.* (1986, 1987) tested the effect of high concentrations of PFOA 0.75 mM (~310000 ng/ml) on antibody production in human (F4 and Hurwitz) and mouse (HPCM2) B-cell lines cultured *in vitro*. At these high concentrations, PFOA had no effect on *in vitro* IgM synthesis or expression of surface IgM. Higher concentrations (0.8 mM or ~330000 ng/ml and above) were associated with decreased cell viability for all three B-cell lines. Messina *et al.* (2007a, 2007b) reported that *in vitro* exposure to high concentrations of the sodium salt of PFOA (0.1-6 mM or 43600 to 2616000 ng/ml) induced conformational changes in human IgG; however, IgG binding or functionality were not tested.

The available assays were considered less informative or indirect evidence for predicting effects on the antibody response because they fail to include antigen stimulation, do not involve necessary cell types to test cell-to-cell interactions, and these unstimulated B-cell cultures are not generally supported as predictive for the antibody response. In addition, the concentrations of PFOA tested are well above typical human exposure range of the general population [e.g., Olsen *et al.* reported a range of 1.9 to 52.3 ng/ml PFOA in serum from American Red Cross blood donors (2003b), Looker *et al.* (2014) reported a range of 0.25 to 2140 ng/ml PFOA in serum of residents of the Ohio Valley as part of the C8 studies and the latest NHANES for 1999-2012 reported mean (95% CI) for serum PFOA was 2.08 ng/ml (1.95-2.22)](CDC 2015). The concentrations tested were even above high occupational exposure levels [serum range from 200 to 91900 ng/ml PFOA (Costa *et al.* 2009)]. Finally, both studies were rated probably high risk of bias for multiple questions including exposure characterization (because purity of PFOA was not reported) and outcome assessment (due to lack of blinding of outcome assessors) (see **Figure A3-4**).

The two *in vitro* assays were not considered to provide strong support or opposition for evaluating the biological plausibility of PFOA effects on the antibody response because the study design does not directly address the antibody response, only high concentrations were tested, and there are multiple risk of bias concerns. No other *in vitro* or mechanistic studies were located that directly test the antibody response. Other mechanistic data relevant for evaluating potential PFOA effects on the antibody response are discussed below in the evidence synthesis section in the context of biological plausibility. Relevant mechanistic data would include PFOA effects on antigen processing and cell activation, cytokines important for cell signaling during the antibody response, and changes in key cell populations including B- and T-cells.

Table 12. Antibody Response Evidence Profile for PFOA										
	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					**Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence**				
INITIAL CONFIDENCE for each body of evidence (# of studies)	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	FINAL CONFIDENCE RATING
PFOA										
Human										
Initial Moderate (4 prospective studies)[a]	---	---	---	---	---	---	---	---	---	**Moderate**
Initial Low (2 cross-sectional studies)[b]	---	---	---	---	---	---	---	---	---	**Low**
Confidence Across Human Bodies of Evidence	No change for considering across study designs									**Moderate**
Animal										
Initial High (7 mammal studies)[c]	↓	---	---	---	---	---	↑	---	---	**High**
References: Human: Granum (2013)[a], Grandjean (2012)[a], Kielsen (2016)[a], Looker (2014)[a], Mogensen (2015)[a], Stein (2016)[b] Animal[c]: DeWitt (2008, 2009a, 2016), Hu (2010), Loveless (2008), Vetvicka (2013), Yang (2002a)										

Evidence Synthesis for Antibody Response for PFOA

Higher serum levels of PFOA are associated with suppression of the antibody response based on the human and animal bodies of evidence (see **Table 12** for confidence ratings summaries for the bodies of evidence). There is moderate confidence that exposure to PFOA is associated with suppression of the antibody response in humans based on consistent suppression in at least one measure of the anti-vaccine antibody response across multiple studies with evidence from prenatal, childhood, and adult exposures to PFOA. There is high confidence that exposure to PFOA is associated with suppression of the antibody response in animals based on consistent suppression of the primary antibody response in mice. These confidence ratings translate directly into level-of-evidence conclusions and support an initial hazard identification conclusion of *presumed to be an immune hazard to humans* or PFOA exposure is presumed to suppress the antibody response in humans.

- **Human body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Animal body of evidence:** High Confidence = High Level of Evidence
- **Initial hazard conclusion (Moderate x High)** = Presumed to be an Immune Hazard to Humans
- **Final hazard conclusion (after consideration of biological plausibility)** = Presumed to be an Immune Hazard to Humans

Taken together, the human and animal bodies of evidence present a consistent pattern of findings that higher prenatal, childhood, or adult serum concentrations of PFOA are associated with suppression of the antibody response. Mechanistic data from *in vitro* or *in vivo* studies can then be used to examine the biological plausibility of PFOA- associated suppression of the antibody response to develop the final hazard identification conclusion.

The antibody response begins with B-cell surface antibody recognizing a specific antigen. Then, for a T-cell dependent antibody response (e.g., most of the experimental animal data on SRBC-specific antibody response), T-cells must also recognize the specific antigen involved (generally after processing by a macrophage, dendritic cell, or other antigen presenting cell). When B- and T-cells both recognize the same antigen, the T-cell activates the B-cell and releases cytokines to help the B-cell multiply and mature into an antibody secreting plasma cells that produces the antigen-specific antibody response. Therefore, relevant mechanistic data would include effects of PFOA at relevant concentrations on key cell populations (B-cells, T-cells, or macrophages as antigen presenting cells), antigen processing and cell activation, or cytokines important for cell signaling during the antibody response.

PFOA-related decrease in B-cell or T-cell numbers would present a possible mechanism for reduced antibody response if it was observed at the same or lower concentrations at which reduced antibody response was observed. However, there is inconsistent evidence of reduced B-cell number (see B cells (B220)) at higher exposure levels for PFOA (≥20 mg/kg) and no change in B-cell or T-cell (CD4 or CD8 subpopulations) numbers observed at lower doses of PFOA (3.75 mg/kg) associated with decreased antibody levels (see cell phenotyping data, spleen and thymus cellularity in **Appendix 5**) (DeWitt *et al.* 2012, DeWitt *et al.* 2016). Similarly, at lower exposure levels of PFOA, there are no changes in percentage or cell numbers of macrophages or other antigen presenting cells (Qazi *et al.* 2009a). Overall changes in leukocyte numbers and cellularity in the spleen and thymus are also not affected at lower doses of PFOA. There is no evidence that PFOA-induced changes in cell populations could explain the reduced antibody response at lower doses.

Cytokine release of interleukin-4 (IL-4), IL-5, and IL-6 by T-cells are important for T-cell dependent antibody response (e.g., to SRBC). There are few studies of the potential effect of PFOA on these cytokines, with most data coming from effects on macrophages from the spleen and peritoneal cavity in mice (see cytokine data in **Appendix 5**). PFOA exposure was associated with increased IL-6 secretion from mouse macrophages in culture (Qazi *et al.* 2009a). As would be expected, the specific effects of PFOA on cytokine secretion vary by dose, cell type, and stimulation conditions.

The effects of *in vitro* exposure to PFOA on IL-4 and IL-6 secretion have also been explored in several studies using whole blood from human volunteers. Plasma levels of PFOA from the human volunteers were associated with increased IL-6 under LPS stimulation suggesting B-cell or monocyte origin (Brieger *et al.* 2011). In contrast, *in vitro* exposure at doses 100 to 100000 ng/ml of PFOA had no impact on IL-6 (Brieger *et al.* 2011). In a separate set of studies using human cells, Corsini *et al.* (2011, 2012) reported that *in vitro* exposure to PFOA reduced IL-6 and IL-4 levels, but only at the highest doses tested (≥10000 ng/ml PFOA). The results also suggest that PFOA-associated changes in cytokines may be peroxisome proliferator-activated receptor alpha (PPARα) dependent based on a role for PPARα in PFOA-mediated inhibition of other cytokines (IL-8 and TNF-α) (Corsini *et al.* 2011). In further tests of potential mechanisms for the observed cytokine changes, Corsini *et al.* (2011, 2012) demonstrated that PFOA inhibited pathways that regulate NF-κB activation, which plays a role in cytokine production as well as apoptosis, inflammation, and other immune functions.

Although there is evidence that PFOA exposure *in vivo* to mice and *in vitro* to human peripheral blood alter levels of IL-4 and IL-6, further study is necessary to demonstrate consistent changes in cytokines at relevant exposure concentrations (e.g., see DeWitt *et al.* 2012 for review). Unfortunately, there are no studies that identify the specific cell types involved or link the cytokine changes to suppression of the antibody response. These cytokines have multiple physiological roles and may reflect inflammation rather than changes in antibody-related cell signaling.

The potential role of peroxisome proliferator-activated receptor alpha (PPARα) in the mechanism for immune effects was considered because PFOA activates mouse PPARα (the primary PPAR expressed in lymphocytes). A number of PPARα-activating compounds cause liver tumors in rodents and the human relevance of these tumors is subject to debate because of lower levels and/or lower activity of PPARα in human liver (NRC 2006, Corton 2010, Post *et al.* 2012). However, uncertainty over the human relevance does not necessarily apply to non-hepatic effects mediated by PPARα (Post *et al.* 2012). Some of the health effects observed in experimental animals have been linked to the ability of PFOA to activate PPARα, and others have been shown to be independent of PPARα. For example, developmental effects of PFOA including neonatal lethality were shown to be PPARα-dependent (Abbott *et al.* 2007), while PFOS induced neonatal lethality and delayed eye opening was independent of PPARα (Abbott *et al.* 2009). PPARα appears to play a role in several immune effects of PFOA in mice, including decreased spleen and thymus weight, reduced spleen and thymus cellularity, and mitogen (ConA and LPS)-induced lymphoproliferation (Yang *et al.* 2002b). Yang *et al.* (2002b) reported that PFOA (0.02% diet or approximately 40 mg/kg/day) resulted in decreased spleen and thymus weight and cellularity in wild-type mice (C57BL/6), but not in PPARα knockout mice (SV/129). The background SV/129 mouse strain for the PPARα knockout used by Yang *et al.* (2002b) appears to be less sensitive to PFOA than others (e.g., C57BL/6); however, PPARα-dependence has been reported for reduced spleen and thymus weight in other strains. Dewitt *et al.* (2016) reported that PFOA (30 mg/kg/day) reduced spleen and thymus weight in wild-type mice (C57BL/6), but not in PPARα knockout mice (C57BL/6).

In contrast to the importance of PPARα for reduced organ weight at high doses of PFOA, targeted immune studies suggest that PFOA- and PFOS-associated suppression of the antibody response in mice are independent of PPARα (reviewed in DeWitt *et al.* 2009b, DeWitt *et al.* 2012). Several studies demonstrated antibody suppression in PPARα knockout mice (DeWitt *et al.* 2009b, DeWitt *et al.* 2016). Dewitt *et al.* (2016) reported PFOA-associated (30 mg/kg/day) suppression of the antibody response in both wild-type (C57BL/6) and PPARα knockout mice (C57BL/6). While PPARα appears to contribute to reduced organ weight and changes in immune cell populations at higher doses (30-40 mg/kg PFOA), there is no indication that PPARα is involved at lower doses associated with reduced antibody response (i.e., 3.75 mg/kg/day PFOA). Although no *in vivo* studies directly examined the role of PPARα in human immune outcomes, *in vitro* studies demonstrated that PFOA only altered cytokines at high doses (100000 ng/ml) and the effects on some cytokines (IL-8 and TNF-α) were PPARα-dependent (Corsini *et al.* 2011).

There is some evidence that immunotoxicity in CD-1 mice may involve stress and systemic effects, particularly at higher doses (10-30 mg/kg/day PFOA) (Loveless *et al.* 2008); however, reduced antibody response at lower doses (3.75 to 15 mg/kg/day PFOA) in C57BL6 mice were not stress or corticosterone mediated (DeWitt *et al.* 2009a).

The mechanisms for PFOA-associated suppression of the antibody response are not fully understood at this time (DeWitt *et al.* 2012 for review) and the mechanistic data were not considered to provide evidence to support or refute biological plausibility of this affect. Furthermore, the animal data indicates that effects of PFOA on the antibody response are independent of PPARα. Hazard conclusions are based on a consistent pattern of findings from both the human and animal bodies of evidence that exposure to PFOA is associated with suppression of the antibody response. Therefore, the antibody data support a final hazard identification conclusion that PFOA is *presumed to be an immune hazard in humans*.

Immunosuppression: Disease Resistance/Infectious Disease Outcomes

Direct measures of infectious disease incidence or severity such as respiratory tract infections, pneumonia or otitis media are clearly relevant for evaluating potential immunotoxicity in humans. In

experimental animals, disease outcomes are generally referred to as "disease resistance", and there are a number of disease resistance models that evaluate the animal's ability to defend against viral, bacterial, or parasitic infections. Endpoints include direct measures of disease resistance such as viral or parasitic load in target tissues, indirect measures such as body weight after infection, and mortality. Disease resistance assays are considered some of the best indicators of immunotoxicity because they generally measure clearly adverse health outcomes and therefore these assays are included in most guidelines or testing requirements for immunotoxicity (ICH 2005, WHO 2012).

Human Infectious Disease Data for PFOA

Summary: There is low confidence that exposure to PFOA is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease). Two of three prospective studies that examined the relationship between maternal PFOA exposure and disease outcomes in offspring reported some evidence of PFOA-associated increases in infectious disease (Fei *et al.* 2010, Granum *et al.* 2013) and no association was found in the third prospective study (Okada *et al.* 2012) or the single adult cross-sectional study (Looker *et al.* 2014). Confidence in the body of evidence for the three prospective studies was decreased for a lack of consistency across studies, and within the Fei *et al.* (2010) study by sex (PFOA was associated with increased hospitalization in girls, not boys) or age group analyzed (PFOA was associated with increased hospitalization in analyses combining ages 0-10, but not for individual age groups), to support a final rating of low confidence (see **Table 13** for list of studies, **Figure D16**, **Figure D17** and **Figure D18** for data figures). As discussed below, the fact that few specific infectious disease endpoints have been examined (e.g., data are restricted to colds, influenza, gastroenteritis and otitis media) contributes to the low confidence for drawing a conclusion on infectious disease in general. In contrast, the findings by Fei *et al.* (2010) of an association between maternal PFOA and what is likely to be a less sensitive measure of disease (i.e., hospitalization for any infectious disease, which would only capture the most severe outcomes and could miss potential associations with individual diseases) contributes to the confidence in the association.

The available epidemiological studies in the human body of evidence that evaluated the association between exposure to PFOA and infectious disease resistance include: (1) three prospective birth cohort studies in Demark, Norway and Japan that evaluated maternal exposure and (2) a cross-sectional analysis of adult residents in the Ohio Valley with higher PFOA in drinking water (part of the C8 studies) (see **Table 13** for study details). Analysis in the adult residents of the Ohio Valley was done on concurrent PFOA levels although the major source of exposure to PFOA occurred in the past. The Danish birth cohort study evaluated hospitalization from infectious diseases (which is of greater severity and likely results in less sensitivity) and the remaining studies looked at any or the frequency of self-reported common infections (the specific infections examined varied across studies). The birth cohorts also varied in the time period between exposure (birth) and measurement of the disease and on their statistical models (e.g., β-coefficients/linear regression, Poisson regression). The Danish birth cohort study and the C8 study evaluated exposure response relationships using quartiles of exposure to PFOA (the lowest exposure group served as the comparison group) and the latter also calculated ORs for continuous log transformed PFOA level. Both the Danish and Japanese study reported findings for males and females separately as well as combined (see **Table 13** for additional details). As discussed in the previous section, the Norway study and the C8 study also reported findings for antibody responses to vaccines.

Table 13. Studies on Infectious Disease in Humans (3 prospective; 1 cross-sectional)						
Study	**Study design (Location/Study) [n cohort]**	**n cases**	**Exposure measure timing**	**Disease assessment timing**	**Analysis**	**Infectious disease outcomes assessed**
Fei (2010)	Prospective (subset of the Danish National Birth Cohort, record linkage) [1400]	363 children (577 hospital-izations)	Maternal	from birth to age 10	Poisson regression IRR; trend; comparison group lowest exposure	hospitalization for infectious disease link to hospital records; different results by age-group analyzed: inconsistent age 0-1, 1-2, 2-4, 4-10 years; age 0-10 years: girls ↑*,†; boys ↓*
Granum (2013)	Prospective (Norwegian/ MoBa birth cohort) [99]	94 common cold; 87 gastro-enteritis; 27 otitis media	Maternal 0-3d post delivery	From birth to age 3	**Any disease:** log-istic regression OR **Frequency:** linear regression β coefficient	self-reported common cold↑*, gastroenteritis↑*, otitis media
Okada (2012)	Prospective (Sapporo Japan birth cohort) [343]	61	Maternal (log transformed)	From birth to age 18 months	Logistic regression OR for 10-fold increase in individual PFAAs (PFOA, PFOS)	self-reported otitis media (any, frequency)
Looker (2014)	Cross-sectional (USA OH/WV residents elevated PFOA in drinking water) [755]	163 flu; 538 colds	Adult: current (quartiles and log transformed)	Adult: previous 12 months	Logistic regression OR	self-reported cold or influenza (any, frequency)
significantly increased (↑) or decreased (↓) associated with (*) PFOA or (†) PFOS exposure						

As described in **Table 14**, two of three prospective studies that examined the relationship between maternal PFOA exposure and disease outcomes in offspring reported evidence of PFOA-associated increase in infectious disease (Fei *et al.* 2010, Granum *et al.* 2013) and no association was found in the single adult cross-sectional study. Higher maternal plasma concentrations of PFOA were associated with increased number of episodes of common cold and gastroenteritis in children up to age 3 from a sub-cohort of the MoBa study (n = 63-93)(Granum *et al.* 2013). The Granum *et al.* (2013) study provides evidence for PFOA-associated increases in infectious disease incidence in children from a study with a relatively small sample size (n = 63-93) (see **Table 14, Figure D16** and **Figure D17**). The number of episodes of common cold and gastroenteritis may be more sensitive measure of disease than incidence data; however, this cannot be assessed in this dataset as no other studies examined number of episodes for disease endpoints in children. Within the Granum *et al.* (2013) study, no associations were detected when examining common cold or gastroenteritis as a binary outcome (yes/no), and incidence of otitis media was not associated with PFOA.

Fei *et al.* (2010) examined the association between maternal PFOA and hospitalizations for infectious diseases in children (n = 1400; from birth to age 10 years) from a subset of the Danish National Birth Cohort (1996-2002) with outcomes based on National Hospital Discharge Register. The Fei *et al.* (2010) study only evaluated infectious disease outcomes that were severe enough to warrant hospitalization and therefore the outcome was likely to be a less sensitive measure of disease incidence than doctor diagnosis or prescription-based measures. The authors state that there were too few hospitalization events to support infection-specific analyses, and therefore potential associations with specific infections could have been missed. Analyses of the association between maternal PFOA and hospitalization by different age groups (0-1, 1-2, 2-4, and 4-10 years of age) were inconclusive without clear increase or decrease in hospitalization. However, in analyses of the combined age group (0-10 years of age) and split by sex, maternal PFOA was associated with increased incidence of hospitalization in girls and decreased hospitalization in boys (**Table 14** and **Figure D18**). This study also evaluated and reported similar results with maternal PFOS.

The third prospective study evaluated otitis media and reported that no relationship was found between maternal PFOA and otitis media in children at 18 months of age in a prospective cohort in Sapporo Japan (n = 343) (Okada *et al.* 2012). Although data were collected on chicken pox, bronchitis, RSV disease, pneumonia and other infectious diseases, the low number of cases (n<20) did not allow analyses of these outcomes. The outcomes in the Okada *et al.* (2012) study were assessed by mothers' self-administered questionnaire. Therefore, outcome assessment was rated probably high risk of bias given there was no evidence that the questionnaire was validated (and the authors did not respond to email request for clarification; see **Figure D21**).

Table 14. Summary of PFOA and Selected Data on Infectious Disease in Humans

Disease	PFOA Results	Possible sources of heterogeneity	Exposure measurement timing	Study
Hospitalization for infectious diseases	**Adj. IRR (95% CI) Highest vs. lowest** All: 0.84 (0.62–1.13) **F: 1.74 (1.06–2.87)** **M: 0.57 (0.38–0.86)** **Trend** All: 0.96 (0.87–1.06) **F: 1.21 (1.04–1.42)** **M: 0.83 (0.73–0.95)**	• lower sensitivity of the outcome (hospitalization vs. incidence) • no measure of specific diseases for incidence or frequency • developmental exposure metric	Maternal	Fei (2010)
Gastroenteritis (No. episodes/ frequency)	**β (95% CI)[a]** **0.31 (0.00-0.61) p=0.048**	• only study of this disease • frequency as measure • developmental exposure metric	Maternal 0-3 day post delivery	Granum (2013)
Otitis media (any)	NR (no association)	• developmental exposure metric • incidence as measure	Maternal 0-3 day post delivery	Granum (2013)
	Adj. OR (95% CI) All: 1.51 (0.45–5.12) F: 0.95 (0.16–5.69) M. 1.92 (0.35–10.40)	• developmental exposure metric • incidence as measure	Maternal	Okada (2012)
Common cold (No. episodes/ frequency)	**β (95% CI) 3 yrs[a]** **0.42 (0.21–0.72) p<0.001**	• developmental exposure metric • frequency as measure	Maternal 0-3d post delivery	Granum (2013)
	Adj. OR (95%CI) (continuous)[b] 0.91 (0.70–1.19)	• developmental exposure metric • frequency as measure	Adult Current	Looker (2014)
Flu (any)	**Adj. OR (95%CI)** (continuous)[b] 0.98 (0.70–1.38)	• outcome in adults	Adult Current	Looker (2014)

[a]multivariate models

[b] no association observed in categorical analyses

F=female; M=male; NR = not reported; bold text indicates statistically significant decreased or increased association.

This table highlights select findings to illustrate data informing the potential relationship with infectious disease; for additional data see **Figure D16**, **Figure D17** and **Figure D18**.

In adults, current serum concentrations of PFOA were not associated with self-reported cold or influenza over the previous 12 months despite evidence of PFOA-associated suppression of the antibody response to influenza A/H3N2 in a cross-sectional analysis of adults (n = 403) from the C8 study living in the Ohio Valley, a region with elevated drinking water PFOA levels (Looker *et al.* 2014). A retrospective mortality study on workers at a US polymer manufacturing facility was identified as it performed some analyses for potential disease relationships with PFOA (Leonard *et al.* 2008); however, that study was not utilized in developing conclusions as the study was not considered to provide reliable data on infectious disease incidence because it relied on cause of death from mortality data rather than measures of disease incidence for infectious disease. Therefore, the Leonard *et al.* (2008) study was rated definitely high risk of bias for outcome assessment. The Leonard *et al.* (2008) study was also rated, definitely high risk of bias for exposure given the lack of any quantitative exposure data and probably high risk of bias for failing to account for important confounders given the lack of adjustment for a number of confounders including smoking, alcohol consumption, BMI, and potential co-exposures given the manufacturing setting of the study (see **Figure D21**).

As discussed in the antibody response section above, exposure characterization risk of bias assessment examined the consistency and reliability of the exposure measures such as use of established test methods (e.g., high pressure liquid chromatography with tandem mass spectrometry) and whether exposure was assessed in a relevant time-window for development of the outcome (see protocol http://ntp.niehs.nih.gov/go/749926 for details). Maternal serum PFOA was used as the exposure metric for all three studies examining potential effects of prenatal exposure on infectious disease in children (Fei *et al.* 2010, Okada *et al.* 2012, Granum *et al.* 2013). Although the studies cannot exclude the potential impact of post-natal exposure directly to children, maternal concentrations of PFOA are considered good measures of fetal exposure due to the strong positive correlations (e.g., Pearson's correlation between 0.79 – 0.93) reported between maternal PFOA and cord PFOA (Glynn *et al.* 2012). Given the slow clearance and long biological half-life of PFOA in humans (2 to 8 years)(Olsen *et al.* 2007a, Kudo 2015), a single sample during pregnancy is considered a good measure of PFOA exposure. Risk-of-bias assessment of important confounders included age, sex, race/ethnicity, smoking, body mass index, alcohol consumption, and variables that represent socioeconomic status based on prior reports of associations with PFOA exposure levels (Calafat *et al.* 2007, Nelson *et al.* 2010) and immune outcomes (WHO 1996, Dallaire *et al.* 2005). Most studies included consideration of all the important confounders listed in the protocol at a minimum but did not adjust for potential effects of other PFAAs. Duration of breastfeeding was considered as a confounder the Granum *et al.* (2013) study because of evidence that breast feeding may support immune function and duration of breastfeeding may affect PFOA exposure as well (Karrman *et al.* 2007). The failure to adjust for breastfeeding in the other studies may result in an underestimation of the association between PFOA and infectious disease incidence. The Fei *et al.* (2010) and Granum *et al.* (2013) studies were rated probably high risk of bias for consideration of confounding for limited ability to differentiate the effects of PFOA from PFOS or other PFAAs. As discussed previously, the Leonard *et al.* (2008) study was not considered in developing conclusions because the study relied on cause of death from mortality registry. All of the studies considered in developing conclusions were rated probably low or definitely low risk of bias for exposure characterization. Most studies were rated probably low risk of bias for infectious disease outcome assessment as methods included questionnaires relying on medical records, assessment by interviewer, or parent's report of doctor diagnosis (see **Figure D20** and **Figure D21**).

Relationship to antibody response data for PFOA

Studies of infectious disease may provide additional insight on health outcomes potentially associated with reduced antibody response. However, only two human studies measured antibody response to vaccination and infectious disease outcomes in the same populations. One study provides support for PFOA-associated decreased antibody response and infectious disease (Granum *et al.* 2013) and the other does not (Looker *et al.* 2014). There is low confidence in this body of evidence and limited ability to support or refute immunosuppression-related health effects that would be expected based on PFOA-associated reduced antibody response.

Maternal plasma concentration of PFOA was associated with increased number of episodes of common cold and gastroenteritis in children up to age three (Granum *et al.* 2013) in the same population that PFOA was associated with lower antibody levels to rubella vaccination. The consistency of finding for these immunosuppression-related outcomes that could be causally related (i.e., reduced antibody response leading to increased incidence of infectious disease) supports both effects, although it is a relatively small study (n = 49 to 93). It may be worth noting that Granum *et al.* (2013) also measured PFOA, PFNA, and PFHxS and reported maternal PFOA, PFNA, and PFHxS were associated with both lower antibody levels to vaccination and increased number of episodes of common cold (PFOA, PFNA, and PFHxS) and gastroenteritis (PFOA, PFHxS) in children. The strength of the association between PFOA (β =-0.40[-0.64, -0.17]), PFNA (β =-1.26[-2.32, -0.20]), and PFHxS (β =-0.38[-0.66, -0.11]) and lower

antibody levels are stronger than the association with PFOS (β =-0.08[-0.14, -0.02]). The weaker association with PFOS and lower antibody levels may explain why the Granum *et al.* (2013) study did not find an association between PFOS and infectious disease. As discussed for the antibody response, the effect of PFAAs on infectious disease resistance appears to be in the same direction (reduced ability to respond to infectious disease or increased incidence of disease). The effects of individual PFAAs on the disease resistance can be considered effect modifiers, rather than true confounders of the association between exposures to individual PFAAs (e.g., PFOA and PFOS) and lower disease resistance.

Although the Looker *et al.* (2014) study reported that elevated serum PFOA was associated with suppression of the antibody response to influenza A/H3N2 vaccination (not influenza B or A/H1N1) in adults, there was no evidence for clinical effect on infectious disease (i.e., no increase in colds or influenza). It is unclear why an association between PFOA and infectious disease incidence was not found as would be expected with decreased antibody response. The authors (2014) note several possibilities including: that the extent of antibody suppression may not have been great enough to detect a change in infectious disease given the small population size; the high background rate of colds (>70%) may have made it harder to detect an increase in infectious disease; and the major strain of virus circulating (A/H1N1) was different from the strain for which the antibody response was lower (A/H3N2).

Animal Disease Resistance Data for PFOA

Summary: There is very low confidence that exposure to PFOA is associated with a change in the ability of animals to respond to infectious disease because there are no experimental studies on disease resistance endpoints in mammals and the wildlife studies have serious risk of bias concerns. PFOA (2.1 mg/kg/day for 8 weeks) had no effect on morbidity or mortality to *E. coli* exposure in Japanese quail (Smits and Nain 2013); however, this study would be downgraded for indirectness if used because it used a non-mammalian research model. Two wildlife studies were identified that reported PFOA exposure levels in relation to disease status. Higher concentrations of PFOA were found in sea otters with clinical signs of disease compared to healthy animals (Kannan *et al.* 2006). There was no difference in PFOA levels between bats in populations with white-nose syndrome compared to a healthy reference population (Kannan *et al.* 2010). Confidence in the body of evidence for the mammalian wildlife studies was decreased because of serious concern for risk of bias to support the final rating of very low confidence (see **Figure D19** for data figure and study details). Both wildlife studies (Kannan *et al.* 2006, Kannan *et al.* 2010) were rated probably high risk of bias for two of the key risk of bias questions: (1) exposure characterization and (2) confounding (**Figure D22**). There were concerns in the sea otter study that exposure measurements taken from the liver of animals found dead (generally the diseased animals) may bias exposure when compared to live animals (Kannan *et al.* 2006); and the bat study was rated probably high risk of bias for exposure because there was no difference in exposure levels between the reference and diseased population (Kannan *et al.* 2010). Both studies were rated probably high risk of bias for confounding for failure to consider important confounders including other immunotoxicants (e.g., PCBs and butyltins) shown to be elevated in the wildlife populations examined.

Evidence Synthesis for Disease Resistance/Infectious Disease Outcomes for PFOA

For PFOA, there are no experimental animal studies in mammalian species and few human epidemiological studies available to evaluate the potential relationship between PFOA exposure and infectious disease resistance. Two of three prospective studies reported that higher maternal plasma concentrations of PFOA were associated with a disease outcome in offspring. There is low confidence from the human body of evidence that exposure to PFOA is associated with increased incidence of infectious disease due to lack of consistency across studies and within the Fei *et al.* (2010) study by sex or age group analyzed. The very low confidence in the animal evidence translates into inadequate level of evidence and the low confidence in the human body of evidence translates directly into a low level of

evidence. Therefore, an evidence profile or detailed discussions of the evidence synthesis were not developed for PFOA and disease resistance and this health effect was not considered for hazard identification conclusions.

Immunosuppression: Natural Killer (NK) Cell Activity

Natural killer (NK) cells are important for resistance against viruses and tumor cells. Successful defense by NK cells involves killing of target cell through release of cytolytic granules or inducing apoptosis (Dietert 2010). Assays for NK cell activity are included in many immunotoxicity testing guidelines as a measure of immune function because they are considered good predictors for overall immunotoxicity (Luster *et al.* 1992, US EPA 1998, ICH 2005, WHO 2012).

Human NK Data for PFOA

No human data were identified on potential association between PFOA and NK cell activity.

Animal NK Data for PFOA

Summary: There is very low confidence that exposure to PFOA is associated with suppression of NK cell activity in animals because the body of evidence is from a single experimental mouse study at a single dose (20 mg/kg/day) with very serious risk of bias concerns that reported PFOA-associated suppression of NK cell activity (Vetvicka and Vetvickova 2013) and a wildlife study in dolphins (Fair *et al.* 2013) that did not find any association between serum PFOA (mean 55ng/ml; range 0.5-561 ng/ml) and NK cell activity. Confidence in the body of evidence was downgraded twice for very serious concern for risk of bias in the Vetvicka and Vetvickova (2013) study and also downgraded because of serious concern for unexplained inconsistency because all of the experimental data are from a single study and a single dose (**Figure D23**) so there is no ability to evaluate consistency across doses or studies to support the final rating of very low confidence.

There were multiple risk of bias concerns to support a very serious risk of bias rating for the Vetvicka and Vetvickova (2013) study: (1) the study was rated as probably high risk of bias for all 3 Key Questions (failure to randomize animals to treatment groups, exposure characterization for use of PFOA with below 98% purity without independent confirmation of purity, and outcome assessment [due to lack of blinding of outcome assessors]); and (2) the study was rated probably high risk of bias for lack of allocation concealment and researcher blinding during study (**Figure D26**).

In vitro /Mechanistic Data on NK Cell Activity for PFOA

One study was identified that evaluated NK cell activity following *in vitro* exposure to PFOA. In a small pilot study of 11 volunteers, Brieger *et al.* (2011) tested the effect of *in vitro* exposure to PFOA on NK cell activity in human cells. Exposure consisted of pre-treatment of peripheral blood cells with 0, 1000, 10000, or 100000 ng/ml PFOA for 24 hours and PFOA had no effect (Brieger *et al.* 2011)(see **Figure D24**). Concentrations used for the NK cell assay had no effect on cell viability. The concentration of PFOA used were well above typical human exposure range of the general population [e.g., Olsen *et al.* (2003b) reported a range of 1.9 to 52.3 ng/ml PFOA in serum from American Red Cross blood donors in 2000-2001 and the latest NHANES data for 1999-2012 reported mean (95% CI) was 2.08 ng/ml (1.95-2.22) for serum PFOA (CDC 2015)].

Evidence Synthesis for NK Cell Activity for PFOA

There is no human evidence to evaluate the potential association between exposure to PFOA and NK cell function because no epidemiological studies of PFOA and NK cell activity were identified. There is very low confidence in the body of evidence for evaluating potential effects of PFOA on NK cell activity

in non-human animals. Both the human and animal evidence translate into inadequate level of evidence. Therefore, an evidence profile table and detailed discussion of the evidence synthesis were not developed for PFOA and NK cell activity and this health effect was not considered for hazard identification conclusions.

Hypersensitivity-related Effects and Outcomes

Sensitization or hypersensitivity-related responses include a number of undesirable or exaggerated immune reactions (e.g., allergies or asthma) to foreign agents. These responses are complicated by the two-phase nature of a hypersensitivity reaction. The first phase, sensitization, is without symptoms and it is during this step that a specific interaction is developed to the sensitizing agent so that the immune system is prepared to react to the next exposure. Once an individual or animal has been sensitized, contact with that same agent leads to the second phase, elicitation, and symptoms of allergic disease. While hypersensitivity responses are mediated by circulating factors such as T-cells, IgE and inflammatory cytokines, many of the health effects associated with hypersensitivity and allergic response are respiratory or dermal (e.g., asthma, airway hyper-responsiveness, and contact dermatitis). Chemicals may exacerbate or promote a hypersensitivity-related outcome without being direct sensitizers.

Human Hypersensitivity Data for PFOA

Summary: There is low confidence that exposure to PFOA during childhood is associated with increased hypersensitivity responses based on the available human studies. Several cross-sectional studies report increased incidence of ever having had a diagnosis of asthma and elevated serum IgE levels in children age 10-19 with higher current serum PFOA concentrations (**Figure D27**). No prospective studies were located that assessed the potential relationship between childhood PFOA exposure and hypersensitivity; however, prospective studies in younger children (birth to age 9) report no association between maternal levels of PFOA and hypersensitivity endpoints (see **Table 15** for list of studies). The low confidence in the body of evidence for studies that evaluated the relationship between childhood PFOA levels and asthma is primarily due to the cross-sectional nature of the studies and uncertainty as to whether exposure levels reflect exposure prior to the development of hypersensitivity. There were no changes in the confidence rating for the body of evidence after considering factors that may increase or decrease confidence (see **Table 17** for confidence ratings summaries for the body of evidence). Heterogeneity in the findings may be explained by differences in the timing of the exposure measures (developmental vs. childhood).

The available epidemiological studies that satisfy the criteria for inclusion in the systematic review and that measured sensitivity or hypersensitivity response in relation to PFOS or PFOA include (1) studies in five birth cohorts conducted in Canada, Norway, Japan, Ukraine, and Taiwan measured maternal exposures or cord blood levels of PFOA and PFOS in relation to outcomes in children from birth through the age of 9; (2) five cross-sectional analyses of children, three of which were NHANES analyses in children ages 12-19, and two studies analyzed children ages 10-15 from the Taiwanese Genetic and Biomarker study for Childhood Asthma, (3) an adult ecological study of communities with elevated drinking water, and (4) a retrospective cohort study of workers exposed to PFOA in the Ohio Valley who were part of the C8 studies (see **Table 15** for study details and **Table 16** for summary of the asthma and IgE data). In addition to study design and age of the population, the studies varied in size (from 49 to 3700), age and timing of the exposure measurement (e.g., maternal or cord blood, or in childhood when hypersensitivity was evaluated), methods for evaluating hypersensitivity/sensitivity response (self-reported physician-diagnosed asthma or allergy (ever or current), total or cord blood IgE, or self-

reported allergic sensitization, food sensitization by specific IgE or self-report), type of statistical analyses (Odds ratios contrasting quartiles or tertiles, or for a shift from 25^{th} to 75^{th} percentile, or for a certain standard deviation change, Log10 quadratic ORs, or β coefficients from linear regression). The prospective birth cohort in Taiwan evaluated different outcomes (IgE in cord blood or at 2 years of age).

Table 15. Studies on Hypersensitivity-related Outcomes in Humans						
Study	**Study design (Location / Study)**	**n**	**Exposure measure timing**	**Disease assessment timing**	**Analysis**	**Hypersensitivity outcomes assessed**
Maternal Exposure (6 prospective)						
Ashley-Martin (2015)	Prospective (Canadian MIREC birth cohort)	1242	Maternal (log transformed)	Birth	ORs for elevated IgE	cord IgE
Granum (2013)	Prospective (Norwegian/ MoBa birth cohort)	63 to 93	Maternal 0-3d post delivery	From birth to age 3	Linear regression (bivariate and multivariate) β coefficient	Eczema/itchiness, wheeze, atopic eczema or asthma
Okada (2012)	Prospective (Sapporo Hospital, Hokkaido Japan birth cohort 2002-2005)	231 to 343	Maternal (log transformed)	From birth to age 18 months	Logistic regression OR for 10-fold increase PFOA or PFOS for symptoms Polynomial regression IgE	food allergy, eczema or wheezing, cord IgE↓*
Okada (2014)	Prospective (One of 37 hospitals, Hokkaido Japan birth cohort 2003-2009)	2062	Maternal (quartiles)	From birth to age 2	Logistic regression OR	wheezing, allergic rhinoconjuctivitis symptoms, eczema↓* total allergic diseases↓*
Smit (2015)	Prospective (Greenland, Ukraine from the INUENDO birth cohort)	1024	Maternal	Children age 5-9	Logistic regression OR (principal component analysis)	asthma, eczema or wheeze↓†
Wang (2011)	Prospective (Taiwan birth cohort)	244	Cord Blood (quartiles)	From birth to age 2	Linear regression β coefficient for log IgE; Logistic regression OR for dermatitis	atopic dermatitis, cord IgE↑*,†, total IgE at 2 years of age
Child Exposure (5 cross-sectional)						
Buser (2016)	Cross-sectional (USA, NHANES 2005-2006 and 2007-2010)	2005-6 (637) 2007-(701)	Child: current (quartiles)	Children (12-19)	Logistic regression OR for food sensitization (1) food-specific IgE or (2) self-reported food allergy	(1) Food allergy indicated by food-specific IgE or (2) self-reported food allergy↑*
Dong (2013) and Zhu (2016)	Case-control (Taiwan, Genetic and Biomarker study for Childhood Asthma)	asthma (231) non (225)	Child: current (quartiles)	Children (10-15)	Logistic regression OR for asthma; Linear regression for continuous measures in children with and without asthma	asthma↑*,†, total IgE↑*,†, eosinophil count↑*,†, eosinophil cationic protein↑*,†; association with asthma stronger in males; males IL-4↑*, IL-5↑*, IL-2, IFN-γ,
Humblet (2014)	Cross-sectional (USA, NHANES 1999-2000 and 2003-2008)	1877	Child: current (linear; ln-linear) (tertiles)	Children (12-19)	Odds of asthma, wheeze with two fold increase individual PFAAs(PFOA, PFOS)	ever having asthma↑*↓†, wheeze↓†
Stein (2016)	Cross-sectional (USA, NHANES 2005-2006)	638	Child: current	Children (12-19)	Linear regression for total IgE per 2-fold increase of individual PFAAs(PFOA, PFOS); Logistic regression OR (shift from 25 to 75 percentile) for effects	current rhinitis↑*, total IgE↑*, current wheeze, current asthma, allergen-specific IgE↓†
Adult Exposure (1 ecological, 1 retrospective cohort)						
Anderson-Mahoney (2008)	Ecological (residents in OH/ WV region with elevated drinking water PFOA levels)	566	None: residence-based comparison to SPR	Current	Standardized Prevalence Ratios (SPRs) computed to compare the exposed group and NHANES 2001-2002	asthma↑*, chronic bronchitis↑*, shortness of breath↑*
Steenland (2015)	Retrospective Cohort (workers exposed to PFOA in OH/ WV part of C8 studies)	3713	Lifetime cumulative estimated (quartiles) (lagged and unlagged)	Self-reported chronic diseases	Cox regression (survival), age as the time variable and time-varying exposure and covariates; Rate ratios (RR) estimated for quartiles	asthma with current medication↓*, COPD
significantly ($p<0.05$) decreased (↓) or increased (↑) associated with (*) PFOA or (†) PFOS exposure						

relative to measures of PFOA/PFOS in cord blood. The study in adults measured self-reported asthma with medication in which the proportion validated was not reported

The available epidemiological studies that satisfy the criteria for inclusion in the systematic review and that measured sensitivity or hypersensitivity response in relation to PFOS or PFOA include (1) studies in five birth cohorts conducted in Canada, Norway, Japan, Ukraine, and Taiwan measured maternal exposures or cord blood levels of PFOA and PFOS in relation to outcomes in children from birth through the age of 9; (2) five cross-sectional analyses of children, three of which were NHANES analyses in children ages 12-19, and two studies analyzed children ages 10-15 from the Taiwanese Genetic and Biomarker study for Childhood Asthma, (3) an adult ecological study of communities with elevated drinking water, and (4) a retrospective cohort study of workers exposed to PFOA in the Ohio Valley who were part of the C8 studies (see **Table 15** for study details and **Table 16** for summary of the asthma and IgE data). In addition to study design and age of the population, the studies varied in size (from 49 to 3700), age and timing of the exposure measurement (e.g., maternal or cord blood, or in childhood when hypersensitivity was evaluated), methods for evaluating hypersensitivity/sensitivity response (self-reported physician-diagnosed asthma or allergy (ever or current), total or cord blood IgE, or self-reported allergic sensitization, food sensitization by specific IgE or self-report), type of statistical analyses (Odds ratios contrasting quartiles or tertiles, or for a shift from 25^{th} to 75^{th} percentile, or for a certain standard deviation change, Log10 quadratic ORs, or β coefficients from linear regression). The prospective birth cohort in Taiwan evaluated different outcomes (IgE in cord blood or at 2 years of age) relative to measures of PFOA/PFOS in cord blood. The study in adults measured self-reported asthma with medication in which the proportion validated was not reported.

Levels of PFOA in pregnant women and hypersensitivity response in children
Maternal serum or plasma concentrations of PFOA were not associated with hypersensitivity across multiple indicators (e.g., asthma or wheeze) in children age 18 months to 9 years across five prospective studies (**Table 15**). Maternal PFOA concentration was not associated with eczema or itchiness, wheeze, or doctor-diagnosed atopic eczema or asthma in 3-year old children from a sub-cohort of the MoBa study (n = 63-93) (Granum *et al.* 2013). Similarly, PFOA was not associated with food allergy, eczema or wheezing in children through 18 months of age from a prospective cohort in Sapporo Hospital Japan (n = 231) (Okada *et al.* 2012). Wang *et al.* (2011) also reported that maternal PFOA was not associated with atopic dermatitis at 2 years of age in a prospective cohort in Taiwan (n = 244). Okada *et al.* (2014) reported that maternal PFOA was not associated with wheezing or allergic rhinoconjuctivitis symptoms; however, there was a significant trend for PFOA-associated reductions in eczema ($p = 0.032$) and total allergic diseases ($p = 0.030$) in children through 2 years of age from a prospective cohort in Hokkaido Japan (n = 2062). In a fifth prospective cohort study that examined children at 5-9 years of age, maternal PFOA was not associated with asthma, eczema or wheeze in children from Greenland or Ukraine (n = 1024) (Smit *et al.* 2015).

The body of evidence with exposure measured during development presents inconclusive results and for most studies with maternal exposure measures there is a lack of an association between maternal PFOA concentrations and hypersensitivity outcomes in children; however, there is conflicting evidence that maternal or cord PFOA is associated with cord blood levels of IgE. Maternal PFOA was associated with decreased cord IgE levels in female infants from the Japanese Sapporo birth cohort (Okada *et al.* 2012). In contrast, cord serum PFOA was associated with increased cord IgE for male infants of the Taiwan Birth Panel cohort (Wang *et al.* 2011). In a third study, there was no association between maternal PFOA and cord IgE in the Canadian MIREC cohort (n = 1242)(Ashley-Martin *et al.* 2015).

Table 16. Summary of PFOA and Selected Data on Hypersensitivity in Humans

Disease	PFOA	Possible sources of heterogeneity	Exposure measurement	Study
Asthma	Adj OR (95% CI) 4th vs. lowest quartile **OR = 4.05 (2.21 – 7.42)** 3rd vs. lowest quartile **OR = 2.67 (1.49 – 4.79)** **Trend p<0.001 (males + females)**	• ever asthma • childhood exposure metric • sig. for males, females, combined	Child: current serum	Dong (2013) and Zhu (2016)
	OR = 3.56 (0.84 – 15.02)	• ever asthma or current • maternal exposure metric	Maternal plasma 0-3d post delivery	Granum (2013)
	Adj OR (95% CI) Ln-linear or linear **p=0.04** **OR = 1.18 (1.01 – 1.39)**	• ever asthma • not sig. for current asthma • childhood exposure metric	Child: current serum	Humblet (2014)
	OR for 1 SD change in serum PFOA OR = 0.93 (0.47 – 1.84)	• current asthma • maternal exposure metric	Maternal: serum	Smit (2015)
	Disease-specific Cox regression not significant **Trend: p=0.05 for inverse association with PFOA exposure via categories**	• adult modeled exposure • not sig. lagged exposure • not sig. cumulative exposure	Adult: modeled	Steenland (2015)
Total IgE	Adj OR log10 PFOA concentration OR = 1.1 (0.6 – 1.9)	• cord blood IgE measure • maternal exposure metric	Maternal: first trimester plasma	Ashley-Martin (2015)
	Mean IgE (95% CI) quartiles PFOA in asthmatics Q1: 512.1 (329.4 – 694.8) Q2: 604.6 (422.1 – 787.1) Q3: 788.2 (274.6 – 537.9) **Q4: 836.4 (652.0 – 1020.8)** **Trend: p=0.005 (males + females)**	• childhood exposure metric • childhood IgE measure • outcome in asthmatics • not sig. in non-asthmatics • sig for females, combined, not males (p trend=0.100)	Total IgE in serum in children (age 12-19)	Dong (2013) and Zhu (2016)
	Log10 quadratic PFOA quadratic polynomial regression coefficient **F: -1.429 (-2.416 – -0.0422)** **M:** 0.227 (-1.584 – 2.037)	• maternal exposure metric • cord blood IgE measure	Maternal serum after the second trimester of pregnancy	Okada (2012)
	Adj % change (95% CI) for doubling of PFOA: **OR = 10.5 (0.17 – 22)**	• childhood exposure metric	Child: Current serum	Stein (2016)
	Adjusted β (S.E.) **β =0.134 (0.115), p=0.047** F: β =0.067 (0.231), p=0.823 **M: β=0.206 (0.165), p=0.025**	• cord exposure metric • cord blood IgE measure • not sig. in childhood IgE measure	Cord blood serum sample measures of PFOA/PFOS (ng/ml)	Wang (2011)
Rhinitis	Adj OR for shift from 25th to 75th percentile (95% CI) of PFOA: **OR = 1.35 (1.10 – 1.66)**	• childhood exposure metric	Child: Current serum	Stein (2016)

Bold text indicates statistically significant decreased or increased association; sig. = significant.

This table highlights select findings to illustrate studies that evaluated asthma (having ever had a diagnosis or some analyses current asthma) and total IgE in different populations relative to PFOA measured in pregnant women, children, and adults; for additional data see **Figure D27**.

Risk-of-bias assessment of the exposure characterization considered the consistency and reliability of the exposure measures such as use of established test methods and whether exposure was assessed in a relevant time-window for development of the outcome (see protocol for more details http://ntp.niehs.nih.gov/go/749926). All of the studies were rated probably low or definitely low risk of bias for exposure (see **Figure D34**). Maternal serum PFOA was used as the exposure metric for five of the studies (Okada *et al.* 2012, Granum *et al.* 2013, Okada *et al.* 2014, Ashley-Martin *et al.* 2015, Smit *et al.* 2015), and cord serum for the sixth (Wang *et al.* 2011). Strong positive correlations (e.g., Pearson's correlation between 0.79 – 0.93) have been reported between maternal PFOA and cord levels as a measure of fetal exposure (Glynn *et al.* 2012). Given the slow clearance and long biological half-life of PFOA in humans (2 to 8 years)(Olsen *et al.* 2007a, Kudo 2015), exposure measured from a single sample/time point is considered a good measure of PFOA exposure. However, Glynn *et al.* (2012) did show that the correlation between maternal and fetal levels was strongest for maternal samples taken closer to delivery (i.e., third trimester or within 3 weeks after delivery), with the weakest correlations for

the first trimester (Pearson's correlations between PFOA in cord blood are 0.78 and 0.93 for first and third trimester respectively). Samples from the first trimester are still highly correlated with cord blood and only the Ashley-Martin *et al.* (2015) study used exposure measured in the first trimester; the other studies measured PFOA in maternal serum from the 3rd trimester, at birth, or from cord blood taken at birth.

Risk-of-bias assessment of potential confounders included age, sex, race/ethnicity, smoking, body mass index, alcohol consumption, parental asthma or allergy, anti-inflammatory medication, and variables that represent socioeconomic status based on prior reports of associations with PFOA exposure levels (Calafat *et al.* 2007, Nelson *et al.* 2010) and immune outcomes (WHO 1996, Dallaire *et al.* 2005) (see protocol http://ntp.niehs.nih.gov/go/749926 for more details). The six studies of hypersensitivity endpoints in children with maternal or cord blood exposure levels considered all of the important confounders other than anti-inflammatory medication and were rated probably low risk of bias for confounding because of lack of information on the validity of the questionnaires used to assess confounding (see **Figure D34**).

Levels of PFOA in children and hypersensitivity response in children
Current serum concentrations of PFOA in children were associated ever having had a diagnosis of asthma, increased total IgE and several other indicators of respiratory hypersensitivity or food allergy in all five studies that investigated the potential association between serum concentrations of PFOA in children and hypersensitivity (**Table 16** and **Figure D27**). These studies included three cross-sectional studies based on NHANES data on children age 12-19 (Humblet *et al.* 2014, Buser and Scinicariello 2016, Stein *et al.* 2016) and two publications from a case-control study of asthma in children age 10-15 in Taiwan (Dong *et al.* 2013, Zhu *et al.* 2016). No prospective studies were located that examined the potential relationship with childhood PFOA exposure and hypersensitivity. An unpublished report from the C8 Science Panel was located that examined asthma and COPD in relation to PFOA exposure in the Ohio Valley, a region associated with elevated PFOA levels in drinking water (Fletcher *et al.* 2012); however, the report did not include full methods or results and was therefore not used in developing conclusions.

Current serum PFOA was associated with a higher odds ratio of doctor diagnosed asthma in a case-control study of 231 asthmatic and 225 nonasthmatic children age 10-15 from Taiwan (Dong *et al.* 2013) (**Figure D27**). PFOA was also positively associated with increased total serum IgE, absolute eosinophil count and eosinophilic cationic protein concentration among the asthmatics. In subsequent analyses of the case-control study of asthmatics in Taiwan, Zhu *et al.* (2016) explored the potential role of TH_1 (IL-2 and IFN-γ) and TH_2 cytokines (IL-4 and IL-5) as a mechanism by which PFOA or other PFAAs may contribute to the development of asthma and the role of the child's sex. There is overlap in cytokine function, but in general IL-4, IL-5 and other TH_2 cytokines are associated with promotion of IgE and eosinophilic responses associated with atopy, asthma and hypersensitivity. In contrast, the IL-2, INFγ and other TH_1 cytokines function in cell-mediated immunity against intracellular pathogens. Zhu *et al.* (2016) reported that in male children with asthma (n = 158), serum PFOA was associated with polarization toward a TH_2 response indicative of hypersensitivity (i.e., increased TH_2 cytokines and non-significant decreases in TH_1 cytokines). However, they did not find a significant PFOA-associated change in TH_1 or TH_2 cytokines in females despite the finding that serum PFOA was associated with diagnosis of asthma when considering males and females together ($p < 0.001$) or split by sex [males ($p = 0.001$) and females ($p = 0.005$)] (Zhu *et al.* 2016).

Humblet *et al.* (2014) reported that increased serum PFOA was associated with higher odds of ever having received a diagnosis of asthma using NHANES data from 1999-2000 and 2003-2008 (n = 1877);

although there was no association with wheezing or current asthma (**Figure D27**). Using a smaller NHANES population restricted to fewer sample years (n = 638; from 2005-2006), Stein *et al.* (2016) reported that PFOA was associated with current rhinitis, and similar to Humblet *et al.* (2014) there was no association with current asthma or wheeze. In addition to respiratory endpoints, Stein *et al.* (2016) examined potential association with total serum IgE and specific IgE levels to common allergens. Serum PFOA concentration was associated with 10% increase in total IgE (see **Table 16**), but not allergen-specific IgE. Elevated serum total IgE is considered a hallmark of atopy or hypersensitivity and the tendency to develop allergic diseases in general. Increased total serum IgE is commonly associated with asthma, and multiple studies have demonstrated the association between total IgE and asthma that is independent of specific IgE levels or other indications of allergy (Sunyer *et al.* 1996, Beeh *et al.* 2000).

Buser and Scinicariello (2016) used multivariate logistic regression to analyze the potential association between current serum PFOA concentrations and two separate indicators of food sensitization: (1) food-specific IgE over a concentration clinically considered to indicated an allergic response (0.35 kU/L) and (2) self-reported food allergies in children ages 12-19 from NHANES. Serum PFOA was associated with an increased OR for self-reported food allergies (OR = 9.09 [95%CI 3.32 to 24.90] for highest quartile vs. lowest; p-trend < 0.001) in children from NHANES 2007-2010. However, PFOA was not associated with food-specific IgE in children from NHANES 2005-2006.

As discussed above, the protocol has details on the risk-of-bias assessment of potential confounders including those considered for hypersensitivity endpoints (http://ntp.niehs.nih.gov/go/749926). The three studies of asthma in children with current exposure levels considered most of the important confounders and were rated probably low risk of bias for confounding (see **Figure D32** and **Figure D33**). Parental history of asthma and duration of breastfeeding were not considered in any study although both of these factors may influence the incidence of asthma in children (Matheson *et al.* 2012, Nurmatov *et al.* 2012) and there is some evidence that duration of breastfeeding may affect PFOA exposure as well (Karrman *et al.* 2007). Humblet *et al.,* (2014) discussed breastfeeding as a potential confounder, but no information is available in NHANES on duration of breastfeeding so its potential influence could not be considered. The failure to adjust for breastfeeding could result in an underestimation of the association between PFOA and hypersensitivity-related effects because breastfeeding has been associated with lower incidence of asthma but elevated child PFOA concentrations.

Risk-of-bias assessment of the exposure characterization considered the consistency and reliability of the exposure measures such as use of established test methods and whether exposure was assessed in a relevant time-window for development of the outcome (see protocol for more details http://ntp.niehs.nih.gov/go/749926). All three of the studies assessing asthma relative to childhood PFOA levels rely on current exposure data, and the long biological half-life of PFOA in humans (2 to 8 years) (Olsen *et al.* 2007a, Kudo 2015) increases the likelihood that these serum measurements represent past exposure that would be biologically relevant for development of asthma or other hypersensitivity responses. Nevertheless, the measurement of outcome and exposure simultaneously is likely to result in some exposure misclassification. The three NHANES studies (Humblet *et al.* 2014, Buser and Scinicariello 2016, Stein *et al.* 2016) were rated probably low risk of bias for exposure characterization due to the long half-life of PFOA (see **Figure D32** and **Figure D33**). In contrast, the relevance of the exposure measurement timing is of particular concern for the Taiwanese case control (Dong *et al.* 2013, Zhu *et al.* 2016) studies where PFOA were measured in serum samples taken from children when they were enrolled in the study (i.e., at age 10-15). However, 52% of the cases were diagnosed with asthma before 5 years of age and exposure was measured 5 to 10 years after the development of asthma. Therefore, the Dong *et al.* (2013) and Zhu *et al.* (2016) studies were rated

probably high risk of bias for exposure because of the high likelihood of exposure misclassification (**Figure D33**).

Confidence in the body of evidence was not downgraded for risk of bias, although there is likely to be some exposure misclassification for all three studies and the Dong study was rated probably high risk of bias for exposure characterization. Care is taken not to double count or downgrade confidence in the body of evidence twice for the same factor. In the OHAT method, the relevance of the exposure assessment for the time-window appropriate to the development of the outcome can be considered in two places: (1) in assessing the risk of bias for the exposure characterization as described above, and (2) in setting the initial confidence in bodies of evidence based on study design factors. When rating confidence in the body of evidence, studies with a cross-sectional study design (e.g., the body of evidence with data on childhood PFOA exposure and asthma) start at a lower initial confidence because cross-sectional exposure sampling cannot assure exposure took place before development of the outcome. Ratings of definitely high risk of bias in the exposure characterization or probably high risk of bias across all studies would have been considered further and may have resulted in further downgrading the confidence in the body of evidence.

Current serum concentrations of PFOA in children were consistently associated with ever having had a diagnosis of asthma, increased total IgE and several other indicators of respiratory hypersensitivity. The data are considered a consistent pattern of findings for PFOA-associated increase in asthma and related indicators of hypersensitivity in children in relation to PFOA levels in the children. The low confidence in this body of evidence is primarily due to the cross-sectional nature of the studies and uncertainty as to whether exposure levels reflect exposure prior to the development of hypersensitivity. There are no prospective studies examining the potential relationship with childhood PFOA exposure and hypersensitivity.

Levels of PFOA in adults and hypersensitivity response in adults
There are two studies in adults living in a region with elevated drinking water PFOA levels, and they present conflicting results. Anderson-Mahoney *et al.* (2008) reported residents had increased prevalence of effects associated with airway hypersensitivity (e.g., asthma, chronic bronchitis) compared to standardized prevalence ratios, whereas Steenland *et al.* (2015) reported PFOA was negatively associated with asthma with medication in a study of workers.

A study of adults (n = 566) living in a region with elevated drinking water PFOA levels found an increase in the reported prevalence of respiratory effects including asthma, chronic bronchitis and shortness of breath on stairs compared to standardized prevalence ratios estimated from NHANES data (Anderson-Mahoney *et al.* 2008). However, the study had a number of serious risk-of-bias concerns and was rated probably high for all three key risk of bias questions: (1) failure to consider most important confounders (e.g., smoking, body mass index, and socioeconomic status), (2) exposure characterization was based on residence in an area with drinking water contamination for at least one year with no information as to how variables such as percent of residents reporting water consumption were used, and (3) disease outcomes were obtained by questionnaire with no indication that the questionnaire had been validated (see **Figure D34**). In addition, the participants were plaintiffs or potential plaintiffs in a lawsuit regarding PFOA exposure of residents near a Teflon manufacturing plant on the Ohio River in West Virginia and therefore likely knew of their exposure and potential health effects.

Steenland *et al.* (2015) reported a significant negative trend (p = 0.05 trend via categories with no lag exposure analysis) for lifetime PFOA exposure and asthma with medication in a study of workers (n = 3713) with established PFOA exposure (Steenland *et al.* 2015). The workers were a subset of a larger cohort (part of the C8 studies) of community residents in Ohio and West Virginia with higher

levels of PFOA in drinking water. Cumulative PFOA exposure was estimated based on plant emissions, residence, work history and modeling during follow-up.

Animal Hypersensitivity Data for PFOA

Summary: There is moderate confidence that exposure to PFOA is associated with increased hypersensitivity responses based on the available animal studies. The results show consistent enhancements in hypersensitivity-related endpoints in two studies of airway hypersensitivity in mice (see **Figure D29** for data figure and study details). PFOA-associated hypersensitivity is further indicated by a study in mice reporting increased histamine release and exacerbation of IgE-dependent allergic reaction in a passive cutaneous anaphylaxis assay following PFOA exposure. A conservative approach was taken to downgrade the confidence rating for the body of evidence for serious risk of bias(see **Table 17** for confidence ratings summary for the body of evidence). Although one of the airway hypersensitivity studies was rated probably low or definitely low risk of bias for the key questions, there were only two airway studies and the other was rated probably high for two of the key risk of bias questions (exposure characterization and outcome assessment)(see **Figure D35** and **Figure D36**).

Both of the available studies that directly tested airway hypersensitivity after exposure to PFOA reported enhancement across multiple airway hypersensitivity-related endpoints in mice (Fairley *et al.* 2007, Ryu *et al.* 2014). The two studies differed substantially in duration (4-day vs. gestational day 2 through 12-weeks) and route of exposure (dermal vs. dietary). The results show enhancement across multiple airway hypersensitivity-related endpoints in mice and the heterogeneity across endpoints may be explained by variation between studies in exposure route (dermal vs dietary) and duration (4-day vs. gestational day 2 through 12 weeks).

Dietary exposure to PFOA (4 mg/kg diet through 12 weeks of age) was associated with increased airway hyperresponsiveness in male and female mice following methacholine challenge characterized by increased airway resistance, greater peripheral tissue resistance, elevated elastance (indicating stiffer lungs requiring greater work for breathing), greater airway sensitivity to methacholine, elevated lung leukocyte and macrophage number in bronchial alveolar lavage fluid (BALF) (Ryu *et al.* 2014) (**Figure D29**). In this dietary exposure mouse model, PFOA exposure alone increased airway hyperresponsiveness consistent with asthma; however, dietary PFOA exposure had no effect on OVA-induced airway hyperresponsiveness. Fairley *et al.* (2007) also reported increased hypersensitivity related-responses with PFOA exposure using a different route of exposure and model of asthma. Fairley *et al.* (2007) tested the hypersensitivity response to OVA in a mouse model of asthma after 4-day dermal exposure to PFOA (0, 0.25, 2.5, 6.25, 12.5, 18.75, 25, or 50 mg/kg) in female mice (**Figure D29**). Dermal exposure to PFOA at higher doses (18.75 to 50 mg/kg/day) enhanced the hypersensitivity response to OVA including increased OVA-specific IgE (**Figure D30**), total IgE (**Figure D31**), and OVA-specific airway hypersensitivity characterized by lung histopathology indicating eosinophilia and mucin production (Fairley *et al.* 2007). There was no indication that PFOA was a sensitizer, as PFOA exposure alone (i.e., without OVA) did not increase total IgE (Fairley *et al.* 2007). PFOA exposure increased the hypersensitivity response to OVA in a mouse model of asthma, suggesting that PFOA may increase hypersensitivity responses to environmental allergens. Singh *et al.* (2012) reported PFOA-associated increases in dye accumulation following 4-day dermal PFOA (0, 10, 50 mg/kg/day on the ear) treatment to mice in the IgE-dependent passive cutaneous anaphylaxis assay using anti-dinitrophenyl-IgE. In a separate experiment, a single intraperitoneal injection (0, 1, 5 mg/kg PFOA) resulted in increased serum histamine 1 hour after treatment (Singh *et al.* 2012).

The confidence in the body of evidence was not increased for evidence of a dose response because the data do not clearly indicate a dose-response for airway hypersensitivity, antigen-specific IgE, or total IgE

(**Figure D29**, **Figure D30**, or **Figure D31**). The Fairley *et al.* (2007) study reported significant effects at higher doses (18.75 to 50 mg/kg/day) but the highest dose did not result in a response that was larger or even significantly elevated relative to control. The Ryu *et al.* (2014) study only tested one dose (4 mg/kg diet), and therefore the results are not informative as to whether or not there is a dose response. Although Singh *et al.* (2012) study only tested two doses; both histamine release and PCA appear to support a dose response. Although both studies reported multiple endpoints that indicate PFOA-associated increase in hypersensitivity, there are differences in the response to the specific antigen OVA that may relate to differences in duration and route of exposure between the studies. The Ryu *et al.* (2014) study included prenatal, juvenile, and adult exposure to PFOA, and therefore does not directly address if developmental-only exposure is, or is not, associated with hypersensitivity in animal models.

The results of animal studies show PFOA-associated enhancement of hypersensitivity across multiple airway hypersensitivity-related endpoints and the clear involvement of IgE in a passive cutaneous anaphylaxis assay in mice. Therefore, the data are considered a consistent pattern of findings for PFOA-associated increases in the hypersensitivity response.

In vitro /Mechanistic Data on Hypersensitivity-related Endpoints for PFOA

Two studies were identified that evaluated hypersensitivity-related endpoints after exposure to PFOA: 1) a study using cells from a human a mast cell line (HMC-1) and 2) a study using cells from a rat basophilic leukemia cell line (RBL2H3). Both studies were modeling potential effects of PFOA on mast cells, a type of leukocyte that plays a central role in asthma, eczema and allergic reactions by rapidly releasing hypersensitivity mediators (e.g., cytokines and histamine) when allergens bind to cell surface IgE (Yamashita 2007, Oh and Lim 2010).

Singh *et al.* (2012) tested the effects of *in vitro* PFOA exposure on histamine release, pro-inflammatory cytokine gene expression, and the role of NF-κB in human mast cells. Treatment of HMC-1 cells with 25-100 μM of PFOA (10350 – 41400 ng/ml) increased histamine release, and higher concentrations (20700 – 165600 ng/ml PFOA) increased intracellular calcium and gene expression (mRNA) for multiple cytokines (TNF-α, IL-1β, IL-6, and IL-8) (Singh *et al.* 2012). The PFOA-associated histamine release by human mast cells *in vitro*, supports a similar finding with *in vivo* exposure to PFOA in mice. Singh (2012) reported PFOA given by intraperitoneal injection (1 – 5 mg/kg) resulted in increased histamine release. The results suggest that *in vitro* PFOA exposure promoted a hypersensitivity response from mast cells via activation of NF-κB and pre-transcriptional increase in inflammatory cytokines (although cytokine secretion was not measured).

In a similar experiment with rat RBL2H3 cells, Yamaki and Yoshino (2010) examined the effects of *in vitro* PFOA exposure on release of histamine and other indicators of degranulation (e.g., β-hexosaminidase). Pre-treatment of RBL2H3 cells with 10-300 μM of PFOA (4140 - 124200 ng/ml) for 20 minutes resulted in the increased release of β-hexosaminidase following activation by antigen (OVA) and IgE (Yamaki and Yoshino 2010). The β-hexosaminidase release was calcium, and antigen/IgE-dependent, suggesting it represented active degranulation characteristic of mast cell response. Histamine was not affected at doses that did not cause cell death.

The release of hypersensitivity mediators from human HMC-1 cells and rat RBL2H3 cells after *in vitro* exposure support PFOA-associated increases in airway hypersensitivity. However, the available *in vitro* assays are limited to evaluations of a single cell type (mast cells) testing degranulation, and cytokine release. The lower dose with the rat RBL2H3 cells (4140 ng/ml) is similar to internal doses from the Ryu *et al.* (2014) hypersensitivity study in mice (4 mg/kg-diet resulted in serum concentrations of 4800 ng/ml PFOA). However, the lowest concentrations (10350 ng/ml PFOA) tested on human mast cells *in vitro* by

Singh *et al.* (2012) are well above typical human exposure range of the general population [e.g., Olsen *et al.* (2003b) reported a range of 1.9 to 52.3 ng/ml PFOA in serum from American Red Cross blood donors and the latest NHANES for 1999-2012 reported mean (95% CI) for serum PFOA was 2.08 ng/ml (1.95-2.22)](CDC 2015). The concentrations tested were within occupational exposure levels (range from approximately 200 to 90000 ng/ml PFOA (Costa *et al.* 2009)). In addition, both studies were rated probably high risk of bias for multiple questions including exposure characterization (because purity of PFOA was not reported or below 98%) and outcome assessment (due to lack of blinding of outcome assessors) (see **Figure A3-4**).

These two *in vitro* assays provided support, but were not considered to provide strong support for the biological plausibility of PFOA-related increase in hypersensitivity. Mast cells are important to allergic reactions, but the *in vitro* database is limited to this one cell type, few endpoints, and only two studies. Although the concentrations used for the human cell data are within occupational exposure levels, there were no data at concentrations reported for the general population. Other mechanistic data relevant for evaluating potential PFOA effects on hypersensitivity are discussed below in the evidence synthesis section in the context of biological plausibility. Relevant mechanistic data would include PFOA-related increases in IgE levels or a change in cytokine production favoring hypersensitivity or a TH_2 response.

Table 17. Hypersensitivity–related Outcomes Evidence Profile for PFOA

INITIAL CONFIDENCE for each body of evidence (# of studies)	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
PFOA										
Human										
Initial Low (3 cross-sectional child exposure studies)[a]	---	---	---	---	---	---	---	---	---	**Low**
Animal										
Initial High (2 mammal studies)[b]	↓	---	---	---	---	---	---	---	---	**Moderate**
References: Human: Dong (2013)[a], Humblet (2014)[a], Stein (2016)[a] Animal: Fairley (2007)[b], Ryu (2014)[b]										

Evidence Synthesis for Hypersensitivity-related Outcomes for PFOA

The body of evidence for increased hypersensitivity associated with PFOA measured during childhood is supported by experimental animal studies of PFOA-associated increases in airway hypersensitivity. The human studies on hypersensitivity-related outcomes differed depending on the exposure window evaluated. There is no evidence of an association between maternal PFOA concentrations and hypersensitivity outcomes in children and few studies in adults.

Current serum concentrations of PFOA in children were consistently associated with ever having had a diagnosis of asthma, increased total IgE and several other indicators of respiratory hypersensitivity in

studies based on analysis of NHANES data on children age 12-19 and a case-control study of asthma in children age 10-15 in Taiwan. Because these studies are cross-sectional in nature, there is low confidence that exposure to PFOA during childhood is associated with increased hypersensitivity based on these studies (see **Table 17** for confidence ratings summaries for the bodies of evidence). The animal data also consistently support PFOA-associated hypersensitivity and there is moderate confidence that PFOA exposure is associated with increased hypersensitivity response based on several studies of airway hypersensitivity and increased IgE in mice. These confidence ratings translate directly into level-of-evidence conclusions and support an initial hazard identification conclusion of *suspected to be an immune hazard to humans* or that PFOA exposure is suspected to increase hypersensitivity-related outcomes in humans.

- **Human body of evidence:** Low Confidence = Low Level of Evidence
- **Animal body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Initial hazard conclusion (Low x Moderate)** = Suspected to be an Immune Hazard to Humans
- **Final hazard conclusion**
  (after consideration of biological plausibility) = Suspected to be an Immune Hazard to Humans

Collectively, the human and animal bodies of evidence present a consistent pattern of findings that higher exposure to PFOA is associated with hypersensitivity based on increased diagnosis of asthma, IgE, and airway hypersensitivity measures. The human data are based on cross-sectional studies with exposure and outcome evaluated in children from 10-19 years of age. Biological plausibility of the association between PFOA and hypersensitivity could be supported by mechanistic data showing PFOA-associated promotion of hypersensitivity such as increased IgE levels or a change in cytokine production favoring a TH_2 response. There is overlap in cytokine function, but in general TH_2 cytokines (e.g., IL-4, 5, 6 and 13) are associated with promotion of IgE and eosinophilic responses associated with atopy and hypersensitivity. TH_1 cytokines (e.g., IL-2 and INFγ) are involved in cell-mediated immunity against intracellular pathogens.

Part of the evidence for PFOA-associated hypersensitivity is based on data reporting increased IgE levels and therefore the data supporting this aspect of mechanistic data have been discussed earlier. Elevated total serum IgE is commonly associated with asthma, and that link may be independent of allergen-specific IgE levels or other indications of allergy (Sunyer *et al.* 1996, Beeh *et al.* 2000). To summarize briefly, there is evidence from human and animal studies that PFOA exposure results in increased total IgE. Serum concentrations of PFOA in children were associated with increased total serum IgE in both of the epidemiological studies that examined this endpoint. A doubling of serum PFOA was associated with 10% increase in total IgE in children ages 12-19 (n = 638; NHANES 2005-2006) (Stein *et al.* 2016). Current serum PFOA was associated with increased total serum IgE (over 60% increase in the highest quartile) in a study of asthma in Taiwanese children ages 10-15 (n = 456) (Dong *et al.* 2013). The link between PFOA, IgE and asthma is further supported in this study because the association was only significant in children diagnosed with asthma, and was not observed in children without asthma. Dong *et al.* (2013) also reported that PFOA and PFOS were both associated with increased serum eosinophil counts and higher eosinophilic cationic protein levels in the children with asthma.

The animal data support increased IgE with PFOA exposure and provide evidence that PFOA-associated hypersensitivity responses are IgE mediated. Fairley *et al.* (2007) reported PFOA-associated elevations in total and OVA-specific IgE in mice following 4-day dermal exposure to PFOA (20 to 50 mg/kg/day). The clear involvement of IgE and exacerbation of IgE-dependent allergic reaction in response to PFOA exposure was shown in a passive cutaneous anaphylaxis assay in mice. Singh *et al.* (2012) reported

PFOA-associated increases in dye accumulation following 4-day dermal PFOA (0, 10, 50 mg/kg/day on the ear) treatment to mice in a passive cutaneous anaphylaxis assay using anti-dinitrophenyl-IgE.

Several studies of mast cells provide another line of mechanistic data supporting PFOA-associated hypersensitivity. Along with IgE, mast cells are key drivers of immediate hypersensitivity and play an important role in promoting and maintain asthma and other allergic diseases (Galli and Tsai 2012). Singh (2012) reported Intraperitoneal injection of PFOA (1 – 5 mg/kg) resulted in increased serum histamine levels. In the same study, *in vitro* PFOA (10350 – 41400 ng/ml) exposure to human MHC-1 mast cells resulted in degranulation characterized by increased histamine release and higher PFOA concentrations (20700 – 165600 ng/ml) increased intracellular calcium and gene expression (mRNA) for multiple inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) (Singh *et al.* 2012). PFOA exposure also resulted in active degranulation (e.g., release of β-hexosaminidase and histamine) in a rat model for mast cells (RBL2H3 basophilic cells) that was calcium and antigen/IgE-dependent (Yamaki and Yoshino 2010). The lowest effective dose (4140 ng/ml PFOA) in the rat mast cell model is similar to internal doses from the Ryu *et al.* (2014) hypersensitivity study in mice (4800 ng/ml PFOA). However, the PFOA concentrations (10350 ng/ml PFOA) tested on human mast cells are well above typical human exposure range of the general population [e.g., Olsen *et al.* (2003b) reported a range of 1.9 to 52.3 ng/ml PFOA in serum from American Red Cross blood donors and the latest NHANES for 1999-2012 reported mean (95% CI) for serum PFOA was 2.08 ng/ml (1.95-2.22)](CDC 2015).

Few studies have examined potential effects of PFOA on general inflammatory response or inflammatory cytokine changes. Zhu *et al.* (2016) reported that in male Taiwanese children with asthma (n = 158), serum PFOA was associated with polarization toward a TH_2 response indicative of hypersensitivity [i.e., increased TH_2 cytokines (IL-4 and IL-5) and non-significant decreases in TH_1 cytokines]. The increase in TH_2 cytokines indicates a potential mechanism for PFOA-associated increases in asthma diagnosis in the same population (Dong *et al.* 2013, Zhu *et al.* 2016). However, they did not find a significant PFOA-associated change in TH_1 or TH_2 cytokines in females despite the association between higher serum PFOA and asthma when considering males and females together ($p < 0.001$) or split by sex [males ($p = 0.001$) and females ($p = 0.005$)] (Zhu *et al.* 2016). Given the limited database it is difficult to evaluate whether or not there is a clear or consistent pattern for changes in inflammation or cytokine signaling after exposure to PFOA (see cytokine data in **Appendix 5**). Taylor *et al.* (2002, 2005) demonstrated that PFOA (at doses ≥100 mg/kg) inhibited paw edema in a carrageenan-induced rat model of cutaneous inflammation. Qazi *et al.* (2010a, 2013) reported that dietary exposure to mice at does as low as 3 mg/kg PFOA resulted in decreased IL-4 levels. The same research group also examined the effect of dietary PFOA in mice on IL-6 in serum and for cell populations from spleen, bone marrow, peritoneal cavity, and liver in culture (Qazi *et al.* 2009a). Under most conditions, PFOA (0.02% diet or approximately 40 mg/kg/day) was associated with increased levels of IL-6 and TNF-α in mice, although results differed by organ and culture conditions (e.g., plasma levels of TNF-α were not increased).

The effects of *in vitro* exposure to PFOA on cytokine production of peripheral blood leukocytes has also been explored in several studies using whole blood from in human volunteers (Brieger *et al.* 2011, Corsini *et al.* 2011, Corsini *et al.* 2012). Similar to the mouse data, plasma levels of PFOA from the human volunteers were associated with increased TNF-α and IL-6 under LPS stimulation (Brieger *et al.* 2011). In contrast, *in vitro* exposure at doses 100 to 100000 ng/ml of PFOA had no impact on IL-6 or TNF-α (Brieger *et al.* 2011). Corsini *et al.* (2011) reported that PFOA exposure at concentrations relevant to human exposure (100-1000 ng/ml PFOA) had no effect on cytokine secretion including several TH_2 cytokines (IL-4, IL-6, or IL-10) from peripheral blood leukocytes. In further studies of mechanistic endpoints using peripheral blood and a human promylocytic cell line THP-1, Corsini *et al.* (2011, 2012) demonstrated that PFOA inhibited pathways that regulate NF-κB activation, which plays a role in

cytokine production, inflammation, and other immune functions. Most of the mechanistic endpoints were significant at concentrations of 100000 ng/ml PFOA; however, inhibition of NF-κB promotor activity was demonstrated at substantially lower concentrations (i.e., 1000 ng/ml PFOA). Although these data present potential mechanisms, there are no data to support PFOA-induced changes in cytokines or NF-κB occur in humans at environmentally relevant exposure levels.

The mechanistic data were not considered to provide evidence to support or refute biological plausibility of this affect. Mechanistic data for PFOA-associated hypersensitivity suggest the response is IgE-mediated and may involve stimulation of mast cells, but a clear pattern of effects on inflammatory cytokines or the role for NF-κB at relevant PFOA concentrations has not been established. Therefore, the hypersensitivity data support a final hazard identification conclusion that PFOA is *suspected to be an immune hazard in humans*.

Autoimmunity-related Effects and Outcomes

Autoimmune disease and related effects are the result of immune responses against self-molecules (WHO 2012). Autoimmune disorders are frequently tissue or organ specific, although they may be systemic when the reaction is to antigens present across multiple tissues. Autoimmune diseases are usually examined in the context of the damaged tissues or organs (e.g., rheumatoid arthritis, thyroiditis, or ulcerative colitis). Tests for chemical induced autoimmunity in animals generally involve model systems developed to evaluate one specific type of autoimmune response. Due to the specificity of the assays, these tests are used when autoimmunity is suspected and not as part of routine screening for autoimmunity.

Human Autoimmunity Data for PFOA

Summary: There is low confidence that exposure to PFOA is associated with ulcerative colitis, an autoimmune disease in the colon and rectum based on the few available human studies. The results of two studies show PFOA-associated increases in the incidence of ulcerative colitis in residents of the Ohio Valley, a region associated with elevated PFOA levels in drinking water and workers from the same population exposed to PFOA. Higher cumulative exposure to PFOA was associated with rheumatoid arthritis in the workers, but not the community residents. The low confidence in the body of evidence is due to the evidence being restricted to studies from a single population. As a result, confidence in the body of evidence was decreased because it was not possible to evaluate consistency across populations to support a final rating of low confidence. There was inconsistent evidence of an association with rheumatoid arthritis in the same studies and no evidence of an association with other autoimmune diseases (see **Table 18** for list of studies, **Figure D37** for data figure).

Higher PFOA exposure was associated with increased incidence of ulcerative colitis in a study of adult residents of the Ohio Valley (n = 32254), a region with elevated PFOA levels in drinking water (Steenland *et al.* 2013) (see **Table 19** for summary and study details). Cumulative PFOA exposure was estimated based on plant emissions, residence, work history and modeling during follow-up. Incidence of self-reported autoimmune disease was confirmed with medical records. Steenland *et al.* (2013) reported an increased incidence of ulcerative colitis (151 validated cases) by quartile of PFOA exposure in retrospective analysis for a combined cohort (total n = 32254) of residents (n = 28541) and workers (n = 3713) with both 10-year lagged and unlagged exposure. There was no evidence of an association with other autoimmune diseases (Crohn's disease, insulin-dependent diabetes, lupus, multiple sclerosis, and rheumatoid arthritis). In a follow-up study of workers (n = 3713) exposed to PFOA that were a subset of the original analysis, there was a significant trend ($p \leq 0.05$) for ulcerative colitis (28 validated

cases) with increasing PFOA exposure level based on unlagged or 10-year lagged exposure at much higher exposure levels (mean serum PFOA in 2005-2006 was 325 ng/ml for workers versus 87 ng/ml in the combined cohort) (Steenland *et al.* 2015).

In contrast to the community study, there is some evidence that rheumatoid arthritis (28 cases) is associated with PFOA exposure in the workers. There was a positive trend for rheumatoid arthritis by quartiles of PFOA exposure; however, only the trend test using midpoint of the quartiles was statistically significant ($p \leq 0.05$), whereas analyses using continuous log transformed cumulative exposure were not significant ($p = 0.54$ and $p = 0.75$ for 10-year lag or no lag exposure) (Steenland *et al.* 2015). The only other autoimmune study located is a pilot study that reported prenatal concentrations of PFOA were not associated with autoantibodies to several neural or non-neural antigens in 7 year old children (n = 38) from the Faroe Island birth cohort (Osuna *et al.* 2014). Although the study did not find an association with PFOA exposure, autoantibody data without support from other related endpoints (e.g., for the neural antigens studied) is not considered to provide clear evidence for or against an effect on autoimmunity (WHO 2012).

Table 18. Studies on Autoimmunity in Humans (2 retrospective cohort, 1 prospective)

Study	Study design (Location / Study)	n	Exposure measure timing	Disease assessment timing	Analyses	Autoimmune disease outcomes assessed
Steenland (2013)	Retrospective Cohort (residents with higher PFOA in drinking water in OH/ WV part of C8 studies) and prospective analysis of ulcerative colitis after 2005-2006 baseline survey (n = 29 cases)	32254	Lifetime cumulative estimated (quartiles) (lagged and unlagged)	Self-reported autoimmune diseases	Cox regression (survival), age as the time variable and time-varying exposure and covariates; Rate ratios (RR) estimated for quartiles	Crohn's disease, lupus, insulin-dependent diabetes, multiple sclerosis, rheumatoid arthritis, ulcerative colitis↑* (self-reported disease confirmed by medical records)
Steenland (2015)	Retrospective Cohort (workers exposed to PFOA in OH/ WV part of C8 studies)	3713	Lifetime cumulative estimated (quartiles) (lagged and unlagged)	Self-reported chronic diseases	Cox regression (survival), age as the time variable and time-varying exposure and covariates; Rate ratios (RR) estimated for quartiles	Autoimmune disease combined, rheumatoid arthritis↑*, ulcerative colitis↑* (self-reported disease confirmed by medical records)
Osuna (2014)	Prospective pilot study (Faroe Islands birth cohort)	38	Maternal Child (age 7)	Children age 7	Linear regressions assessed associations of autoantibody levels with chemical exposures	Autoantibodies to neural and non-neural antigens, only anti-actin IgG associated with maternal exposure ↓†

significantly ($p<0.05$) increased (↑) or decreased (↓) associated with (*) PFOA or (†) PFOS exposure

The available epidemiological studies that evaluated the association between exposure to PFOA and that satisfy the criteria for inclusion in the systematic review include (1) two publications of overlapping cohorts that reported the incidence of self-reported autoimmune diseases (from 1952 to 2008/2011) in adult residents in the Ohio Valley with higher PFOA in drinking water (part of the C8 studies) and (2) a pilot study using data from the Faroe Island birth cohort that evaluated the association between maternal and childhood PFOA levels and autoimmune antibodies at age 7. The C8 studies consist of a large community-based cohort of individuals that worked or lived in PFOA contaminated water districts and a sub cohort of the same PFOA exposed workers. Both studies assessed lifetime cumulative exposure retrospectively from estimated yearly serum PFOA levels based on historical data and ecological modeling (community) or a JEM combined with residual exposure (workers). Rate ratios for autoimmune disease were calculated using Cox regression (lagged and 10 year lagged) for quartile of cumulative exposure, using the lowest exposure group as the reference group and test for trends were based on cumulative exposure modeled as continuous variable (both studies) or via categories (workers). The community study also conducted a prospective analysis of autoimmune cases that

developed after base-line (2005-2006). Although stratified analyses were not reported for the community members only, it seems unlikely that the findings for ulcerative colitis could be explained by the worker population because there were only 28 reported in the worker cohort and 596 cases reported in the residential and worker combined cohort.

Table 19. Summary of PFOA and Select Data on Autoimmune Diseases in humans

Disease	Changes in incidence with PFOA[a]	Possible sources of heterogeneity	Exposure measurement	Study
Rheumatoid Arthritis	Adj. OR (95% CI) Highest vs. lowest -10 yr. lag OR = 1.35 (0.87-2.11) Trend p = 0.73	• combined community and worker population	Estimated cumulative exposure: Job exposure matrix for workers and an environmental fate and transport model for community exposure	Steenland (2013)
	Adj. RR (95% CI) Highest vs. lowest - 10 yr. lag RR = 2.62 (0.47 to 14.7) Trend p=.75 **Trend categories p = 0.06**	• analyses (trend by cumulative and quartiles of exposure) • worker only population	Estimated cumulative exposure: Job exposure matrix for workers plus consideration of residential component with fate and transport model; and quartiles	Steenland (2015)
Ulcerative colitis	Adj. OR (95% CI) Highest vs. lowest -10 yr. lag **OR = 3.05 (1.56 to 5.96)** **Trend p < .0001**	• combined community and worker population	Estimated cumulative exposure: Job exposure matrix for workers and an environmental fate and transport model for community exposure	Steenland (2013)
	Adj. RR (95% CI), Highest vs. lowest – 10 yr. lag **RR = 6.57 (1.47 to 29.40)** **Trend = 0.05** **Trend categories p = 0.05**	• worker only population • analyses (trend by cumulative and quartiles of exposure)	Estimated cumulative exposure: Job exposure matrix for workers plus consideration of residential component with fate and transport model; and quartiles	Steenland (2015)
Auto-antibodies specific to neural and non-neural antigens	-22% change in actin IgG concentration per 2-fold increase in PFOS exposure (**p<0.05**)	• low number of participants • autoantibodies without additional indications of autoimmunity considered inconclusive evidence	Prenatal cord blood	Osuna (2014)

[a]linear regression (β or % change in antibody per 2-fold increase of PFOA unless noted as OR or RR).
Bold text indicates statistically significant decreased or increased association or trend.
This table highlights select findings to illustrate data informing the potential relationship with autoimmune disease; for additional data see **Figure D37**.

Risk-of-bias assessment of the exposure characterization considered the consistency and reliability of the exposure measures such as use of established test methods and whether exposure was assessed in a relevant time-window for development of the outcome (see protocol for more details http://ntp.niehs.nih.gov/go/749926). Both studies were rated definitely low risk of bias for exposure because the exposure modeling methods have been previously published with a moderate to high degree of correlation between predicted and observed serum PFOA concentrations (e.g., Spearman's rank correlation coefficients from 0.67 to 0.82 for residents in 2005-2006) (see **Figure D38** and **Figure D39**). Cumulative PFOA exposure of residents, and the non-occupational portion of workers, was derived from estimates of annual mean serum PFOA levels during follow-up, which were based on plant emissions, residential and work history, and a fate-transport model (Shin *et al.* 2011, Woskie *et al.* 2012). Worker PFOA exposure was modeled based on job category, cumulative years worked, and change in process/plan emissions over time (Woskie *et al.* 2012).

Risk-of-bias assessment of potential confounders included age, sex, race/ethnicity, smoking, body mass index, alcohol consumption, and variables that represent socioeconomic status based on prior reports of associations with PFOA levels (Calafat *et al.* 2007, Nelson *et al.* 2010) and immune outcomes (WHO 1996, Dallaire *et al.* 2005) (see protocol http://ntp.niehs.nih.gov/go/749926 for more details). Although the general resident study (Steenland *et al.* 2013) was rated probably low risk of bias for confounding, the worker study (Steenland *et al.* 2015) was rated probably high risk of bias because other potential

workplace exposures in the fluoropolymer manufacturing plant were not considered (see **Figure D38** and **Figure D39**).

The data from both the community resident study and the worker analysis support a dose-response relationship between PFOA exposure and increased incidence of ulcerative colitis; however, because the workers were included as part of the combined cohort, the analyses are not independent. Steenland *et al.* (2013) reported a positive trend ($p < 0.001$) for ulcerative colitis by quartile of exposure in retrospective analysis for a combined cohort (total n = 32254) of residents (n = 28541) and workers (n = 3713). However, prospective analysis of ulcerative colitis diagnosed (n = 29) after the 2005-2006 baseline presented a non-significant trend ($p = 0.21$). In the subsequent analysis of the worker subset of the population (n = 3713), a positive trend was also reported ($p \leq 0.05$) for ulcerative colitis. Confidence in the body of evidence was not increased for dose-response because the dose-response was observed in a single study/population.

The two C8 studies of residents and workers in the Ohio Valley (Steenland *et al.* 2013, Steenland *et al.* 2015) report PFOA-associated increases in the incidence of ulcerative colitis in this population. There is inconsistent evidence of an association between PFOA and rheumatoid arthritis, and no evidence of an association with other autoimmune diseases. The major limitation of the body of evidence is that the studies are from the same population that had been exposed to high levels of PFOA, and that the analysis in the larger study of residents also included the workers and therefore there are no independent results from a separate population.

Animal Autoimmunity for PFOA
No animal studies were identified on potential association between PFOA and autoimmunity.

In vitro /Mechanistic Data on Autoimmunity-related Endpoints for PFOA
No *in vitro* exposure studies were identified on the potential association between PFOA and autoimmunity.

Evidence Synthesis for Autoimmunity Data for PFOA
Few studies of PFOA exposure and autoimmunity were located. The body of evidence is based on two epidemiological studies (two C8 studies) of residents and workers in the Ohio Valley and these studies report an association between PFOA exposure and increased incidence of ulcerative colitis. There is no animal evidence to evaluate the potential association between exposure to PFOA and autoimmunity because no studies of PFOA in animal models of autoimmunity were identified.

There is low confidence that exposure to PFOA is associated with ulcerative colitis, an autoimmune disease in the colon and rectum based on the available human studies. The results of two epidemiological studies show PFOA-associated increases in the incidence of ulcerative colitis in residents of the Ohio Valley, a region associated with elevated PFOA levels in drinking water, and workers exposed to PFOA that were a subset of the original analysis (Steenland *et al.* 2013, Steenland *et al.* 2015). The low confidence in the body of evidence is because the studies are from the same population, and that the analysis in the larger study of residents also included the workers, and therefore the findings lack replication in a second population. The lack of experimental animal studies translates into inadequate level of evidence and the low confidence in the human body of evidence translates directly into a low level of evidence. Given the low level of evidence from human studies and inadequate level of evidence from animal studies, autoimmunity was not considered for hazard identification conclusions of PFOA.

Although there is low confidence in the human body of evidence, data that may inform the biological plausibility for ulcerative colitis were considered because the human data were from a large study

population. Biological plausibility of the association between PFOA and ulcerative colitis could be supported by mechanistic data showing PFOA-associated promotion of hypersensitivity such as a change in cytokine production favoring a TH_2 response. There is overlap in cytokine function, but in general TH_2 cytokines are associated with promotion of inflammatory or hypersensitivity responses that may contribute to ulcerative colitis. There are few studies to evaluate the role of cytokines, although the Zhu *et al.* (2016) study of children with asthma in Taiwan reported that serum PFOA was associated with polarization toward a TH_2 response in males but not in the female children (i.e., increased TH_2 cytokines [IL-4 and IL-5] and decreased in TH_1 cytokines [IL-2 and INFγ]). It is important to note that the C8 epidemiological studies report an association between PFOA and ulcerative colitis (which is restricted to the colon and rectum) and the data do not support an association between PFOA and Crohn's disease (which can occur in any part of the gastrointestinal tract). This suggests that the mechanism is more specific than increased inflammation or hypersensitivity of the gastrointestinal tract. Steenland *et al.* (2015) hypothesize that the mechanism may involve effects of PFOA on bacterial exposure unique to the lower digestive tract as well as inflammatory-based mechanisms. There are few studies that have examined potential PFOA-associated cytokine changes in humans or experimental animals, and none of them have evaluated the colon or rectum. For example, there is evidence that IL-13 is involved in the inflammatory response that promotes ulcerative colitis (Mannon and Reinisch 2012); but no evidence as to whether or not PFOA influences IL-13 levels. Given the limited database and the lack of organ-specific data, it is difficult to evaluate whether PFOA exposure would result in a pro-inflammatory or pro-hypersensitivity cytokine pattern relevant to the development of ulcerative colitis (see cytokine data in **Appendix 5**).

Similarly, there are no data to evaluate whether or not PFOA affects disease resistance in bacterial exposure models in mammals. PFOA (2.1 mg/kg/day for 8 weeks) had no effect on morbidity or mortality to *E. coli* exposure in Japanese quail (Smits and Nain 2013). However, these data are from a non-mammalian model and the exposure was route was subcutaneous injection and therefore not from oral exposure that would be more relevant to ulcerative colitis.

The mechanistic data were not considered to provide evidence to support or refute biological plausibility of the reported association between PFOA exposure and ulcerative colitis.

PFOS Immune Evidence

The sections below on each primary immune health effect begin with a brief description of the health effect, followed by a summary of the human evidence and the confidence rating of the body of evidence from human studies. A similar summary of the animal evidence and confidence rating in the animal body of evidence is then presented. *In vitro* or mechanistic studies are then summarized. Then evidence synthesis was conducted in a three-part process for each outcome. First, the confidence ratings were translated into level-of-evidence of health effects conclusions using the procedure outlined in **Figure 3**. Next, initial hazard identification conclusions were reached by integrating the level-of-evidence conclusions for the human and animal evidence streams using the procedure outlined in **Figure 4**. Finally, the degree of support from mechanistic data was considered and discussed in reaching final hazard identification conclusions for each of the major immune health outcomes.

Immunosuppression: Antibody Response

The development of specific antibodies in response to an immune challenge (e.g., injection with sheep red blood cells or SRBC) is a well-accepted measure of immune function included in many guidelines or testing requirements for immunotoxicity (US EPA 1998, ICH 2005, WHO 2012). Antibodies are proteins found in blood and other body fluids that bind to antigens (generally proteins on the cell surface of infectious agents such as viruses or bacteria) and thereby identify them for destruction or removal. The production, release, and increase in circulating levels of antigen-specific antibodies are important for protection against the infectious agent and preventing or reducing severity of influenza, respiratory infection, colds, and other diseases as part of the humoral immune response. Reduced antibody production is an indication of decreased immune function or immunosuppression that may indicate a greater risk of disease. There are 5 antibody or immunoglobulin (Ig) classes in mammals: IgM, IgG, IgA, IgD, and IgE that differ in structure and function.

Antibody assays for immunosuppression generally measure IgM or IgG. IgM is important for the early or primary response after a single antigen challenge and IgG is a later response that is important in recognizing the antigen following re-exposure. Antigen-specific IgM to a T-cell-dependent antigen (e.g., SRBC) is considered one of the most predictive measures of overall immune function because proper response requires cooperation between T-cells, B-cells, and antigen-presenting cells to develop an antibody response (Luster *et al.* 1992). This antibody response can be examined by measuring antigen-specific antibody levels after vaccination in humans and after challenge with SRBC or other antigens in laboratory animals. Measurement of total immunoglobulin levels (rather than antigen-specific IgM or IgG) is considered observational data that is less predictive for immunotoxicity (WHO 2012).

Human Antibody Response Data for PFOS

Summary: There is moderate confidence that exposure to PFOS is associated with suppression of the antibody response based on the available human studies. The results show consistent PFOS-associated suppression in at least one measure of the anti-vaccine antibody response across multiple studies with evidence from developmental, childhood, and adult exposures (see **Table 10** for list of studies). There were no changes in confidence rating for the body of evidence after considering factors that may increase or decrease confidence (see **Table 20** for confidence ratings summaries for the body of evidence). Heterogeneity in the findings may be explained by variation between studies in the different vaccinations tested, time between vaccination and measurement of the antibody response, and analyses or ways to measure the antibody response.

The human body of evidence for PFOA and PFOS on the antibody response is based on the same six epidemiological studies with very similar results and findings for both chemicals. The confidence ratings for the human data are the same for PFOA and PFOS. Therefore, to avoid repetition, the reader is

referred to **Human Antibody Response Data** for discussion of the human data on the antibody response for both PFOA and PFOS.

As was the case for PFOA, the available studies (**Table 10**) provide evidence that higher developmental, childhood, or adult serum concentrations of PFOS are associated with lower specific antibody response to one or more commonly used vaccine in each study with the exception of a study of adults living in the Ohio Valley (as part of the C8 studies), a region with elevated drinking water PFOA levels. The residents have much higher serum PFOA than the general US population and serum levels of PFOA were associated with a reduced rise in antibody levels in response to influenza A/H3N2 vaccination (Looker *et al.* 2014). Interestingly, this is the only human study that did not report an effect of PFOS on antibody levels and it is the only study where PFOA concentrations were higher than PFOS (in all other studies mean PFOS concentrations are 5x PFOA levels or more). It is unknown why PFOS was not associated with changes in the antibody response for any of the vaccines in this study; however, effects of PFOA on the antibody response and the high concentrations of PFOA in this population may have obscured any relationship. The data are considered a consistent pattern of findings for PFOA and PFOS-associated antibody suppression in humans.

Animal Antibody Response Data for PFOS

Summary: There is high confidence that exposure to PFOS is associated with suppression of the antibody response based on the available animal studies. The results show consistent suppression of the primary antibody response (see **Figure D8** for data figure and **Table 20** for confidence ratings summary for the body of evidence). Confidence in the body of evidence was decreased because of serious concern for risk of bias and increased for evidence of dose-response observed across multiple studies (**Figure D8**) to support the final rating of high confidence. All studies were rated probably high risk of bias for outcome assessment due to lack of blinding of outcome assessors, one of the Key Questions. In addition, all of the studies were rated probably high risk of bias for lack of allocation concealment and lack of researcher blinding during the study (see **Figure D14** and **Figure D15**).

There is consistent evidence that PFOS exposure results in suppression of the primary antibody response as determined by antigen-specific IgM antibody production to single challenge with T-cell specific antigens (SRBC) in male and female mice (Keil *et al.* 2008, Peden-Adams *et al.* 2008, Dong *et al.* 2009b, Zheng *et al.* 2009, Qazi *et al.* 2010b, Dong *et al.* 2011, Vetvicka and Vetvickova 2013) with support from a study in chickens (Peden-Adams *et al.* 2009)(**Figure D8**) at oral doses from 0.00166 to 40 mg/kg/day. Antibody suppression in the lower dose range (0.00166 to 5 mg/kg/day PFOS) takes place without changes in body weight, spleen or thymus cellularity, or other signs of overt toxicity.

The primary antibody response data from mice demonstrate a consistent pattern of findings to support PFOS-associated suppression, and no data were located testing the primary response in rats. There are two sources of heterogeneity in the data including differences in antigen (T-cell dependent SRBC vs. T-cell independent TNP) and outcome (primary vs secondary response). The response to T-cell dependent antigens was consistently suppressed with PFOS exposure; however, the effect of PFOS on antibody response to T-cell independent antigens is less clear. Two of the three studies that tested the response to T-cell independent antigens did not find an effect of PFOS exposure on antibody response to this type of antigen (Peden-Adams *et al.* 2008, Qazi *et al.* 2010b, Vetvicka and Vetvickova 2013). There is limited data on the secondary IgG response from which to draw a conclusion on the effect of PFOS exposure; it was not suppressed in the one study testing it in mice (Dong *et al.* 2011), or the one rat study (Lefebvre *et al.* 2008), but it was suppressed in chicken exposed during development (Peden-Adams *et al.* 2009) (**Figure D9**). The LOAEL (5 mg/kg) in mice from the one developmental exposure study (Keil *et al.* 2008), is higher than the LOAEL from several adult exposure studies (0.00166 to 0.08 mg/kg/day). The lower

susceptibility (i.e., high dose to suppress the antibody response) in a developmental exposure study without explanation is unusual for an immune endpoint.

As discussed for PFOA, the primary antibody response to T-cell specific antigens as determined by antigen-specific IgM is considered among the most predictive measures of immunotoxicity and part of multiple testing guidelines. Therefore, the data are considered a consistent pattern of findings for PFOS-associated antibody suppression.

In Vitro /Mechanistic Data on Antibody Response for PFOS

No *in vitro* studies were located that tested potential effects of PFOS on antibodies or antibody production [e.g., using a Mishell-Dutton assay with antigen (e.g., SRBC) challenge].

Table 20. Antibody Response Evidence Profile for PFOS

INITIAL CONFIDENCE for each body of evidence (# of studies)	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
PFOS										
Human										
Initial Moderate (4 prospective studies)[a]	---	---	---	---	---	---	---	---	---	**Moderate**
Initial Low (2 cross-sectional studies)[b]	---	---	---	---	---	---	---	---	---	**Low**
Confidence Across Human Bodies of Evidence	No change for considering across study designs									**Moderate**
Animal										
Initial High (8 mammal studies)[c]	↓	---	---	---	---	---	↑	---	---	**High**

References:

Human: Granum (2013)[a], Grandjean (2012)[a], Kielsen (2016)[b], Looker (2014)[a], Mogensen (2015)[a], Stein (2016)[b]

Animal[c]: Dong (2009b, 2011), Keil (2008), Lefebvre (2008), Peden-Adams (2008), Qazi (2010b), Vetvicka (2013), Zheng (2009)

Evidence Synthesis for Antibody Response for PFOS

Higher serum levels of PFOS are associated with suppression of the antibody response based on the human and animal bodies of evidence (see **Table 20** for confidence ratings summaries for the bodies of evidence). There is moderate confidence that exposure to PFOS is associated with suppression of the antibody response in humans based on consistent suppression in at least one measure of the anti-vaccine antibody response across multiple studies with evidence from prenatal, childhood, and adult exposures to PFOS. There is high confidence that exposure to PFOS is associated with suppression of the antibody response in animals based on consistent suppression of the primary antibody response in mice. These confidence ratings translate directly into level-of-evidence conclusions and support an initial

hazard identification conclusion of *presumed to be an immune hazard to humans* or PFOS exposure is presumed to suppress the antibody response in humans.

- **Human body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Animal body of evidence:** High Confidence = High Level of Evidence
- **Initial hazard conclusion (Moderate x High)** = Presumed to be an Immune Hazard to Humans
- **Final hazard conclusion (after consideration of biological plausibility)** = Presumed to be an Immune Hazard to Humans

Taken together, the human and animal bodies of evidence present a consistent pattern of findings that higher prenatal, childhood, or adult serum concentrations of PFOS are associated with suppression of the antibody response. Mechanistic data from *in vitro* or *in vivo* studies can then be used to examine the biological plausibility of PFOS-associated suppression of the antibody response to develop the final hazard identification conclusion.

The antibody response begins with B-cell surface antibody recognizing a specific antigen. Then, for a T-cell dependent antibody response (e.g., most of the experimental animal data on SRBC-specific antibody response), T-cells must also recognize the specific antigen involved (generally after processing by a macrophage, dendritic cell, or other antigen presenting cell). When B- and T-cells both recognize the same antigen, the T-cell activates the B-cell and releases cytokines to help the B-cell multiply and mature into an antibody secreting plasma cells that produces the antigen-specific antibody response. Therefore, relevant mechanistic data would include effects of PFOS at relevant concentrations on key cell populations (B-cells, T-cells, or macrophages as antigen presenting cells), antigen processing and cell activation, or cytokines important for cell signaling during the antibody response.

PFOS-related decrease in B-cell or T-cell numbers would present a possible mechanism for reduced antibody response if it was observed at the same or lower concentrations at which reduced antibody response was observed. However, there is inconsistent evidence of reduced B-cell number (see B cells (B220)) at higher exposure levels for PFOS (≥2 mg/kg) and no change in B-cell or T-cell (CD4 or CD8 subpopulations) numbers observed at lower doses PFOS (0.00166 to 0.08 mg/kg) associated with decreased antibody levels (see cell phenotyping data, spleen and thymus cellularity in **Appendix 5**) (Dong *et al.* 2009a, Zheng *et al.* 2009, Fair *et al.* 2011). Similarly, at lower exposure levels of PFOS, there are no changes in percentage or cell numbers of macrophages or other antigen presenting cells (Qazi *et al.* 2009a, Fair *et al.* 2011, Dong *et al.* 2012). Overall changes in leukocyte numbers and cellularity in the spleen and thymus are also not affected at lower doses of PFOS. There is no evidence that PFOS-induced changes in cell populations could explain the reduced antibody response at lower doses.

Cytokine release of interleukin-4 (IL-4), IL-5, and IL-6 by T-cells are important for T-cell dependent antibody response (e.g., to SRBC). In studies designed to examine these cell signaling pathways in mice, PFOS exposure *in vivo* resulted in increased IL-6 secretion from B-cells under culture conditions designed to test cytokine communication necessary for the antibody response (Fair *et al.* 2011) (i.e., including stimulation of the CD40 cell surface protein critical to IL-6 stimulation of IgM secretion) (Baccam *et al.* 2003, Bishop and Hostager 2003). Under these same conditions, PFOS did not affect IL-4, IL-5, or IL-6 secretion by T-cells in mice (Fair *et al.* 2011) (see cytokine data in **Appendix 5**). In a separate set of studies, PFOS exposure was associated with increased secretion of IL-4 and IL-6 in mixed cultures of splenocytes from mice exposed to higher doses (0.833 to 20 mg/kg/day), doses that are at or above PFOS doses associated with decreased antibody response (Dong *et al.* 2011, Mollenhauer *et al.* 2011, Zheng *et al.* 2011). Similarly, PFOS exposure was associated with increased IL-6 secretion in cultures of peritoneal macrophages from mice (Qazi *et al.* 2009a, Mollenhauer *et al.* 2011, Dong *et al.* 2012). As

would be expected, the specific effects of PFOS on cytokine secretion vary by dose, cell type, and stimulation conditions.

The effects of *in vitro* exposure to PFOS on IL-4 and IL-6 secretion have also been explored in several studies using whole blood from human volunteers. Plasma levels of PFOS from the human volunteers were associated with increased IL-6 under LPS stimulation suggesting B-cell or monocyte origin (Brieger *et al.* 2011). In contrast, *in vitro* exposure at doses 100 to 100000 ng/ml of PFOS had no impact on IL-6 (Brieger *et al.* 2011). In a separate set of studies using human cells, Corsini *et al.* (2011, 2012) reported that PFOS exposure at doses from (100 to 10000 ng/ml) resulted in lower IL-6 and IL-4 levels in cultures of whole blood that were not dependent on PPARα (Corsini *et al.* 2011). In further tests of potential mechanisms for the observed cytokine changes, Corsini *et al.* (2011, 2012) demonstrated that PFOS inhibited pathways that regulate NF-κB activation, which plays a role in cytokine production as well as apoptosis, inflammation, and other immune functions.

Although there is evidence that PFOS exposure *in vivo* to mice and *in vitro* to human peripheral blood both alter levels of IL-4 and IL-6, further study is necessary to demonstrate consistent changes in cytokines at relevant exposure concentrations (e.g., see Fair *et al.* 2011, and DeWitt *et al.* 2012 for review). Furthermore, other than the PFOS-associated increase in IL-6 from B-cells (Fair *et al.* 2011), the studies do not identify the specific cell types involved or link the cytokine changes to suppression of the antibody response. These cytokines have multiple physiological roles and may reflect inflammation rather than changes in antibody-related cell signaling.

The potential role of PPARα in the mechanism for immune effects was considered because PFOS activates mouse PPARα (the primary PPAR expressed in lymphocytes). A number of PPARα-activating compounds cause liver tumors in rodents and the human relevance of these tumors is subject to debate because of lower levels and/or lower activity of PPARα in human liver (NRC 2006, Corton 2010, Post *et al.* 2012). However, uncertainty over the human relevance does not necessarily apply to non-hepatic effects mediated by PPARα (Post *et al.* 2012). Some of the health effects observed in experimental animals have been linked to the ability of PFOA and PFOS to activate PPARα, and others have been shown to be independent of PPARα. For example, developmental effects of PFOA including neonatal lethality were shown to be PPARα-dependent (Abbott *et al.* 2007), while PFOS induced neonatal lethality and delayed eye opening was independent of PPARα (Abbott *et al.* 2009). PPARα appears to play a role in PFOS-associated changes in immune organ weight and cellularity at high doses of PFOS. Qazi *et al.* (2009a) reported that PFOS-associated (0.02% diet or approximately 40 mg/kg/day) changes in cellularity of the spleen and thymus were partially dependent on PPARα. In contrast to the importance of PPARα for reduced organ weight at high doses PFOS, targeted immune studies suggest that PFOS-associated suppression of the antibody response in mice are independent of PPARα (reviewed in DeWitt *et al.* 2009b). While PPARα appears to contribute to reduced organ weight and changes in immune cell populations at higher doses (30-40 mg/kg PFOS), there is no indication that PPARα is involved at lower doses associated with reduced antibody response (i.e., 0.00166 mg/kg/day PFOS). Although no *in vivo* studies directly examined the role of PPARα in human immune outcomes, *in vitro* studies demonstrated that PFOS effects (100 ng/ml) on cytokine secretion (including IL-4 and IL-6 that may be relevant for antibody production) from human whole blood and human THP-1 cells (a promyelocytic cell line) were PPARα-independent (Corsini *et al.* 2011).

The mechanisms for PFOS- associated suppression of the antibody response are not well understood at this time (DeWitt *et al.* 2012 for review) and the mechanistic data were not considered to provide evidence to support or refute biological plausibility of this effect. Furthermore, the animal data suggests that effects of PFOS on the antibody response are independent of PPARα. Hazard conclusions are based

on a consistent pattern of findings from both the human and animal bodies of evidence that exposure to PFOS are associated with suppression of the antibody response. Therefore, the antibody data support a final hazard identification conclusion that PFOS is *presumed to be an immune hazard in humans*.

Immunosuppression: Disease Resistance/Infectious Disease Outcomes

Direct measures of infectious disease incidence or severity such as respiratory tract infections, pneumonia or otitis media are clearly relevant for evaluating potential immunotoxicity in humans. In experimental animals disease outcomes are generally referred to as "disease resistance", and there are a number of disease resistance models that evaluate the animal's ability to defend against viral, bacterial, or parasitic infections. Endpoints include direct measures of disease resistance such as viral or parasitic load in target tissues, indirect measures such as body weight after infection, and mortality. Disease resistance assays are considered some of the best indicators of immunotoxicity because they generally measure clearly adverse health outcomes and therefore these assays are included in most guidelines or testing requirements for immunotoxicity (ICH 2005, WHO 2012).

Human Infectious Disease Data for PFOS

Summary: There is low confidence that exposure to PFOS is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease). One of three prospective studies (Fei *et al.* 2010) that examined the relationship between maternal PFOS exposure and disease outcomes in offspring reported some evidence of PFOS-associated increase in infectious disease and no association was found in the single adult cross-sectional study (Looker *et al.* 2014). Confidence in the body of evidence for the three prospective studies (Fei *et al.* 2010, Okada *et al.* 2012, Granum *et al.* 2013) was decreased for a lack of consistency across studies and within the Fei *et al.* (2010) study by sex (PFOS was associated with increased hospitalization for infectious disease in girls, but not in boys) and age group analyzed (PFOS was associated with increased hospitalization in analyses combining ages 0-10, but not for individual age groups), to support a final rating of low confidence (see **Table 13** for list of studies, **Figure D16**, **Figure D17** and **Figure D18** for data figure and **Table 22** for confidence ratings summary for the body of evidence). As discussed below, the fact that few specific infectious disease endpoints have been examined (e.g., data are restricted to colds, influenza, gastroenteritis and otitis media) contributes to the low confidence for drawing a conclusion on infectious disease in general. In contrast, the findings by Fei *et al.* (2010) of an association between maternal PFOS and what is likely to be a less sensitive measure of disease (i.e., hospitalization for any infectious disease, which would only capture the most severe outcomes and could miss potential associations with individual diseases) contributes to the confidence in the association.

The available epidemiological studies in the human body of evidence that evaluated the association between exposure to PFOS and infectious disease resistance include: (1) three prospective birth cohort studies in Demark, Norway and Japan that evaluated maternal exposure and (2) a cross-sectional analysis of adult residents in the Ohio Valley with higher PFOA in drinking water (part of the C8 studies) (see **Table 13** for study details). The Danish birth cohort study evaluated hospitalization from infectious diseases (which is of greater severity and likely results in less sensitivity) and the remaining studies looked at any infection or the frequency of self-reported common infections (the specific infections examined varied across studies). The birth cohorts also varied in the time period between exposure (birth) and measurement of the disease and on their statistical models (e.g., β-coefficients/linear regression, Poisson regression). The Danish birth cohort study and the C8 study evaluated exposure response relationships using quartiles of exposure to PFOS (the lowest exposure group served as the comparison group) and the latter also calculated ORs for continuous log transformed PFOS level. Both

the Danish and Japanese study reported findings for males and females separately as well as combined (see **Table 13** for additional details).

Table 21. Summary of PFOS and Selected Data on Infectious Disease in Humans

Disease	PFOS Results	Possible sources of heterogeneity	Exposure measurement timing	Study
Hospitalization for infectious diseases	**Adj. IRR (95% CI) Highest vs. lowest** All: 1.00 (0.76–1.32) **F: 1.59 (1.02–2.49)** M: 0.77 (0.54–1.12) **Trend** All: 1.0 (0.91–1.09) **F: 1.18 (1.03, 1.36)** M: 0.90 (0.80–1.02)	• lower sensitivity of the outcome (hospitalization vs. incidence) • no measure of specific diseases for incidence or frequency • developmental exposure metric	Maternal	Fei (2010)
Gastroenteritis (No. episodes/ frequency)	**β (95% CI)**[a] 0.03 (-0.04-0.10)	• only study of this disease • frequency as measure • developmental exposure metric	Maternal 0-3 day post delivery	Granum (2013)
Otitis media (any)	NR (no association)	• developmental exposure metric • incidence as measure	Maternal 0-3 day post delivery	Granum (2013)
	Adj.OR (95% CI) All: 1.40 (0.33–6.00) F: 1.43 ((0.17–12.30) M: 1.38 (0.18–10.60)	• developmental exposure metric • incidence as measure	Maternal	Okada (2012)
Common cold (No. episodes/ frequency)	**β (95% CI) 3 yrs** 0.01 (-0.02–0.05)	• developmental exposure metric • frequency as measure	Maternal 0-3d post delivery	Granum (2013)
	Adj. OR (95%CI) (continuous)[b] 0.89 (0.60–1.33)	• developmental exposure metric • frequency as measure	Adult Current	Looker (2014)
Flu (any)	**Adj. OR (95%CI)** (continuous)[b] 0.97 (0.58–1.63)	• outcome in adults	Adult Current	Looker (2014)

[a]multivariate models; [b] no association observed in categorical analyses

F=female; M=male; NR = not reported; bold text indicates statistically significant decreased or increased association.

This table highlights select findings to illustrate data informing the potential relationship with infectious disease; for additional data see **Figure D16**, **Figure D17**, **Figure D18**.

As illustrated in **Table 21**, one of three prospective studies that examined the relationship between maternal PFOS exposure and disease outcomes in offspring reported evidence of PFOS-associated increase in infectious disease (Fei *et al.* 2010) and no association was found in the single adult cross-sectional study (**Figure D16**, **Figure D17** and **Figure D18**). Fei *et al.* (2010) examined the association between maternal PFOS and hospitalizations for infectious diseases in children (n = 1400; from birth to age 10 years) from a subset of the Danish National Birth Cohort (1996-2002) with outcomes based on National Hospital Discharge Register. The Fei *et al.* (2010) study only evaluated infectious disease outcomes that were severe enough to warrant hospitalization and therefore the outcome was likely to be a less sensitive measure of disease incidence than doctor diagnosis or prescription-based measures. Potential associations with specific infections could have been missed because there were too few hospitalization events to support infection-specific analyses. Analyses of the association between maternal PFOS and hospitalization by different age groups (0-1, 1-2, 2-4, and 4-10 years of age) were inconclusive. However, in analyses of the entire age group (0-10 years of age) and split by sex, maternal PFOS was associated with increased rate of hospitalization in girls (**Figure D18**). This study also evaluated and reported similar results with maternal PFOA.

Maternal plasma concentrations of PFOS were not associated with common cold, otitis media, or gastroenteritis in children up to age 3 from a sub-cohort of the MoBa study (n = 63-93)(Granum *et al.* 2013). Okada *et al.* (2012) reported that no relationship was found between maternal PFOS and otitis media at age of 18 months in a prospective cohort in Sapporo Japan (n = 343). Although data were

collected on chicken pox, bronchitis, RSV disease, pneumonia and other infectious diseases, the low number of cases (n<20) did not allow analysis of these outcomes.

In adults, current serum concentrations of PFOS were not associated with self-reported cold or influenza over the previous 12 months in a cross-sectional analysis of adults (n = 403) living in the Ohio Valley (C8 study), a region with elevated drinking water PFOA levels (Looker *et al.* 2014).

As discussed in the antibody response section above, exposure characterization risk of bias assessment examined consistency and reliability of the exposure measures and whether exposure was assessed in a relevant time-window for development of the outcome. Maternal serum PFOS were used as the exposure metric for all three studies examining potential effects of prenatal exposure on infectious disease in children (Fei *et al.* 2010, Okada *et al.* 2012, Granum *et al.* 2013). Although the studies cannot exclude the potential impact of post-natal exposure directly to children, maternal concentrations of PFOS are considered good measures of fetal exposure due to the strong positive correlations (e.g., Pearson's correlation between 0.79 – 0.93) reported between maternal PFOS and cord blood PFOS (Glynn *et al.* 2012). Given the slow clearance and long biological half-life of PFOS in humans (2 to 8 years)(Olsen *et al.* 2007a, Kudo 2015), a single sample during pregnancy is considered a good measure of PFOS exposure. Risk-of-bias assessment of important confounders included age, sex, race/ethnicity, smoking, body mass index, alcohol consumption, and variables that represent socioeconomic status based on prior reports of associations with PFOS exposure levels (Calafat *et al.* 2007, Nelson *et al.* 2010) and immune outcomes (WHO 1996, Dallaire *et al.* 2005). Most studies included consideration of all the important confounders listed in the protocol at a minimum but did not adjust for potential effects of PFOA or other PFAAs. Duration of breastfeeding was considered as a confounder the Granum *et al.* (2013) study because of evidence that breast feeding may support immune function and duration of breastfeeding may affect PFAA exposure as well (Karrman *et al.* 2007). The failure to adjust for breastfeeding in the other studies may result in an underestimation of the association between PFOS and infectious disease incidence. The Fei *et al.* (2010) and Granum *et al.* (2013) studies were rated probably high risk of bias for consideration of confounding for limited ability to differentiate the effects of PFOS from PFOA or other PFAAs. The risk of bias of the infectious disease outcome assessment was rated probably low for most studies as their methods included questionnaires relying on medical records, assessment by interviewer, or parent's report of doctor diagnosis. Outcome assessment in the Okada *et al.* (2012) study was rated probably high risk of bias because it was completed using a mothers' self-administered questionnaire with no evidence that the questionnaire was validated (and the authors did not respond to email request for clarification). All of the studies were rated probably low or definitely low risk of bias for exposure characterization (see **Figure D20** and **Figure D21**).

Relationship to antibody response data for PFOS

Studies of infectious disease may provide additional insight on health outcomes potentially associated with reduced antibody response. However, only two human studies measured antibody response to vaccination and infectious disease outcomes in the same populations. Neither study provided evidence for PFOS-associated increases in infectious disease. The Looker *et al.* (2014) study did not find an association between PFOS and the antibody response to influenza in adults from the Ohio Valley (part of the C8 study in a region with elevated drinking water PFOA levels) and therefore would not have been expected to report an association between PFOS and influenza or colds. The Granum *et al.* (2013) study did report that maternal PFOS was associated with decreased antibody levels to rubella vaccination; however, they did not find an association between PFOS and episodes of common cold and gastroenteritis in children up to age three. It may be worth noting that Granum *et al.* (2013) also measured PFOA, PFNA, and PFHxS and reported maternal PFOA, PFNA, and PFHxS were associated with both lower antibody levels to vaccination and increased number of episodes of common cold (PFOA,

PFNA, and PFHxS) and gastroenteritis (PFOA, PFHxS) in children. The strength of the association between PFOA (β =-0.40[-0.64, -0.17]), PFNA (β =-1.26[-2.32, -0.20]), and PFHxS (β =-0.38[-0.66, -0.11]) and lower antibody levels are stronger than the association with PFOS (β =-0.08[-0.14, -0.02]). The weaker association with PFOS and lower antibody levels may explain why the study did not find a PFOS association with infectious disease. As discussed for the antibody response, the effect of PFAAs on infectious disease resistance appears to be in the same direction (reduced ability to respond to infectious disease or increased incidence of disease). The effects of individual PFAAs on the disease resistance can be considered effect modifiers, rather than true confounders of the association between exposures to individual PFAAs (e.g., PFOA and PFOS) and lower disease resistance.

Animal Disease Resistance Data for PFOS

Summary: There is moderate confidence that exposure to PFOS is associated with reduced ability of animals to respond to infectious disease based on available animal studies. The body of evidence is from a single experimental study in female mice (Guruge *et al.* 2009) and two wildlife studies (Kannan *et al.* 2006, Kannan *et al.* 2010). Mice exposed to PFOS (0.005-0.05 mg/kg/day via gavage) for 21 days had reduced resistance to influenza A virus challenge as indicated by lower body weight 10 days post challenge, increased mortality and reduced survival rate (Guruge *et al.* 2009). There was evidence for a dose-response with increasing PFOS exposure across multiple measures of disease resistance. For example, the increased mortality was dose-dependent by trend test (p = 0.014), and a dose effect on decreased survival rate was evident (46%, 30%, and 17% in the control, 0.005 and 0.05 mg/kg/day PFOS groups respectively and significant at the high dose p = 0.035). The wildlife studies provided mixed evidence of an effect of PFOS on disease resistance and therefore the experimental study (Guruge *et al.* 2009) forms the basis of the conclusion for this body of evidence. Confidence in this study (as the only experimental study comprising the body of evidence for experimental studies of PFOS on disease resistance) was decreased because of serious concern for risk of bias to support the final rating of moderate confidence (see **Figure D19** for data figure and study details and **Table 22** for confidence ratings summary for the body of evidence).

There serious concern for risk of bias was based on ratings for the Guruge *et al.* (2009) study of: (1) probably high risk of bias for one of the Key Questions, outcome assessment (due to lack of blinding of outcome assessors), and (2) probably high risk of bias ratings for lack of allocation concealment, concern for potential attrition due to unexplained changes in animal numbers, and lack of researcher blinding during study (**Figure D22**). Potential changes in the confidence rating for the body of evidence were also considered for inconsistency and dose response. Although there was evidence for a dose response across multiple measures of disease resistance, confidence was not upgraded for dose response because the body of evidence was limited to a single study. Similarly, confidence in the body of evidence was not downgraded for inconsistency, although the body of evidence for experimental studies was from a single study and consistency across studies could not be evaluated. In the OHAT method, care is taken not to double count or downgrade confidence in the body of evidence twice for the same factor. Thus, because the data are from a single study, confidence was not upgraded for dose-response, but not downgraded for inconsistency.

The wildlife studies provided mixed evidence of an effect. Higher concentrations of PFOS were found in sea otters with clinical signs of disease compared to healthy animals (Kannan *et al.* 2006). In contrast, there was no difference in PFOS levels between bats in a population with white-nose syndrome compared to a healthy reference population (Kannan *et al.* 2010). As discussed previously for PFOA, both wildlife studies (Kannan *et al.* 2006, Kannan *et al.* 2010) were rated probably high risk of bias for two of the key risk of bias questions: (1) exposure characterization and (2) confounding (**Figure D22**).

Table 22. Disease Resistance /Infectious Disease Evidence Profile for PFOS										
	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					**Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence**				
INITIAL CONFIDENCE for each body of evidence (# of studies)	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	FINAL CONFIDENCE RATING
PFOS										
Human										
Initial Moderate (3 prospective studies)[a]	---	↓	---	---	---	---	---	---	---	**Low**
Initial Low (1 cross-sectional study)[b]	---	---	↓	---	---	---	---	---	---	**Very Low**
Confidence Across Human Bodies of Evidence	No change for considering across study designs									**Low**
Animal										
Initial High (1 mammal study)[c]	↓	---	---	---	---	---	---	---	---	**Moderate**
Initial Low (2 wildlife studies)[d]	↓	---	---	---	---	---	---	---	---	**Very Low**
References: Human: Granum (2013)[a], Fei (2010)[a], Okada (2012)[a], Looker (2014)[b] Animal PFOS: Guruge (2009)[c], Kannan (2006, 2010)[d]										

Evidence Synthesis for Disease Resistance/Infectious Disease Outcomes for PFOS:
There are few human epidemiological studies of PFOS and infectious disease. One of three prospective studies that examined the relationship between maternal PFOS exposure and disease outcomes in offspring reported some evidence of PFOS-associated increase in infectious disease and no association was found in the single adult cross-sectional study. There is low confidence from the human body of evidence that exposure to PFOS is associated with increased incidence of infectious disease due to lack of consistency across studies and within the Fei *et al.* (2010) study by sex (PFOS was only associated with increased hospitalization for infectious disease in girls) or by age group analyzed (PFOS was only associated with increased hospitalization in analyses combining ages 0-10) (see **Table 22** for confidence ratings summary for the body of evidence). The fact that few specific infectious disease endpoints have been examined contributes to the low confidence for drawing a conclusion on infectious disease in general. In contrast, the reported association between maternal PFOS and increased hospitalization in girls (ages 0-10) by Fei *et al.* (2010) using a less sensitive measure (i.e., hospitalization for any infectious disease, which would only capture the most severe outcomes and could miss potential associations with individual diseases) contributes to the confidence in the association. There is moderate confidence that exposure to PFOS is associated with reduced ability of animals to respond to infectious disease based on available animal studies. The moderate confidence in the animal body of evidence translates into a moderate level of evidence and the low confidence in the human body of evidence translates into a low level of evidence. These level-of-evidence conclusions support an initial hazard identification conclusion of *suspected to be an immune hazard to humans* based on the disease resistance data or PFOS exposure is suspected to suppress resistance to infectious disease in humans.

- **Human body of evidence:** Low Confidence = Low Level of Evidence
- **Animal body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Initial hazard conclusion (Low x Moderate)** = Suspected to be an Immune Hazard to Humans
- **Final hazard conclusion**
  (after consideration of biological plausibility) = Suspected to be an Immune Hazard to Humans

The hazard conclusion for PFOS (0.005-0.025 mg/kg/day) is primarily based on an experimental study of disease resistance to influenza A virus challenge in female mice. There is limited support from a wildlife study reporting higher PFOS concentrations in sea otters with clinical signs of disease compared to healthy animals, but there are serious risks of bias concerns for these studies. There is also evidence from a single prospective study reporting an association between maternal PFOS and increased hospitalization for infectious disease in girls (ages 0-10). Mechanistic data can then be used to examine the biological plausibility of PFOS-associated suppression of disease resistance to develop the final hazard identification conclusion. Disease resistance involves multiple components of the immune system, and successful immune response to viral challenge (as in Guruge *et al.* 2009) includes rapid responses from the innate immune system (e.g., NK cell activity and the cytokine IFN-γ) as well as humoral immunity (antibody mediated responses). There is high confidence that exposure to PFOS is associated with suppression of the antibody response and moderate confidence that exposure to PFOS is associated with changes in NK cell activity in animals (see **Table 20** and **Table 23** for confidence ratings summaries in the bodies of evidence). Therefore, the demonstration of PFOS-associated reductions in antibody response in mice at similar exposure levels (0.00166 to 40 mg/kg/day) and NK cell activity in mice at higher exposure levels (0.833 to 40 mg/kg/day) supports the biological plausibility of PFOS-associated suppression of disease resistance.

Two studies reported PFOS-associated reductions in IFN-γ in mice at the same doses that NK cell activity is reduced (0.833 to 20 mg/kg/day) (see cytokine data in **Appendix 5**) (Dong *et al.* 2011, Zheng *et al.* 2011, Dong *et al.* 2012). The effects of PFOS exposure on INF-γ secretion has also been tested in a pair of studies examining the effect of *in vitro* exposure to PFOS or PFOA on multiple cytokines in cultures of whole blood from human volunteers (Corsini *et al.* 2011, Corsini *et al.* 2012). The results indicated that PFOS exposure at concentrations relevant to human exposure (100-10000 ng/ml PFOS, including the lowest dose tested) reduced secretion of multiple cytokines including INF-γ (with PHA stimulation). The effects of PFOS on cytokine production were independent of PPARα and potentially related to interference with NF-κB (Corsini *et al.* 2011).

Unfortunately, there is only one experimental study of disease resistance and the study did not investigate antibody response, NK cell activity, cytokines, or other mechanistic data in the same animals that might be related to the reduced disease resistance reported. In addition, the database is limited to a single disease resistance model (influenza A). Potential mechanisms for PFOS- associated suppression of disease resistance include reduced antibody response, NK cell activity, and production of INF-γ. However, the mechanistic data were not considered to provide strong evidence to support or refute biological plausibility of PFOS-associated suppression of disease resistance because none of the studies established a link between mechanisms such as reduced NK cell activity and the disease resistance endpoints observed. Therefore, the disease resistance data support a final hazard identification conclusion that PFOS is *suspected to be an immune hazard in humans*.

Immunosuppression: Natural Killer (NK) Cell Activity

Natural killer (NK) cells are important for resistance against viruses and tumor cells. Successful defense by NK cells involves killing of target cell through release of cytolytic granules or inducing apoptosis (Dietert 2010). Assays for NK cell activity are included in many immunotoxicity testing guidelines as a measure of immune function because they are considered good predictors for overall immunotoxicity (Luster *et al.* 1992, US EPA 1998, ICH 2005, WHO 2012).

Human NK Data for PFOS

No human data were identified on potential association between PFOS and NK cell activity.

Animal NK Data for PFOS

Summary: There is moderate confidence that exposure to PFOS is associated with changes in NK cell activity in animals. There is consistent evidence that PFOS exposure results in suppression of NK cell activity in mice at doses from 0.833 to 40 mg/kg/day PFOS (Keil *et al.* 2008, Dong *et al.* 2009b, Zheng *et al.* 2009, Vetvicka and Vetvickova 2013). However, at lower doses (0.0166 to 0.166 mg/kg/day), the results are mixed, including no effect of PFOS (Keil *et al.* 2008, female mice in Peden-Adams *et al.* 2008) or increased NK cell activity (male mice in Peden-Adams *et al.* 2008, Dong *et al.* 2009b). The LOAEL after developmental exposure (1 mg/kg/day) (Keil *et al.* 2008) is similar to reported LOAEL's following exposure of juveniles or adults. There was also a wildlife study that reported no association between high serum levels of PFOS (mean 1420 ng/ml; range 317-6257 ng/ml) and NK cell activity in dolphins (Fair *et al.* 2013). Confidence in the body of evidence for experimental studies of PFOS on NK cell activity was decreased because of serious concern for risk of bias to support the final rating of moderate confidence (see **Figure D23** for data figure and study details and **Table 23** for confidence ratings summary for the body of evidence). The serious concern for risk of bias was based on: (1) most studies were rated probably high risk of bias for one of the Key Questions, outcome assessment (due to lack of blinding of outcome assessors), and (2) all studies were rated probably high risk of bias for lack of allocation concealment, and lack of researcher blinding during study (**Figure D25** and **Figure D26**).

The suppression of NK activity at doses above 0.833 mg/kg/day PFOS was considered a consistent pattern of findings and not downgraded for inconsistent effects at lower doses. Reduced NK cell activity in the lower dose range (0.833 to 5 mg/kg/day PFOS) takes place without changes in body weight, spleen or thymus cellularity, or other signs of overt toxicity. The conclusions are restricted to the evidence of PFOS-associated suppression; although some authors suggest the data support an inverted U-shaped dose response with increased NK activity at low doses of PFOS (0.017 to 0.166 mg/kg/day) and decreased NK cell activity at higher doses (Wirth *et al.* 2014). Additional data would be necessary to further characterize the shape of the dose-response curve at lower doses. Although NK activity was suppressed at doses above 0.833 mg/kg/day PFOS, confidence was not increased for dose response as the evidence for dose response was unclear. Several studies showed the same or lower magnitude of effect at higher doses (e.g., male mice show 42% reduction in NK activity at 1 mg/kg dose, but only a 28% reduction at 5 mg/kg in Keil *et al.* 2008) (see **Figure D23**).

In vitro /Mechanistic Data on NK Cell Activity for PFOS

Two studies were identified that evaluated NK cell activity using cells of different origins and *in vitro* exposure to PFOS: 1) a study using human peripheral blood and 2) a study using bottlenose dolphin peripheral blood as well as mouse spleen cells. In a small pilot study of 11 volunteers, Brieger *et al.* (2011) tested the effect of *in vitro* exposure to PFOS on NK cell activity. Exposure consisted of pre-treatment of peripheral blood cells with 0, 1000, 10000, or 100000 ng/ml PFOS for 24 hours. Exposure to PFOS at the high dose decreased NK cell activity by 32% (Brieger *et al.* 2011)(see **Figure D24**). Concentrations used for the NK cell assay had no effect on cell viability.

Wirth *et al.* (2014) tested NK cell activity of mouse spleen cells and peripheral blood leukocytes from bottlenose dolphins exposed to PFOS at concentrations from 10 to 5000 ng/ml. NK cell activity from the mouse spleen cells (n = 15 mice per dose group) was decreased by 30-45% at PFOS concentrations from 10-1000 ng/ml and increased 450% at 5000 ng/ml PFOS (see **Figure D24**). The suppression of NK cell activity with *in vitro* exposure to PFOS (10-1000 ng/ml) is consistent with the *in vivo* exposure studies in mice that have reported decreased NK cell activity at higher PFOS doses (≥0.833 mg/kg/day and serum levels of 65000 ng/ml). However, there was no evidence of a dose response for the concentrations that resulted in decreased NK cell activity, and the authors provide no explanation as to why the highest dose resulted in increased activity (cell viability was not affected by PFOS at the concentrations tested). PFOS exposure had no effect on NK cell activity in cells from the bottlenose dolphins (n = 12 per dose group) at the concentrations tested. Although the animals used for this study were "managed-care" dolphins from captive populations, serum PFOS levels of the dolphins used as sources of NK cells were not tested and background levels of PFOS may have decreased the sensitivity of the assay and ability to detect a possible effect of PFOS. Serum PFOS levels in dolphins can be very high due in part to their diet; wild-caught dolphins have very high reported serum levels of PFOS (from 317 to 6257 ng/ml) (Fair *et al.* 2013).

Although *in vitro* NK cell activity assays have not been shown to fully predict *in vivo* toxicity (e.g., assays may be lacking in some relevant cell populations), they are considered good evidence and support for potential effects on NK cells *in vivo*. The two *in vitro* studies support suppression of NK cell activity with PFOS exposure using cultures of human and mouse cells. The PFOS concentrations used in the mouse studies are within exposure ranges of the general population; however, the concentrations of PFOS used for the human cells are well above typical human exposure range of the general population [e.g., Olsen *et al.* (2003b) reported a range of 4.3 to 1656 ng/ml PFOS in serum from American Red Cross blood donors in 2000-2001 and the latest NHANES data for 1999-2012 reported mean (95% CI) was 6.31 ng/ml (5.84-6.82) for serum PFOS (CDC 2015)]. The PFOS concentration that resulted in decreased NK cell activity was 10 times greater than high occupational exposure levels [maximum reported levels approximately 90000 ng/ml PFOA and 10000 ng/ml PFOS (Olsen *et al.* 2003a, Costa *et al.* 2009)]. Both *in vitro* studies were well conducted, and rated probably low or definitely low risk of bias for all questions including exposure characterization and outcome assessment that included blinding of outcome assessors (see **Figure A3-4**).

The two *in vitro* assays provided support, but were not considered to provide strong support for the biological plausibility of PFOS effects on NK cell cytotoxicity because the mouse cell data are from a single study lacking evidence of a dose response, and the human cell data only reported effects at PFOS at concentrations above human exposure levels. Other mechanistic data relevant for evaluating potential PFOA or PFOS effects on the NK cell activity are discussed below in the evidence synthesis section in the context of biological plausibility. Relevant mechanistic data would include effects of PFOS on changes in NK cell populations, cell signaling, or NK cell activation by target cells.

Table 23. NK Cell Activity Evidence Profile for PFOS										
	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					**Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence**				
INITIAL CONFIDENCE for each body of evidence (# of studies)	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	FINAL CONFIDENCE RATING
PFOS										
Animal										
Initial High (5 studies)[a]	↓	---	---	---	---	---	---	---	---	**Moderate**
Note: no human studies of NK cell activity **References:** Animal[a]: Dong (2009b), Keil (2008), Peden-Adams (2008), Vetvicka (2013), Zheng (2009)										

Evidence Synthesis for NK Cell Activity for PFOS

The animal studies provide the basis for developing hazard conclusions on the relationship between PFOS exposure and NK cell activity. There is moderate confidence that exposure to PFOS is associated with changes in NK cell activity based on experimental animal data including consistent evidence for PFOS-associated suppression of NK cell activity in mice at doses from 0.833 to 40 mg/kg/day (see **Table 23** for confidence ratings summaries for the bodies of evidence). There is no human evidence to evaluate the potential association between exposure to PFOS and NK cell function because no epidemiological studies of PFOS and NK cell activity were identified. The lack of human studies translates into inadequate level of evidence and the moderate confidence in the animal body of evidence translates directly into a moderate level of evidence for PFOS effects on NK cell activity. These level-of-evidence conclusions support an initial hazard identification conclusion of *suspected to be an immune hazard to humans* based on the NK cell activity data or PFOS exposure is suspected to suppress the NK cell activity in humans.

- **Human body of evidence:** Inadequate Confidence/no evidence = Inadequate Level of Evidence
- **Animal body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Initial hazard conclusion (Inadequate x Moderate)** = Suspected to be an Immune Hazard to Humans
- **Final hazard conclusion (after consideration of biological plausibility)** = Suspected to be an Immune Hazard to Humans

The hazard conclusion for PFOS is based on the consistent pattern of findings from the animal body of evidence that PFOS doses from 0.833 to 40 mg/kg/day are associated with suppression of NK cell activity in mice. Mechanistic data from *in vitro* or *in vivo* studies can then be used to examine the biological plausibility of PFOA- and PFOS- associated suppression of NK cell activity to develop the final hazard identification conclusion. Two *in vitro* studies were located that directly tested NK cell activity with *in vitro* exposure to PFOS. These *in vitro* NK assays provided support, but were not considered to provide strong support for the biological plausibility of PFOS effects on NK cell cytotoxicity because the mouse data are from a single study lacking evidence of a dose response, and the human data only reported effects at PFOS concentrations above human exposure levels (100000 ng/ml PFOS).

NK cell activity is a rapid response to viral-infected cells and tumor cells that is considered part of innate immunity because it does not require antibodies to recognize infected cells. NK cells are stimulated by cytokines released by viral-infected cells, thus the cytokine activation is linked to the presence of viral pathogens. Multiple cytokines have been implicated in activation of NK cells including IL-2, IL-12, IL-15, and IL-18. Activation, in turn, leads to the release of cytolytic granules as well as release of INF-γ by NK cells (Dietert 2010). Therefore, the most relevant mechanistic data would include effects of PFOS at relevant concentrations on NK cell numbers, cytokines important for NK cell activation, or release of cytolytic granules and INF-γ from NK cells.

Only one study was located that evaluated potential effects of PFOS on NK cell numbers (Qazi *et al.* 2009b); and the data do not provide consistent evidence of PFOS-associated changes (see cell phenotyping data in **Appendix 5**). Reduced NK cell activity in the lower dose range (0.833 to 5 mg/kg/day PFOS) takes place without signs of overt toxicity (i.e., NK activity is suppressed at doses without changes in cellularity of the spleen, spleen weight, or body weight). Although there are no data on potential PFOS effects on most of the cytokines associated with NK cell activation (e.g., IL-12, IL-15, and IL-18), a set of studies reported changes in IL-2 and IFN-γ in mice at the same doses that NK cell activity is reduced (0.833 to 20 mg/kg/day) (see cytokine data in **Appendix 5**) (Dong *et al.* 2011, Zheng *et al.* 2011, Dong *et al.* 2012). Oral exposure of PFOS in mice was associated with reductions in the number of IL-2 positive T-cells and reduced INF-γ secretion by splenocytes in culture.

The effects of PFOS exposure on INF-γ secretion has also been tested in a pair of studies examining the effect of *in vitro* exposure to PFOS on multiple cytokines in cultures of whole blood from human volunteers (Corsini *et al.* 2011, Corsini *et al.* 2012). The results indicated that PFOS exposure at concentrations relevant to human exposure (100-10000 ng/ml PFOS, including the lowest dose tested) reduced secretion of multiple cytokines including INF-γ (with PHA stimulation). The effects of PFOS on cytokine production were independent of PPARα and potentially related to interference with NF-κB (Corsini *et al.* 2011). The Corsini *et al.* (2011, 2012) studies demonstrated that PFOS inhibited pathways that regulate NF-κB activation, which play a role in cytokine production as well as apoptosis, inflammation, proliferation and other aspects of immune function.

The studies reporting PFOS-associated decreases of INF-γ from mouse splenocytes and human peripheral blood add to the biological plausibility of effects on NK cell activity. Unfortunately, none of the experiments with human or mouse cells differentiated whether or not reduced INF-γ was from NK cells or other cell types and INF-γ is also produced by various T-cell populations and macrophages.

Potential mechanisms for PFOS- associated suppression of NK cell activity include reduced IL-2 mediated NK cell activation, reduced production of INF-γ by NK cells, and a role for NF-κB. However, the mechanistic data were not considered to provide strong evidence to support or refute biological plausibility of PFOS-associated suppression of NK cell activity. Therefore, the NK cell activity data support a final hazard identification conclusion that PFOS is *suspected to be an immune hazard to humans*.

Hypersensitivity-related Effects and Outcomes

Sensitization or hypersensitivity-related responses include a number of undesirable or exaggerated immune reactions (e.g., allergies or asthma) to foreign agents. These responses are complicated by the two-phase nature of a hypersensitivity reaction. The first phase, sensitization, is without symptoms and it is during this step that a specific interaction is developed to the sensitizing agent so that the immune

system is prepared to react to the next exposure. Once an individual or animal has been sensitized, contact with that same agent leads to the second phase, elicitation, and symptoms of allergic disease. While hypersensitivity responses are mediated by circulating factors such as T-cells, IgE, and inflammatory cytokines, many of the health effects associated with hypersensitivity and allergic response are respiratory or dermal (e.g., asthma, airway hyper-responsiveness, and contact dermatitis). Chemicals may exacerbate or promote a hypersensitivity-related outcome without being direct sensitizers.

Human Hypersensitivity Data for PFOS

Summary: There is very low confidence that exposure to PFOS during childhood is associated with changes in the hypersensitivity responses in children based on the available human studies. The results of several cross-sectional studies present inconsistent association between current PFOS concentrations in children and asthma and other airway hypersensitivity-related endpoints (see **Figure D28** for data figures). No prospective studies were located that assessed hypersensitivity relative to childhood PFOS exposure. However, prospective studies in younger children (birth to age 9) report no association between maternal levels of PFOS and hypersensitivity endpoints (see **Table 15** for list of studies and **Table 24** for select data on asthma and IgE). Confidence in the body of evidence was downgraded for unexplained inconsistency. There was no clear explanation for the heterogeneity in the findings across the childhood exposure studies.

Levels of PFOS in pregnant women and hypersensitivity response in children

Maternal serum or plasma concentrations of PFOS were not associated with hypersensitivity across multiple indicators (e.g., asthma or eczema) in children age 18 months to 9 years across five prospective studies. Maternal PFOS concentrations were not associated with eczema or itchiness, wheeze, or doctor-diagnosed atopic eczema or asthma in 3-year old children from a sub-cohort of the MoBa study (n = 63-93) (Granum *et al.* 2013). Similarly, maternal PFOS was not associated with food allergy, eczema or wheezing in children through 18 months of age from a prospective cohort in Sapporo Japan (n = 231) (Okada *et al.* 2012). Wang *et al.* (2011) also reported that maternal PFOS was not associated with atopic dermatitis at 2 years of age in a prospective cohort in Taiwan (n = 244). In a fourth prospective cohort study, maternal PFOS was not associated with total allergic diseases, eczema, wheezing or allergic rhinoconjuctivitis symptoms in children through 2 years of age from a prospective cohort in Hokkaido Japan (n = 2062) (Okada *et al.* 2014). In a fifth prospective cohort study, maternal PFOS was associated with decreased wheeze in the Ukraine population (n = 492), but not in the Greenland population (n = 532) or with asthma or eczema in either group of children at 5-9 years of age (Smit *et al.* 2015).

The body of evidence with exposure measured during development presents inconclusive results and there is a lack of association between maternal PFOS concentrations and hypersensitivity outcomes in children, and there is inconsistent evidence that maternal PFOS is associated with cord blood levels of IgE. There was no association between maternal PFOS and cord IgE in either the Japanese Sapporo cohort or Canadian MIREC cohort (n = 1242) (Okada *et al.* 2012, Ashley-Martin *et al.* 2015). However, cord blood PFOS was associated with increased cord IgE for male offspring of the Taiwan Birth Panel cohort (Wang *et al.* 2011). Although White *et al.* (2011) only measured IgE in maternal serum, not cord blood, the authors reported PFOS-associated decreased serum levels of IgE in nursing mothers in the MAMMA study.

The risk of bias considerations for exposure characterization and confounding for hypersensitivity related outcomes are the same for PFOS as were discussed above for PFOA and therefore not repeated here (see discussion above and protocol for more details on risk of bias considerations

http://ntp.niehs.nih.gov/go/749926). All of the studies were rated probably low or definitely low risk of bias for exposure and confounding (see **Figure D34**).

Table 24. Summary of PFOS and Selected Data on Hypersensitivity in Humans

Disease	PFOS	Possible sources of heterogeneity	Exposure measurement	Study
Asthma	Adj OR (95% CI) Highest vs. lowest quartile OR = 2.63 (1.48 – 4.69) **Trend p = 0.003 (males + females)**	• ever asthma • childhood exposure metric • sig. for males, combined, not females p trend=0.899)	Child: current serum	Dong (2013) and Zhu (2016)
	OR = 1.22 (0.89 – 1.66)	• ever asthma or current • maternal exposure metric	Maternal plasma 0-3d post delivery	Granum (2013)
	Adj OR (95% CI) Ln-linear (p=0.13) or linear (p=0.07) OR = 0.88 (0.74 – 1.04)	• ever asthma • not sig. for current asthma • childhood exposure metric	Child: current serum	Humblet (2014)
	OR for 1 SD change in serum PFOS OR = 0.75 (0.39 – 1.42)	• current asthma • maternal exposure metric	Maternal: serum	Smit (2015)
Total IgE	Adj OR log10 PFOS concentration OR = 1.1 (0.6 – 1.9)	• cord blood IgE measure • maternal exposure metric	Maternal: first trimester plasma	Ashley-Martin (2015)
	Mean IgE (95% CI) quartiles PFOS in asthmatics Q1: 517.9 (336.7 – 699.2) Q2: 686.2 (501.3 – 871.1) Q3: 658.1 (475.2 – 841.1) **Q4: 877.3 (695.2 – 1059.5)** **Trend: p=0.008 (males + females)**	• childhood exposure metric • childhood IgE measure • outcome in asthmatics • not sig. in non-asthmatics • sig for females, combined, not males (p trend=0.082)	Total IgE in serum in children (age 12-19)	Dong (2013) and Zhu (2016)
	Log10 quadratic PFOS quadratic polynomial regression coefficient **F:** -0.681 (-2.500 – 1.137) **M**: 0.911 (-1.101 – 2.922)	• maternal exposure metric • cord blood IgE measure	Maternal serum after the second trimester of pregnancy	Okada (2012)
	Adjusted β (S.E.) **β =0.161 (0.147), p=0.017** F: β=0.151 (0.165), p=0.616 **M: β=0.175 (0.179), p=0.053**	• cord exposure metric • cord blood IgE measure • not sig. in childhood IgE measure	Cord blood serum sample measures of PFOA/PFOS (ng/ml)	Wang (2011)

Bold text indicates statistically significant decreased or increased association; sig. = significant.

This table highlights select findings to illustrate studies that evaluated asthma (having ever had a diagnosis or some analyses current asthma) and total IgE in different populations relative to PFOS measured in pregnant women, children, and adults; for additional data see **Figure D28**.

Levels of PFOS in children and hypersensitivity response in children

Five studies with data on the potential association between current levels of PFOS in children and indicators of hypersensitivity present inconsistent results. Two of the cross-sectional studies based on NHANES data on children age 12-19 present data that suggest PFOS in children may generally be associated with decreased airway hypersensitivity (Humblet *et al.* 2014, Stein *et al.* 2016) and the third indicates that PFOS may be associated with increased odds ratio for self-reported food allergies (Buser and Scinicariello 2016). Humblet *et al.* (2014) reported an inverse relationship that was borderline significant for current PFOS concentrations and asthma (p = 0.13 and p = 07 ln-linear and linear models respectively) or wheeze (p = 0.08 and p = 37 ln-linear and linear models respectively) using NHANES data from 1999-2000 and 2003-2008 (n = 1877). Stein *et al.* (2016) examined potential association with total serum IgE and specific IgE levels to common allergens using a smaller sample size restricted to fewer sample years (n = 638; from 2005-2006). Serum PFOS concentration was associated with lower allergen-specific IgE levels for several allergens including plants and cockroaches/shrimp. However, PFOS was associated with increased IgE levels to mold suggesting that the response to different allergens may be impacted differently by PFOS.

In contrast to the generally negative relationship with airway-hypersensitivity markers from the NHANES data, current serum PFOS was associated with a higher odds ratio of doctor diagnosed asthma in a study of 231 asthmatic and 225 nonasthmatic children age 10-15 from Taiwan (Dong *et al.* 2013) (**Figure D28**). PFOS was also positively associated with total serum IgE, absolute eosinophil count and eosinophilic cationic protein concentration among asthmatics. In subsequent analyses of the case-control study of asthmatics in Taiwan, Zhu *et al.* (2016) explored the potential role of TH_1 (IL-2 and IFN-γ) and TH_2 cytokines (IL-4 and IL-5) as a mechanism by which PFOS or other PFAAs may contribute to the development of asthma and the role of the child's sex. There is overlap in cytokine function, but in general IL-4, IL-5 and other TH_2 cytokines are associated with promotion of IgE and eosinophilic responses associated with atopy, asthma and hypersensitivity. In contrast, the IL-2, INFγ and other TH_1 cytokines function in cell-mediated immunity against intracellular pathogens. Zhu *et al.* (2016) reported that in male children with asthma (n = 158), serum PFOS was associated with polarization toward a TH_2 response indicative of hypersensitivity; although the increased IL-4 and IL-5 cytokines were borderline significant in males ($p = 0.059$ and $p = 0.064$ respectively) and not significant in females ($p = 0.350$ and $p = 0.940$ respectively). The limited evidence of a pro-TH_2 cytokine shift that was stronger in males was similar to the finding that serum PFOS was associated with diagnosis of asthma when considering males and females together ($p < 0.001$) and for males alone ($p = 0.001$), that was not significant when evaluating PFOS and females alone ($p = 0.899$) (Zhu *et al.* 2016).

Buser and Scinicariello (2016) used multivariate logistic regression to analyze the potential association between current serum PFOS concentrations and two separate indicators of food sensitization: (1) food-specific IgE over a concentration clinically considered to indicated an allergic response (0.35 kU/L) and (2) self-reported food allergies in children ages 12-19 from NHANES. Serum PFOS was associated with an increased OR for self-reported food allergies (OR = 2.95 [95%CI 3.32 to 24.90] for highest quartile vs. lowest; p-trend <0.27) in children from NHANES 2007-2010. However, PFOS was not associated with food-specific IgE in children from NHANES 2005-2006.

The risk-of-bias assessment of the exposure characterization for these studies was discussed previously above in the context of PFOA and similar reasoning applies for PFOS. In brief, exposure misclassification is of concern because of the cross-sectional study design, although this is mitigated by the long biological half-life of PFOS in humans (2 to 8 years) (Olsen *et al.* 2007a, Kudo 2015). As a result, the three NHANES studies (Humblet *et al.* 2014, Buser and Scinicariello 2016, Stein *et al.* 2016) were rated probably low risk of bias for exposure characterization (see **Figure D32** and **Figure D33**). In contrast, the Taiwanese studies were rated probably high risk of bias for exposure because of the high likelihood of exposure misclassification (over half of the cases were diagnosed with asthma before 5 years of age and exposure was measured 5 to 10 years after the development of asthma). Although the greater likelihood of exposure misclassification in the Taiwanese studies may explain some of the heterogeneity in response, it is not clear how exposure misclassification would result in a positive association with PFOS. Therefore, the body of evidence was downgraded for unexplained inconsistency in the association between PFOS levels in children and measures of hypersensitivity across studies.

Confidence in the body of evidence was not further downgraded for risk of bias, because care is taken not to double count or downgrade confidence in the body of evidence twice for the same factor. In the OHAT method, the relevance of the exposure assessment for the time-window appropriate to the development of the outcome can be considered in two places: (1) in assessing the risk of bias for the exposure characterization as described above, and (2) in setting the initial confidence in bodies of evidence based on study design factors. When rating confidence in the body of evidence, studies with a cross-sectional study design (e.g., the body of evidence with data on childhood PFOS exposure and hypersensitivity) start at a lower initial confidence because cross-sectional exposure sampling cannot

assure exposure took place before development of the outcome. Ratings of definitely high risk of bias in the exposure characterization would have been considered further and may have resulted in further downgrading the confidence in the body of evidence.

Animal Hypersensitivity Data for PFOS

Summary: There is low confidence that exposure to PFOS is associated with a change in the hypersensitivity response in animals because the results are inconsistent from a single study that directly tested airway hypersensitivity and a second study that examined antigen-specific IgE in mice (Dong *et al.* 2011, Ryu *et al.* 2014). Dietary exposure to PFOS (4 mg/kg diet through 12 weeks of age) was associated with greater airway sensitivity to methacholine; however, the association was not consistent as other airway measures did not support hyperresponsiveness (e.g., no effect on airway resistance, tissue resistance, or elastance) and some results suggested suppression (e.g., blunted OVA-induced rise in leukocytes and macrophages in BALF) (Ryu *et al.* 2014). Dong *et al.* (2011) reported that oral PFOS exposure (0.8333 mg/kg/day via gavage) for 60 days was associated with increased antigen-specific IgE levels following SRBC challenge.

Confidence in the body of evidence was downgraded twice for very serious concern for unexplained inconsistency because the results within each study are inconsistent and do not present clear association for hypersensitivity and PFOS exposure. In addition, there is only one airway hypersensitivity study and one study of the antigen-specific IgE so there is no ability to evaluate consistency across studies. The inconsistency and inability to evaluate consistency across studies support the final rating of very low confidence (see **Figure D29** and **Figure D30** for data figures and study details and **Table 17** for confidence ratings summary for the body of evidence). The Ryu et al. (2014) study included prenatal, juvenile, and adult exposure to PFOS, and therefore does not directly address if developmental-only exposure is or is not associated with hypersensitivity in animal models.

In Vitro /Mechanistic Data on Hypersensitivity-related Endpoints for PFOS

The Yamaki and Yoshino (2010) study discussed earlier in the context of PFOA also included exposure to PFOS, and was the only PFOS *in vitro* exposure study of hypersensitivity-related endpoints identified. A more detailed discussion is included above and only briefly outlined here. The increased release of hypersensitivity mediators from rat basophils after *in vitro* exposure to PFOS (5000 - 150000 ng/ml PFOS) was similar to that reported with PFOA treatment. However, this *in vitro* study simply adds more data at a relatively high exposure level and does not support other studies because the human and animal data on PFOS-associated hypersensitivity are inconsistent.

Evidence Synthesis for Hypersensitivity-related Outcomes for PFOS

The human studies on hypersensitivity-related outcomes differed depending on the exposure window evaluated. There is no evidence of an association between maternal PFOS concentrations and hypersensitivity outcomes in children and few studies in adults.

For PFOS, there is low confidence in the animal body of evidence for hypersensitivity because there are few experimental animal studies of PFOS and hypersensitivity-related endpoints and the results of these studies are inconsistent. There is very low confidence from the human body of evidence that exposure to PFOS is associated with changes in hypersensitivity responses due to inconsistent association between current PFOS concentrations in children and asthma and other airway hypersensitivity-related endpoints. The very low confidence in the human body of evidence translates into inadequate level of evidence and the low confidence in the animal data translates into a low level of evidence. Therefore, an evidence profile or detailed discussions of the evidence synthesis were not developed for PFOS and

hypersensitivity-related outcomes and this health effect was not considered for hazard identification conclusions.

Autoimmunity-related Effects and Outcomes

Autoimmune disease and related effects are the result of immune responses against self-molecules (WHO 2012). Autoimmune disorders are frequently tissue or organ specific, although they may be systemic when the reaction is to antigens present across multiple tissues. Autoimmune diseases are usually examined in the context of the damaged tissues or organs (e.g., rheumatoid arthritis, thyroiditis, or ulcerative colitis). Tests for chemical induced autoimmunity in animals generally involve model systems developed to evaluate one specific type of autoimmune response. Due to the specificity of the assays, these tests are used when autoimmunity is suspected and not as part of routine screening for autoimmunity.

Human Autoimmunity Data for PFOS

The only study located that tested for potential PFOS-associated autoimmunity is a pilot study that reported prenatal concentrations of PFOS were negatively associated with anti-actin IgG in a test for antibodies to several neural or non-neural antigens in 7 year old children from the Faroe Island birth cohort (Osuna *et al.* 2014). A change in autoantibodies without support from other related endpoints (e.g., for the neural antigens studied) is not considered to provide clear evidence for or against an effect on autoimmunity (WHO 2012). Therefore, the body of evidence based on this single pilot study was considered inadequate and there is very low confidence in the body of evidence from human studies for evaluating the potential association between PFOS exposure and autoimmunity.

Animal Autoimmunity for PFOS

No animal studies were identified on potential association between PFOS and autoimmunity.

In vitro /Mechanistic Data on Autoimmunity-related Endpoints for PFOS

No *in vitro* exposure studies were identified on the potential association between PFOS and autoimmunity.

Evidence Synthesis for Autoimmunity Data for PFOS

No experimental animal studies were located testing PFOS exposure in autoimmune animal models and the human body of evidence was restricted to a single pilot study examining the potential association between maternal PFOS exposure and autoantibodies in children. Both the human and animal evidence translate into inadequate level of evidence. Therefore, an evidence profile table and detailed discussion of the evidence synthesis were not developed for PFOS and autoimmunity and this health effect was not considered for hazard identification conclusions.

DISCUSSION

Based on a systematic review of the evidence, the NTP concludes that both PFOA and PFOS are *presumed to be immune hazards to humans*. The health effects data for PFOA and PFOS were considered separately in developing these conclusions.

The NTP concludes that exposure to PFOA is ***presumed to be an immune hazard to humans*** based on a high level of evidence that PFOS suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans (**Table 7**. Although the strongest evidence for an effect of PFOA on the immune system is for suppression of the antibody response and increased hypersensitivity, there is additional, although weaker, evidence that is primarily from epidemiological studies that PFOA reduced infectious disease resistance, increased hypersensitivity-related effects, and increased autoimmune disease. The bodies of evidence indicating that PFOA affects multiple aspects of the immune system add to the overall confidence that PFOA alters immune function in humans. However, the mechanism(s) of PFOA-associated immunotoxicity are not clearly understood and effects on diverse endpoints such as suppression of the antibody response and increased hypersensitivity may be unrelated.

The NTP concludes that exposure to PFOS is ***presumed to be an immune hazard to humans*** based on a high level of evidence that PFOS suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans (**Table 9**). Although the strongest evidence for an effect of PFOS on the immune system is for suppression of the antibody response, there is additional, although weaker, evidence that is primarily from studies in experimental animals that PFOS suppresses disease resistance and natural killer (NK) cell activity. The NTP also concludes that PFOS is suspected to suppress infectious disease resistance and NK cell activity in humans, and these conclusions are based on moderate level of evidence from animal studies and low or inadequate level of evidence from human studies. The bodies of evidence indicating that PFOS suppresses multiple aspects of the immune system add to the overall confidence that PFOS alters immune function in humans. Although the mechanism(s) by which PFOS suppresses these immune functions are not clearly understood, suppression of the antibody response and NK cell function are both potential mechanisms by which PFOS may reduce disease resistance.

The majority of data and the strongest bodies of evidence for both the human and animal studies to inform the evaluation of both PFOA- and PFOS-associated immunotoxicity are on suppression of the antibody response.

The moderate level of evidence from human studies is supported by ratings of moderate confidence in the bodies of evidence from human studies of PFOA and PFOS on the antibody response. These confidence ratings are based on results of epidemiological studies that consistently reported an association between higher prenatal, childhood, or adult serum PFOA or PFOS concentrations and lower anti-vaccine antibody levels. The data present a consistent pattern of findings across different populations (e.g., NHANES and the Ohio Valley in the United States, Norway, and the Faroe Islands) and multiple vaccinations. However, the use of different vaccinations also presents a challenge in comparing results across studies, because different studies generally tested the antibody response to different vaccinations. Thus the consistent demonstration that higher maternal, childhood, and adult PFOA or PFOS concentrations are associated with lower anti-diphtheria antibodies levels are studies from a single research group (Grandjean *et al.* 2012, Kielsen *et al.* 2016). Although studies often evaluated the antibody response to more than one vaccine, antibody levels were only associated with PFOA or PFOS concentrations for a subset of the vaccines examined. However, different responses are expected as

different vaccines may stimulate different components of the immune system and the strength and length of an antibody response is known to differ across vaccines so this heterogeneity was not considered a reason to downgrade the evidence for inconsistency.

Where direct comparisons across studies and researchers were possible (e.g., rubella), the results were considered a consistent pattern of findings to support the association between elevated serum levels of both PFOA and PFOS and suppression of the antibody response. The heterogeneity in the findings may be explained by differences between the vaccinations tested and variation in the timing of the exposure measure (maternal vs. childhood) or method to measure the antibody response (antibody level at some time separated from vaccination or antibody level falling below a clinical value considered to offer protection). Exposure to PFOA or PFOS is not associated with changes in the antibody response to measles for example. In contrast, higher maternal or childhood PFOA or PFOS serum concentrations are associated with lower anti-rubella antibodies in both of the studies that examined the response to rubella vaccination. Stein *et al.* (2016) demonstrated that current serum concentrations of both PFOA and PFOS in children age 12-19 were associated with lower anti-rubella antibodies using US NHANES data. Similarly, Granum *et al.* (2013) reported maternal plasma concentrations of both PFOA and PFOS were associated with lower anti-rubella antibodies in 3-year old children in Norway.

The high level of evidence from animal studies is supported by ratings of high confidence in the bodies of evidence from animal studies of both PFOA and PFOS on the antibody response. These confidence ratings are based on results that consistently show that both compounds suppress the primary antibody response in mice (at oral doses from 3.75 to 30 mg/kg/day PFOA and 0.00166 to 40 mg/kg/day PFOS). Not only is there high confidence in the body of evidence from animal studies that PFOS suppresses the antibody response, but the animal data also demonstrate suppression at PFOS serum levels that are relevant to general human exposure levels. The serum PFOS levels in mice associated with the lowest dose that suppressed the antibody response [92 ng/ml PFOS (Peden-Adams *et al.* 2008)] are below occupational exposure levels (range 145 to 3490 ng/ml PFOS)(Olsen *et al.* 2007a) and approximately 3x higher than the upper end of serum PFOS levels of the general population (range 4.3 to 36.9 ng/ml PFOS)(Olsen *et al.* 2007b). In contrast, the serum concentrations of PFOA associated with suppression of the antibody response in animal studies are above most human exposure levels. The serum PFOA levels in mice associated with the lowest dose that suppressed the antibody response [~74000 ng/ml PFOA (Dewitt *et al.* 2008)] are above most occupational exposure levels [range 200 to 91900 ng/ml PFOA (Costa *et al.* 2009)].

Most of the epidemiological studies reported suppression in the antibody response at exposure levels similar to that of the general US population (mean serum concentrations of PFOA and PFOS were 1-4 ng/mL and 16-27 ng/mL respectively), although serum PFOA levels (mean 33.74 ng/ml) in the Looker *et al.* (2014) study of Ohio Valley residents are roughly 5x general population levels. Therefore, concern for suppression in the antibody response at exposure levels of the general US population is indicated by the epidemiological studies of both PFOA and PFOS. Furthermore, the hazard and dose concerns are supported by animal studies using internal dose metrics (i.e., serum levels) for PFOS. While the animal data support the hazard conclusion for PFOA, the serum levels associated with suppression in the animal studies are much higher.

Serum or other internal dose metrics may be particularly useful when evaluating the consistency of human and animal evidence because of substantial species differences in the elimination rates for PFOA and PFOS. The results of non-human animal studies show clear species differences with mice being sensitive to a range of immune effects of both PFOA and PFOS, and few effects observed in rats or monkeys [although the immune outcomes examined in monkeys are limited to spleen and thymus

weight and histology (Griffith and Long 1980, Butenhoff *et al.* 2002)]. The relative lack of sensitivity of rats for immune effects of PFOA may be a result of rapid clearance in rats relative to mice or humans (e.g., estimated half-life for PFOA in female rats is 2-4 hours compared to 20-30 days in mice and 2-5 years in humans Rodriguez *et al.* 2009).

Animal studies, particularly studies in mammals, are considered relevant models for predicting human health effects in the absence of evidence to the contrary (e.g., if effects in the animal model are mediated by a mechanism that is not present in humans). The mechanism(s) by which PFOA and PFOS decrease the antibody response is not completely understood, and data from animal models and *in vitro* studies have not identified consistent evidence for disruption of key cell populations (e.g., B- or T-cells numbers) or cytokine signaling at the lower exposure levels associated with changes in antibody response. The potential role of PPARα for immune effects in experimental animals was considered. PPARα appears to play a role in several immune effects of PFOA in mice, including decreased spleen and thymus weight, reduced spleen and thymus cellularity, and mitogen (ConA and LPS)-induced lymphoproliferation (Yang *et al.* 2002b). However, studies suggest that many immune effects of both PFOA and PFOS, particularly suppression of the antibody response in mice, are partially or wholly independent of PPARα (reviewed in DeWitt *et al.* 2009b, DeWitt *et al.* 2012) including demonstration of PFOA-associated suppression of the antibody response in PPARα knockout mice (DeWitt *et al.* 2016).

The epidemiological studies reported small to moderate reductions in antibody levels to various vaccines [e.g., 6 to 15% reduction in antibody levels to mumps and rubella for a doubling of PFOA or PFOS in US children ages 12-19 using NHANES data and 25 to 38% reduction in antibody levels to diphtheria for a doubling of PFOA or PFOS in children from the Faroe Islands birth cohort (Grandjean *et al.* 2012, Stein *et al.* 2016)]. It is unclear if this level of antibody reduction would affect the immune response to a viral or bacterial challenge for these individuals. However, any lowering of the antibody response may be considered adverse on a population level such that individuals with lower antibody levels may be less able to mount a defense against viruses or bacteria (WHO 2012). Studies of infectious disease or disease resistance could provide additional evidence of the potential impact of reduced antibody response for health outcomes. However, there are few studies of PFOA or PFOS and infectious disease and only two human studies measured antibody response to vaccination and infectious disease outcomes in the same populations. One study provides support for PFOA-associated decreased antibody response and infectious disease (increased episodes of common cold and gastroenteritis in children also showing decreased antibody levels to rubella Granum *et al.* 2013) and the other does not (no change in self-reported influenza or colds in adults showing decreased antibody response to influenza A/H3N2 Looker *et al.* 2014).

For PFOS, the animal data provide consistent evidence for immunosuppression that includes high confidence in the body of evidence for suppression of the antibody response and moderate confidence for suppression of the disease resistance and NK cell activity. Disease resistance involves multiple components of the immune system, and successful immune response to viral challenge (as in Guruge *et al.* 2009) includes rapid responses from the innate immune system (e.g., NK cell activity and the cytokine IFN-γ) as well as humoral immunity (antibody mediated responses). Therefore, the demonstration of PFOS-associated reductions in antibody response in mice at 92 ng/ml PFOS (Peden-Adams *et al.* 2008) and disease resistance at 189 ng/ml PFOS (Guruge *et al.* 2009) increase the support for biological plausibility of PFOS-associated suppression of immune function.

Limitations of the Evidence Base

There are several limitations in the body of evidence from human studies that apply across the different immune outcomes. The major limitation in the epidemiological studies is the lack of control for other

exposures that may also be immunomodulatory, particularly other PFAAs. For example, the Granum *et al.* (2013) study of the MoBa birth cohort reported suppression of the antibody response to rubella with higher serum concentrations of both PFOA and PFOS, but also with serum levels of perfluorohexane sulfonate (PFHxS) and perfluorononanoate (PFNA). Within the Granum *et al.* (2013) study the different PFAAs were not highly correlated (r=0.26-0.60), and the analyses were not performed to correct for potential effects of other PFAAs. A wider range of correlations were reported across PFAAs in the Faroe Island birth cohort (r=0.01 to 0.78) for PFOA, PFOS, PFHxS, PFNA and perfluorodecanoate (PFDA); and all of the different PFAAs were associated with reduced antibody response in at least one vaccine/analysis (e.g., diphtheria or tetanus relative to maternal serum or age 5 serum PFAAs) (Grandjean *et al.* 2012). In further analyses, the authors (Grandjean *et al.* 2012, Mogensen *et al.* 2015) examined the antibody response to diphtheria and tetanus and a combined exposure model to a single variable in a joint latent exposure model for PFAAs that included PFOA, PFOS and PFHxS. The combined variable showed the strongest association and a 57.5% (95% CI 21.2–77.0) decrease in anti-diphtheria antibodies for a doubling of PFAA. However, when adjusting the model for the impact of individual PFAAs, the results were no longer significant. The authors conclude that for this dataset, none of the individual PFAAs were the primary explanation of the reduced antibody levels. While the effect of co-exposure to other PFAAs cannot be ruled out, this co-exposure has been considered in the risk-of-bias assessment and in the evidence integration with animal studies that demonstrate effects of PFOA and PFOS individually. Therefore, it is considered unlikely that a single other PFAA is driving the association with antibody suppression observed with either PFOA or PFOS.

While the association between both PFOA and PFOS and the antibody response is relatively well studied, additional epidemiological studies that address the dose-response relationship and can control for effects of other PFAAs would increase confidence in the bodies of evidence. Additional studies that examine the antibody response to the same vaccine across multiple populations would also increase confidence. For other measures of the immune system, there are no human data, including NK cell activity and DTH response. Few specific infectious disease endpoints have been examined in epidemiological studies and the study with the most power/largest sample size used a potentially less sensitive measure (i.e., hospitalization for any infectious disease, which would only capture the most severe outcomes and could miss potential associations with individual diseases) (Fei *et al.* 2010).

The limitations of the epidemiological data on asthma and hypersensitivity are typical of studies with cross-sectional study design. Although the long half-life of PFOA in humans (2 to 8 years) (Olsen *et al.* 2007a, Kudo 2015) increases the likelihood that current serum measurements represent past exposure that would be biologically relevant for development of asthma, there is likely to be some exposure misclassification. Prospective studies that evaluate asthma, IgE and other hypersensitivity-related outcomes in children relative to early childhood exposures could increase confidence in this body of evidence.

The results of two epidemiological studies show PFOA-associated increases in the incidence of ulcerative colitis in residents of the Ohio Valley, a region associated with elevated PFOA levels in drinking water, and workers exposed to PFOA that were a subset of the original analysis (Steenland *et al.* 2013, Steenland *et al.* 2015). There is low confidence in this body of evidence because the studies are from a single population and therefore there are no independent results from a separate population. Given the low confidence in the human body of evidence and the absence of animal studies, the data are inadequate to classify whether or not PFOA exposure is associated with the incidence of ulcerative colitis. These are the only studies of the potential association between PFOA or PFOS and autoimmunity. Studies of PFOA in animal models of ulcerative colitis [e.g., (Low *et al.* 2013)] or epidemiological studies of ulcerative colitis in other populations would increase confidence in this body of evidence.

Limitations of the Systematic Review

The hazard identification conclusions in this evaluation were developed for immunotoxicity of both PFOA and PFOS based on integrating levels of evidence from human and animal studies with consideration of the degree of support from mechanistic data. In general, the mechanisms for PFOA- or PFOS-associated immune effects are not fully understood at this time (DeWitt *et al.* 2012 for review); however, mechanistic data from *in vitro* or *in vivo* studies were used to examine the biological plausibility of the primary health outcomes considered in developing specific hazard conclusions (e.g., on the antibody response). The literature search was focused on mechanistic data that was clearly relevant for evaluating the biological plausibility of immune outcomes reported from *in vivo* studies in animals or humans. Therefore, the immune outcomes used to search for *in vitro*/ mechanistic data were similar to the outcomes considered for *in vivo* human and animal studies (e.g., antibody response, NK cell activity, phagocytosis, or cytokine production). This focused approach may have missed mechanistic studies of earlier events such as activation of NF-κB that may inform the overall evaluation of potential immunotoxicity associated with exposure to PFOA or PFOS.

CONCLUSION

The NTP concludes that PFOA and PFOS are *presumed to be immune hazards to humans* and to alter immune function in humans. Exposures to PFOA and PFOS are associated with changes in multiple immune outcomes in both experimental animal and epidemiological studies. The strongest bodies of evidence to inform the evaluation of PFOA- and PFOS-associated immunotoxicity are on the antibody response.

The NTP concludes that exposure to PFOA is *presumed to be an immune hazard to humans* based on a high level of evidence that PFOS suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans (**Table 7**). Although the strongest evidence for an effect of PFOA on the immune system is for suppression of the antibody response and increased hypersensitivity, there is additional, although weaker, evidence from epidemiological studies that PFOA reduced infectious disease resistance, increased hypersensitivity-related effects, and increased autoimmune disease. The evidence indicating that PFOA affects multiple aspects of the immune system supports the overall conclusion that PFOA alters immune function in humans. However, the mechanism(s) of PFOA-associated immunotoxicity is not well understood and effects on diverse endpoints such as suppression of the antibody response and increased hypersensitivity may be unrelated.

The NTP concludes that exposure to PFOS is *presumed to be an immune hazard to humans* based on a high level of evidence that PFOS suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans (**Table 9**). Although the strongest evidence for an effect of PFOS on the immune system is for suppression of the antibody response, there is additional, although weaker, evidence that is primarily from studies in experimental animals that PFOS suppresses disease resistance and natural killer (NK) cell activity. The evidence indicating that PFOS suppresses multiple aspects of the immune system supports the overall conclusion that PFOS alters immune function in humans. Although the mechanism(s) of PFOS-associated immunotoxicity is not well understood, suppression of the antibody response and NK cell function are both potential mechanisms by which PFOS may reduce disease resistance.

REFERENCES

Abbott BD, Wolf CJ, Schmid JE, Das KP, Zehr RD, Helfant L, Nakayama S, Lindstrom AB, Strynar MJ, Lau C. 2007. Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha. *Toxicol Sci* 98(2): 571-581.

Abbott BD, Wolf CJ, Das KP, Zehr RD, Schmid JE, Lindstrom AB, Strynar MJ, Lau C. 2009. Developmental toxicity of perfluorooctane sulfonate (PFOS) is not dependent on expression of peroxisome proliferator activated receptor-alpha (PPAR alpha) in the mouse. *Reprod Toxicol* 27(3-4): 258-265.

Anderson-Mahoney P, Kotlerman J, Takhar H, Gray D, Dahlgren J. 2008. Self-reported health effects among community residents exposed to perfluorooctanoate. *New solutions : a journal of environmental and occupational health policy* 18(2): 129-143.

Ashley-Martin J, Dodds L, Levy AR, Platt RW, Marshall JS, Arbuckle TE. 2015. Prenatal exposure to phthalates, bisphenol A and perfluoroalkyl substances and cord blood levels of IgE, TSLP and IL-33. *Environ Res* 140: 360-368.

ATSDR (Agency for Toxic Substances and Disease Registry). 2009. *Draft Toxicological Profile for Perfluoroalkyls*. Atlanta, GA: Division of Toxicology and Environmental Medicine/Applied Toxicology Branch. US Department of Health and Human Services: 404. Available: http://www.atsdr.cdc.gov/toxprofiles/tp200.pdf.

ATSDR (Agency for Toxic Substances and Disease Registry). 2015. *Draft Toxicological Profile for Perfluoroalkyls*. Atlanta, GA: Division of Toxicology and Environmental Medicine/Applied Toxicology Branch. US Department of Health and Human Services: 574. Available: http://www.atsdr.cdc.gov/toxprofiles/tp200.pdf.

Baccam M, Woo SY, Vinson C, Bishop GA. 2003. CD40-mediated transcriptional regulation of the IL-6 gene in B lymphocytes: involvement of NF-kappa B, AP-1, and C/EBP. *J Immunol* 170(6): 3099-3108.

Beeh KM, Ksoll M, Buhl R. 2000. Elevation of total serum immunoglobulin E is associated with asthma in nonallergic individuals. *Eur Respir J* 16(4): 609-614.

Bishop GA, Hostager BS. 2003. The CD40-CD154 interaction in B cell-T cell liaisons. *Cytokine & growth factor reviews* 14(3-4): 297-309.

Brieger A, Bienefeld N, Hasan R, Goerlich R, Haase H. 2011. Impact of perfluorooctanesulfonate and perfluorooctanoic acid on human peripheral leukocytes. *Toxicol In Vitro* 25(4): 960-968.

Buser MC, Scinicariello F. 2016. Perfluoroalkyl substances and food allergies in adolescents. *Environ Int* 88: 74-79.

Butenhoff J, Costa G, Elcombe C, Farrar D, Hansen K, Iwai H, Jung R, Kennedy G, Jr., Lieder P, Olsen G, Thomford P. 2002. Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. *Toxicol Sci* 69(1): 244-257.

Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Tully JS, Needham LL. 2007. Serum concentrations of 11 polyfluoroalkyl compounds in the u.s. population: data from the national health and nutrition examination survey (NHANES). *Environ Sci Technol* 41(7): 2237-2242.

CDC (Centers for Disease Control and Prevention). 2015. *Fourth National Report on Human Exposure to Environmental Chemicals: Updated Tables, February 2015*. Atlanta, GA: U.S. Department of Health and Human Services. Available: http://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Feb2015.pdf [accessed 11 March 2015].

Corsini E, Avogadro A, Galbiati V, dell'Agli M, Marinovich M, Galli CL, Germolec DR. 2011. In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). *Toxicol Appl Pharmacol* 250(2): 108-116.

Corsini E, Sangiovanni E, Avogadro A, Galbiati V, Viviani B, Marinovich M, Galli CL, Dell'Agli M, Germolec DR. 2012. In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). *Toxicol Appl Pharmacol* 258(2): 248-255.

Corton J. 2010. Mode of action analysis and human relevance of liver tumors induced by PPAR-alpha activation. In: *Cancer Risk Assessment: Chemical Carcinogenesis from Biology to Standards Quantification*. Hsu C-H, Stedeford T, eds. Hoboken, NJ.

Costa G, Sartori S, Consonni D. 2009. Thirty years of medical surveillance in perfluorooctanoic acid production workers. *J Occup Environ Med* 51(3): 364-372.

Dallaire F, Dewailly E, Ayotte P. 2005. Approaches to Immunotoxicology in Human Population Studies. In: *Investigative Immunotoxicology*. Tryphonas H, Fournier M, Blakeley BR, Smits JE, Brousseau P, eds. New York, NY, Taylor and Francis: 247-265.

Dewitt JC, Copeland CB, Strynar MJ, Luebke RW. 2008. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. *Environ Health Perspect* 116(5): 644-650.

DeWitt JC, Copeland CB, Luebke RW. 2009a. Suppression of humoral immunity by perfluorooctanoic acid is independent of elevated serum corticosterone concentration in mice. *Toxicol Sci* 109(1): 106-112.

DeWitt JC, Shnyra A, Badr MZ, Loveless SE, Hoban D, Frame SR, Cunard R, Anderson SE, Meade BJ, Peden-Adams MM, Luebke RW, Luster MI. 2009b. Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. *Crit Rev Toxicol* 39(1): 76-94.

DeWitt JC, Peden-Adams MM, Keller JM, Germolec DR. 2012. Immunotoxicity of perfluorinated compounds: recent developments. *Toxicol Pathol* 40(2): 300-311.

DeWitt JC, Williams WC, Creech NJ, Luebke RW. 2016. Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPARalpha and T- and B-cell targeting. *J Immunotoxicol* 13(1): 38-45.

Dietert RR, ed. 2010. *Immunotoxicity Testing: Methods and Protocols*. New York, NY: Hummana Press.

Dong G, Zheng L, Jin Y, Lu X, Liu J, He Q. 2009a. Effect of oral exposure to Perfluorooctane Sulfonate (PFOS) on spleen T cell subsets CD4+/CD8+ of C57BL/6 mice. *Huanjing Kexue Xuebao / Acta Scientiae Circumstantiae* 29(1): 181-185.

Dong GH, Zhang YH, Zheng L, Liu W, Jin YH, He QC. 2009b. Chronic effects of perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/6 mice. *Arch Toxicol* 83(9): 805-815.

Dong GH, Liu MM, Wang D, Zheng L, Liang ZF, Jin YH. 2011. Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. *Arch Toxicol* 85(10): 1235-1244.

Dong GH, Zhang YH, Zheng L, Liang ZF, Jin YH, He QC. 2012. Subchronic effects of perfluorooctanesulfonate exposure on inflammation in adult male C57BL/6 mice. *Environ Toxicol* 27(5): 285-296.

Dong GH, Tung KY, Tsai CH, Liu MM, Wang D, Liu W, Jin YH, Hsieh WS, Lee YL, Chen PC. 2013. Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. *Environ Health Perspect* 121(4): 507-513, 513e501-508.

EFSA (European Food Safety Authority). 2008. *Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA) and their Salts*. The EFSA Journal 653: 1-131. Available: http://www.efsa.europa.eu/en/scdocs/doc/653.pdf.

Fair PA, Driscoll E, Mollenhauer MA, Bradshaw SG, Yun SH, Kannan K, Bossart GD, Keil DE, Peden-Adams MM. 2011. Effects of environmentally-relevant levels of perfluorooctane sulfonate on clinical parameters and immunological functions in B6C3F1 mice. *J Immunotox* 8(1): 17-29.

Fair PA, Romano T, Schaefer AM, Reif JS, Bossart GD, Houde M, Muir D, Adams J, Rice C, Hulsey TC, Peden-Adams M. 2013. Associations between perfluoroalkyl compounds and immune and clinical chemistry parameters in highly exposed bottlenose dolphins (Tursiops truncatus). *Environ Toxicol Chem* 32(4): 736-746.

Fairley KJ, Purdy R, Kearns S, Anderson SE, Meade B. 2007. Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. *Toxicol Sci* 97(2): 375-383.

Fei C, McLaughlin JK, Lipworth L, Olsen J. 2010. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. *Environ Res* 110(8): 773-777.

Fischer A, Koeper LM, Vohr HW. 2011. Specific antibody responses of primary cells from different cell sources are able to predict immunotoxicity in vitro. *Toxicol In Vitro* 25(8): 1966-1973.

Fletcher T, Steenland K, Savitz D. 2012. *Probable link evaluation for Non-infectious Lung Disease (Asthma and Chronic Obstructive Pulmonary Disease - COPD)*. C8 Science Panel. 1-7. Available: http://www.c8sciencepanel.org/pdfs/Probable_Link_C8_Respiratory_Disease_30Jul2012.pdf.

Fu R, Gartlehner G, Grant M, Shamliyan T, Sedrakyan A, Wilt TJ, Griffith L, Oremus M, Raina P, Ismaila A, Santaguida P, Lau J, Trikalinos TA. 2011. Conducting quantitative synthesis when comparing medical interventions: AHRQ and the Effective Health Care Program. *J Clin Epidemiol* 64(11): 1187-1197.

Galli SJ, Tsai M. 2012. IgE and mast cells in allergic disease. *Nat Med* 18(5): 693-704.

Germolec D. 2009. *Explanation of Levels of Evidence for Immune System Toxicity*. Research Triangle Park, NC. Available: http://ntp.niehs.nih.gov/go/9399.

Glynn A, Berger U, Bignert A, Ullah S, Aune M, Lignell S, Darnerud PO. 2012. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996-2010. *Environ Sci Technol* 46(16): 9071-9079.

Grandjean P, Andersen EW, Budtz-Jorgensen E, Nielsen F, Molbak K, Weihe P, Heilmann C. 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA : the journal of the American Medical Association* 307(4): 391-397.

Granum B, Haug LS, Namork E, Stolevik SB, Thomsen C, Aaberge IS, van Loveren H, Lovik M, Nygaard UC. 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotox* 10(4): 373-379.

Griffith FD, Long JE. 1980. Animal toxicity studies with ammonium perfluorooctanoate. *Am Ind Hyg Assoc J* 41(8): 576-583.

Guruge KS, Hikono H, Shimada N, Murakami K, Hasegawa J, Yeung LW, Yamanaka N, Yamashita N. 2009. Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F1 mice. *J Toxicol Sci* 34(6): 687-691.

Guyatt GH, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, Norris S, Falck-Ytter Y, Glasziou P, Debeer H, Jaeschke R, Rind D, Meerpohl J, Dahm P, Schunemann HJ. 2011. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol* 64(4): 383-394.

Hartung T, Corsini E. 2013. Immunotoxicology: challenges in the 21st century and in vitro opportunities. *Altex* 30(4): 411-426.

Higgins J, Green S. 2011. Cochrane Handbook for Systematic Reviews of Interventions, The Cochrane Collaboration.

Hu Q, Strynar MJ, DeWitt JC. 2010. Are developmentally exposed C57BL/6 mice insensitive to suppression of TDAR by PFOA? *J Immunotoxicol* 7(4): 344-349.

Humblet O, Diaz-Ramirez LG, Balmes JR, Pinney SM, Hiatt RA. 2014. Perfluoroalkyl Chemicals and Asthma among Children 12-19 Years of Age: NHANES (1999-2008). *Environ Health Perspect* 122(10): 1129-1133.

ICH (International Confereence on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use). 2005. *Immunotoxicity Studies for Human Pharmaceuticals S8* 11. Available: http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html.

Johnson P, Sutton P, Atchley D, Koustas E, Lam J, Robinson K, Sen S, Axelrad D, Woodruff T. 2013. Applying the Navigation Guide: Case Study #1. The Impact of Developmental Exposure to Perfluorooctanoic Acid (PFOA) On Fetal Growth. A Systematic Review of the Human Evidence - Protocol.

Kakoulidou M, Forsgren M, Lewensohn-Fuchs I, Johansen K. 2010. Serum levels of rubella-specific antibodies in Swedish women following three decades of vaccination programmes. *Vaccine* 28(4): 1002-1007.

Kannan K, Perrotta E, Thomas NJ. 2006. Association between perfluorinated compounds and pathological conditions in southern sea otters. *Environ Sci Technol* 40(16): 4943-4948.

Kannan K, Yun SH, Rudd RJ, Behr M. 2010. High concentrations of persistent organic pollutants including PCBs, DDT, PBDEs and PFOS in little brown bats with white-nose syndrome in New York, USA. *Chemosphere* 80(6): 613-618.

Karrman A, Ericson I, van Bavel B, Darnerud PO, Aune M, Glynn A, Lignell S, Lindstrom G. 2007. Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden. *Environ Health Perspect* 115(2): 226-230.

Keil DE, Mehlmann T, Butterworth L, Peden-Adams MM. 2008. Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. *Toxicol Sci* 103(1): 77-85.

Kielsen K, Shamim Z, Ryder LP, Nielsen F, Grandjean P, Budtz-Jorgensen E, Heilmann C. 2016. Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. *J Immunotoxicol* 13(2): 270-273.

Koustas E, Lam J, Sutton P, Johnson P, Atchley D, Robinson K, Sen S, Axelrad D, Woodruff T. 2013. Applying the Navigation Guide: Case Study #1. The Impact of Developmental Exposure to Perfluorooctanoic Acid (PFOA) On Fetal Growth. A Systematic Review of the Non-Human Evidence - Protocol.

Kudo N. 2015. Metabolism and Pharmacokinetics. In: *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*. DeWitt JC, ed. New York, Springer: 151-175.

Landsteiner A, Huset C, Johnson J, Williams A. 2014. Biomonitoring for perfluorochemicals in a Minnesota community with known drinking water contamination. *J Environ Health* 77(5): 14-19.

Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol Sci* 99(2): 366-394.

Lefebvre DE, Curran I, Armstrong C, Coady L, Parenteau M, Liston V, Barker M, Aziz S, Rutherford K, Bellon-Gagnon P, Shenton J, Mehta R, Bondy G. 2008. Immunomodulatory effects of dietary potassium perfluorooctane sulfonate (PFOS) exposure in adult Sprague-Dawley rats. *J Toxicol Environ Health A* 71(23): 1516-1525.

Leonard RC, Kreckmann KH, Sakr CJ, Symons JM. 2008. Retrospective cohort mortality study of workers in a polymer production plant including a reference population of regional workers. *Ann Epidemiol* 18(1): 15-22.

Levitt D, Liss A. 1986. Toxicity of perfluorinated fatty acids for human and murine B cell lines. *Toxicol Appl Pharmacol* 86(1): 1-11.

Levitt D, Liss A. 1987. Perfluorinated fatty acids alter merocyanine 540 dye binding to plasma membranes. *J Toxicol Environ Health* 20(3): 303-316.

Li L, Zhai Z, Liu J, Hu J. 2015. Estimating industrial and domestic environmental releases of perfluorooctanoic acid and its salts in China from 2004 to 2012. *Chemosphere* 129: 100-109.

Lindstrom AB, Strynar MJ, Libelo EL. 2011. Polyfluorinated Compounds: Past, Present, and Future. *Environ Sci Technol* 45(19): 7954-7961.

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

Loveless SE, Hoban D, Sykes G, Frame SR, Everds NE. 2008. Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate. *Toxicol Sci* 105(1): 86-96.

Low D, Nguyen DD, Mizoguchi E. 2013. Animal models of ulcerative colitis and their application in drug research. *Drug design, development and therapy* 7: 1341-1357.

Luster MI, Portier C, Pait DG, White KL, Jr., Gennings C, Munson AE, Rosenthal GJ. 1992. Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. *Fundam Appl Toxicol* 18(2): 200-210.

Mannon P, Reinisch W. 2012. Interleukin 13 and its role in gut defence and inflammation. *Gut* 61(12): 1765-1773.

Matheson MC, Allen KJ, Tang ML. 2012. Understanding the evidence for and against the role of breastfeeding in allergy prevention. *Clin Exp Allergy* 42(6): 827-851.

Messina PV, Prieto G, Sabín J, Blanco E, Varela C, Dodero V, Ruso JM, Sarmiento F. 2007a. A potentiometrie and spectroscopic study on the interaction between human immunoglobulin G and sodium perfluorooctanoate in aqueous solution. *Macromol Symp* 251: 103-111.

Messina PV, Prieto G, Salgado F, Varela C, Nogueira M, Dodero V, Ruso JM, Sarmiento F. 2007b. The influence of sodium perfluorooctanoate on the conformational transitions of human immunoglobulin. *The journal of physical chemistry. B* 111(28): 8045-8052.

Miyano Y, Tsukuda S, Sakimoto I, Takeuchi R, Shimura S, Takahashi N, Kusayanagi T, Takakusagi Y, Okado M, Matsumoto Y, Takakusagi K, Takeuchi T, Kamisuki S, Nakazaki A, Ohta K, Miura M, Kuramochi K, Mizushina Y, Kobayashi S, Sugawara F, Sakaguchi K. 2012. Exploration of the binding proteins of perfluorooctane sulfonate by a T7 phage display screen. *Bioorg Med Chem* 20(13): 3985-3990.

Mogensen UB, Grandjean P, Heilmann C, Nielsen F, Weihe P, Budtz-Jorgensen E. 2015. Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. *Environ Health* 14: 47.

Mollenhauer MA, Bradshaw SG, Fair PA, McGuinn WD, Peden-Adams MM. 2011. Effects of perfluorooctane sulfonate (PFOS) exposure on markers of inflammation in female B6C3F1 mice. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 46(2): 97-108.

Nelson JW, Hatch EE, Webster TF. 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. *Environ Health Perspect* 118(2): 197-202.

NRC (National Research Council). 2006. *Assessing the human health risk of trichloroethylene: Key scientific issues. Appendix E: Peroxisome proliferators and liver cancer.* . Available: http://www.nap.edu/read/11707/chapter/20 [accessed 20 May 2016].

NTP (National Toxicology Program). 2015a. *OHAT Risk of Bias Rating Tool for Human and Animal Studies - January 2015*. RTP, NC: Office of Health Assessment and Translation. Available: http://ntp.niehs.nih.gov/go/38673 [accessed 25 Jan 2015].

NTP (National Toxicology Program). 2015b. *Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration - January 9 2015*. RTP, NC: Office of Health Assessment and Translation. Available: http://ntp.niehs.nih.gov/go/38673 [accessed 25 Jan 2015].

Nurmatov U, Nwaru BI, Devereux G, Sheikh A. 2012. Confounding and effect modification in studies of diet and childhood asthma and allergies. *Allergy* 67(8): 1041-1059.

OECD (Organisation for Economic Cooperation and Development). 2015. *Working towards a global emission inventory of PFASs: focus on PFCAs - status quo and the way forward*. OECD Environment, Health and Safety Publications Series on Risk Management No. 30. Paris. Available: http://www.oecd.org/chemicalsafety/risk-management/Working%20Towards%20a%20Global%20Emission%20Inventory%20of%20PFASS.pdf.

Oh PS, Lim KT. 2010. Modulatory effects of phytoglycoprotein (75 kDa) on allergic inflammatory cytokines in Di(2-ethylhexyl) phthalate (DEHP)-stimulated RBL-2H3 cells. *Journal of cellular biochemistry* 109(1): 124-131.

Okada E, Sasaki S, Saijo Y, Washino N, Miyashita C, Kobayashi S, Konishi K, Ito YM, Ito R, Nakata A, Iwasaki Y, Saito K, Nakazawa H, Kishi R. 2012. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. *Environ Res* 112: 118-125.

Okada E, Sasaki S, Kashino I, Matsuura H, Miyashita C, Kobayashi S, Itoh K, Ikeno T, Tamakoshi A, Kishi R. 2014. Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. *Environ Int* 65: 127-134.

Olsen GW, Burris JM, Burlew MM, Mandel JH. 2003a. Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. *Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine* 45(3): 260-270.

Olsen GW, Church TR, Miller JP, Burris JM, Hansen KJ, Lundberg JK, Armitage JB, Herron RM, Medhdizadehkashi Z, Nobiletti JB, O'Neill EM, Mandel JH, Zobel LR. 2003b. Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors. *Environ Health Perspect* 111(16): 1892-1901.

Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, Zobel LR. 2007a. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ Health Perspect* 115(9): 1298-1305.

Olsen GW, Mair DC, Reagen WK, Ellefson ME, Ehresman DJ, Butenhoff JL, Zobel LR. 2007b. Preliminary evidence of a decline in perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations in American Red Cross blood donors. *Chemosphere* 68(1): 105-111.

Osuna CE, Grandjean P, Weihe P, El-Fawal HA. 2014. Autoantibodies Associated with Prenatal and Childhood Exposure to Environmental Chemicals in Faroese Children. *Toxicol Sci* 142(1): 158-166.

Peden-Adams MM, Keller JM, Eudaly JG, Berger J, Gilkeson GS, Keil DE. 2008. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicol Sci* 104(1): 144-154.

Peden-Adams MM, Stuckey JE, Gaworecki KM, Berger-Ritchie J, Bryant K, Jodice PG, Scott TR, Ferrario JB, Guan B, Vigo C, Boone JS, McGuinn WD, DeWitt JC, Keil DE. 2009. Developmental toxicity in white leghorn chickens following in ovo exposure to perfluorooctane sulfonate (PFOS). *Reprod Toxicol* 27(3-4): 307-318.

Post GB, Cohn PD, Cooper KR. 2012. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: A critical review of recent literature. *Environ Res* 116: 93-117.

Qazi MR, Bogdanska J, Butenhoff JL, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2009a. High-dose, short-term exposure of mice to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) affects the number of circulating neutrophils differently, but enhances the inflammatory responses of macrophages to lipopolysaccharide (LPS) in a similar fashion. *Toxicology* 262(3): 207-214.

Qazi MR, Xia Z, Bogdanska J, Chang SC, Ehresman DJ, Butenhoff JL, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2009b. The atrophy and changes in the cellular compositions of the thymus and spleen observed in mice subjected to short-term exposure to perfluorooctanesulfonate are high-dose phenomena mediated in part by peroxisome proliferator-activated receptor-alpha (PPARalpha). *Toxicology* 260(1-3): 68-76.

Qazi MR, Abedi MR, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2010a. Dietary exposure to perfluorooctanoate or perfluorooctane sulfonate induces hypertrophy in centrilobular hepatocytes and alters the hepatic immune status in mice. *Int Immunopharmacol* 10(11): 1420-1427.

Qazi MR, Nelson BD, Depierre JW, Abedi-Valugerdi M. 2010b. 28-Day dietary exposure of mice to a low total dose (7 mg/kg) of perfluorooctanesulfonate (PFOS) alters neither the cellular compositions of the thymus and spleen nor humoral immune responses: does the route of administration play a pivotal role in PFOS-induced immunotoxicity? *Toxicology* 267(1-3): 132-139.

Qazi MR, Hassan M, Nelson BD, Depierre JW, Abedi-Valugerdi M. 2013. Sub-acute, moderate-dose, but not short-term, low-dose dietary pre-exposure of mice to perfluorooctanoate aggravates concanavalin A-induced hepatitis. *Toxicol Lett* 219(1): 1-7.

Rodriguez CE, Setzer RW, Barton HA. 2009. Pharmacokinetic modeling of perfluorooctanoic acid during gestation and lactation in the mouse. *Reprod Toxicol* 27(3-4): 373-386.

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, Thayer KA. 2014. Systematic Review and Evidence Integration for Literature-Based Environmental Health Science Assessments. *Environ Health Perspect* 122(7): 711-718.

Ryu MH, Jha A, Ojo OO, Mahood TH, Basu S, Detillieux KA, Nikoobakht N, Wong CS, Loewen M, Becker AB, Halayko AJ. 2014. Chronic exposure to perfluorinated compounds: impact on airway hyperresponsiveness and inflammation. *Am J Physiol Lung Cell Mol Physiol*.

Shin HM, Vieira VM, Ryan PB, Steenland K, Bartell SM. 2011. Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid concentrations for participants in the C8 Health Project. *Environ Health Perspect* 119(12): 1760-1765.

Singh TS, Lee S, Kim HH, Choi JK, Kim SH. 2012. Perfluorooctanoic acid induces mast cell-mediated allergic inflammation by the release of histamine and inflammatory mediators. *Toxicol Lett* 210(1): 64-70.

Smit LA, Lenters V, Hoyer BB, Lindh CH, Pedersen HS, Liermontova I, Jonsson BA, Piersma AH, Bonde JP, Toft G, Vermeulen R, Heederik D. 2015. Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. *Allergy* 70(6): 653-660.

Smits JE, Nain S. 2013. Immunomodulation and hormonal disruption without compromised disease resistance in perfluorooctanoic acid (PFOA) exposed Japanese quail. *Environ Pollut* 179: 13-18.

Steenland K, Fletcher T, Savitz DA. 2010. Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). *Environ Health Perspect* 118(8): 1100-1108.

Steenland K, Zhao L, Winquist A, Parks C. 2013. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the mid-Ohio valley. *Environ Health Perspect* 121(8): 900-905.

Steenland K, Zhao L, Winquist A. 2015. A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). *Occup Environ Med* 72(5): 373-380.

Stein CR, McGovern KJ, Pajak AM, Maglione PJ, Wolff MS. 2016. Perfluoroalkyl and Polyfluoroalkyl Substances and Indicators of Immune Function in Children Aged 12 - 19 years: National Health and Nutrition Examination Survey. *Pediatr Res* 79(2): 348-357.

Sunyer J, Anto JM, Castellsague J, Soriano JB, Roca J. 1996. Total serum IgE is associated with asthma independently of specific IgE levels. The Spanish Group of the European Study of Asthma. *Eur Respir J* 9(9): 1880-1884.

Swartz TA, Saliou P, Catznelson E, Blondeau C, Gil I, Peled T, Havkin O, Fletcher M. 2003. Immune response to a diphtheria and tetanus toxoid administration in a three-dose diphtheria tetanus whole-cell pertussis/enhanced inactivated poliovirus vaccination schedule: a 7-year follow up. *Eur J Epidemiol* 18(8): 827-833.

Taylor BK, Dadia N, Yang CB, Krishnan S, Badr M. 2002. Peroxisome proliferator-activated receptor agonists inhibit inflammatory edema and hyperalgesia. *Inflammation* 26(3): 121-127.

Taylor BK, Kriedt C, Nagalingam S, Dadia N, Badr M. 2005. Central administration of perfluorooctanoic acid inhibits cutaneous inflammation. *Inflamm Res* 54(6): 235-242.

US EPA (US Environmental Protection Agency). 1996a. *Biochemicals Test Guidelines: OPPTS 880.3550 Immunotoxicity*. EPA/712/C-96/280. Washington, DC: Office of Prevention Pesticides and Toxic Substances. 1-14. Available: http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series880.htm.

US EPA (US Environmental Protection Agency). 1996b. *Biochemicals Test Guidelines: OPPTS 880.3800 Immune Response*. EPA/712/C-96/281. Washington, DC: Office of Prevention Pesticides and Toxic Substances. US Environmental Protection Agency: 1-8. Available: http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series880.htm.

US EPA (US Environmental Protection Agency). 1998. *Health Effects Test Guidelines: OPPTS 870.7800 Immunotoxicity*. EPA/712/C-98/351. Washington, DC: Office of Prevention Pesticides and Toxic Substances. 1-11. Available: http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm.

US EPA (US Environmental Protection Agency). 2005. *Draft risk assessment of the potential human health effects associated with exposure to perfluorooctanoic acid and its salts*. Washington, DC: Office of Pollution Prevention and Toxics. US Environmental Protection Agency. Available: http://www.epa.gov/opptintr/pfoa/pubs/pfoarisk.pdf.

US EPA (US Environmental Protection Agency). 2006. *2010/2015 PFOA Stewardship Program*. US Environmental Protection Agency. Available: http://www.epa.gov/oppt/pfoa/pubs/stewardship/index.html.

US EPA (US Environmental Protection Agency). 2009. *Long-Chain Perfluorinated Chemicals (PFCs) Action Plan*. Available: http://www.epa.gov/opptintr/existingchemicals/pubs/actionplans/pfcs.html.

US EPA (US Environmental Protection Agency). 2013. *Perfluorooctanoic Acid (PFOA) and Fluorinated Telomers*. Available: http://www.epa.gov/oppt/pfoa/.

US EPA (US Environmental Protection Agency,). 2014a. *Draft Health Effects Document for Perfluorooctane acid (PFOA)*. Office of Water. Available: https://peerreview.versar.com/epa/pfoa/pdf/Health-Effects-Document-for-Perfluorooctanoic-Acid-(PFOA).pdf.

US EPA (US Environmental Protection Agency). 2014b. *Emerging Contaminants - Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA)*. Available: http://www2.epa.gov/sites/production/files/2014-04/documents/factsheet_contaminant_pfos_pfoa_march2014.pdf.

US EPA (US Environmental Protection Agency,). 2014c. *Draft Health Effects Document for Perfluorooctane sulfonate (PFOS)*. Office of Water. Available: https://peerreview.versar.com/epa/pfoa/pdf/Health-Effects-Document-for-Perfluorooctane-Sulfonate-(PFOS).pdf.

US EPA (US Environmental Protection Agency). 2015. *2014 Annual Progress Reports to Meet Phase-out Goals of the 2010/2015 PFOA Stewardship Program*. Available: http://epa.gov/oppt/pfoa/pubs/stewardship/preports8.html [accessed 10 March 2015].

US EPA (US Environmental Protection Agency,). 2016a. *Health Effects Support Document for Perfluorooctane sulfonate (PFOS)*. EPA 822-R-16-002. Office of Water.

US EPA (US Environmental Protection Agency,). 2016b. *Health Effects Support Document for Perfluorooctane Acid (PFOA)*. EPA 822-R-16-003. Office of Water.

Vandermeulen C, Mathieu R, Geert LR, Pierre VD, Karel H. 2007. Long-term persistence of antibodies after one or two doses of MMR-vaccine. *Vaccine* 25(37-38): 6672-6676.

Vetvicka V, Vetvickova J. 2013. Reversal of perfluorooctanesulfonate-induced immunotoxicity by a glucan-resveratrol-vitamin C combination. *Oriental Pharmacy and Experimental Medicine* 13(1): 77-84.

Wang IJ, Hsieh WS, Chen CY, Fletcher T, Lien GW, Chiang HL, Chiang CF, Wu TN, Chen PC. 2011. The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. *Environ Res* 111(6): 785-791.

Wang Z, Cousins IT, Scheringer M, Buck RC, Hungerbühler K. 2014. Global emission inventories for C4–C14 perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and emissions from quantifiable sources. *Environ Int* 70: 62-75.

White SS, Fenton SE, Hines EP. 2011. Endocrine disrupting properties of perfluorooctanoic acid. *The Journal of steroid biochemistry and molecular biology* 127(1-2): 16-26.

WHO (International Programme on Chemical Safety, World Health Organization). 1996. *Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. IPCS Environmental Health Criteria 180*. IPCS Environmental Health Criteria 180. Geneva: 390. Available: http://www.inchem.org/documents/ehc/ehc/ehc180.htm.

WHO (International Programme on Chemical Safety, World Health Organization). 1999. *Principles for the assessment of risks to human health from exposure to chemicals. IPCS Environmental Health Criteria 210*. IPCS Environmental Health Criteria 210. Geneva: 312. Available: http://www.inchem.org/documents/ehc/ehc/ehc210.htm.

WHO (International Programme on Chemical Safety, World Health Organization). 2012. *Guidance for Immunotoxicity Risk Assessment for Chemicals*. IPCS Harmonization Project No. 10. Geneva. Available: http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf.

Wirth JR, Peden-Adams MM, White ND, Bossart GD, Fair PA. 2014. In vitro PFOS exposure on immune endpoints in bottlenose dolphins (*Tursiops truncatus*) and mice. *J Appl Toxicol* 34(6): 658-666.

Woskie SR, Gore R, Steenland K. 2012. Retrospective exposure assessment of perfluorooctanoic acid serum concentrations at a fluoropolymer manufacturing plant. *Ann Occup Hyg* 56(9): 1025-1037.

Yamaki K, Yoshino S. 2010. Enhancement of FcvarepsilonRI-mediated degranulation response in the rat basophilic leukemia cell line RBL2H3 by the fluorosurfactants perfluorooctanoic acid and perfluorooctane sulfonate. *Environ Toxicol Pharmacol* 29(2): 183-189.

Yamashita M. 2007. Peroxisome proliferator-activated receptor alpha-independent effects of peroxisome proliferators on cysteinyl leukotriene production in mast cells. *Eur J Pharmacol* 556(1-3): 172-180.

Yang J, Wang C, Nie X, Shi S, Xiao J, Ma X, Dong X, Zhang Y, Han J, Li T, Mao J, Liu X, Zhao J, Wu Q. 2015. Perfluorooctane sulfonate mediates microglial activation and secretion of TNF-alpha through Ca(2+)-dependent PKC-NF-small ka, CyrillicB signaling. *Int Immunopharmacol* 28(1): 52-60.

Yang Q, Abedi-Valugerdi M, Xie Y, Zhao XY, Moller G, Nelson BD, DePierre JW. 2002a. Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. *Int Immunopharmacol* 2(2-3): 389-397.

Yang Q, Xie Y, Alexson SE, Nelson BD, DePierre JW. 2002b. Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. *Biochem Pharmacol* 63(10): 1893-1900.

Zheng L, Dong GH, Jin YH, He QC. 2009. Immunotoxic changes associated with a 7-day oral exposure to perfluorooctanesulfonate (PFOS) in adult male C57BL/6 mice. *Arch Toxicol* 83(7): 679-689.

Zheng L, Dong GH, Zhang YH, Liang ZF, Jin YH, He QC. 2011. Type 1 and Type 2 cytokines imbalance in adult male C57BL/6 mice following a 7-day oral exposure to perfluorooctanesulfonate (PFOS). *J Immunotoxicol* 8(1): 30-38.

Zhu J, Qian W, Wang Y, Gao R, Wang J, Xiao H. 2015. Involvement of mitogen-activated protein kinase and NF-kappaB signaling pathways in perfluorooctane sulfonic acid-induced inflammatory reaction in BV2 microglial cells. *J Appl Toxicol* 35(12): 1539-1549.

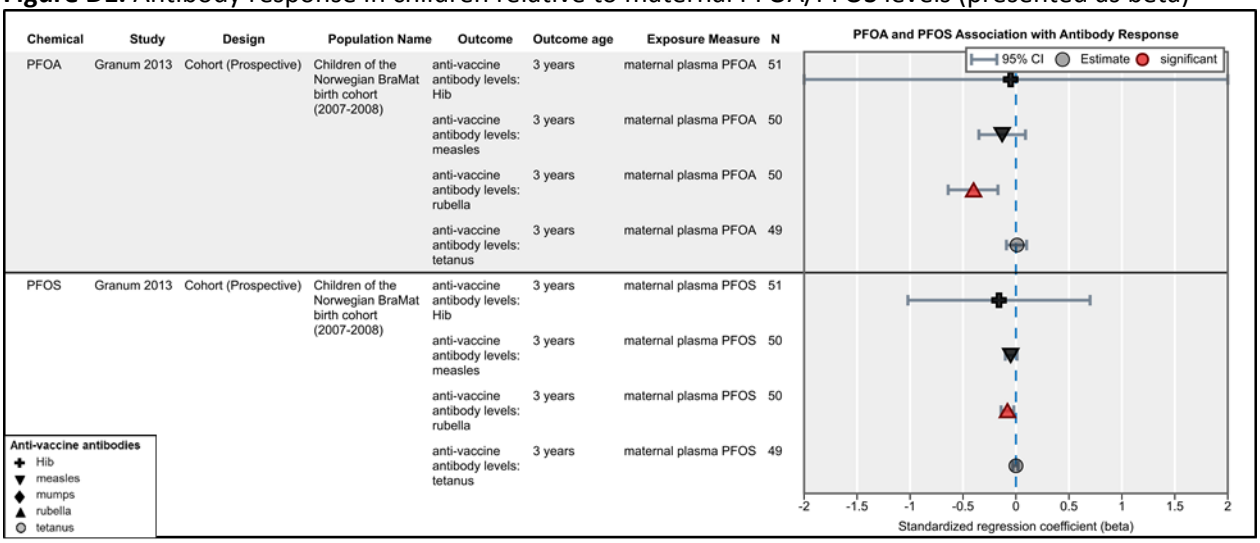
Zhu Y, Qin X-D, Zeng X-W, Paul G, Morawska L, Su M-W, Tsai C-H, Wang S-Q, Lee YL, Dong G-H. 2016. Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: By gender and asthma status. *Sci Total Environ* 559: 166-173.

DATA FIGURES

Immunosuppression-related Effects and Outcomes

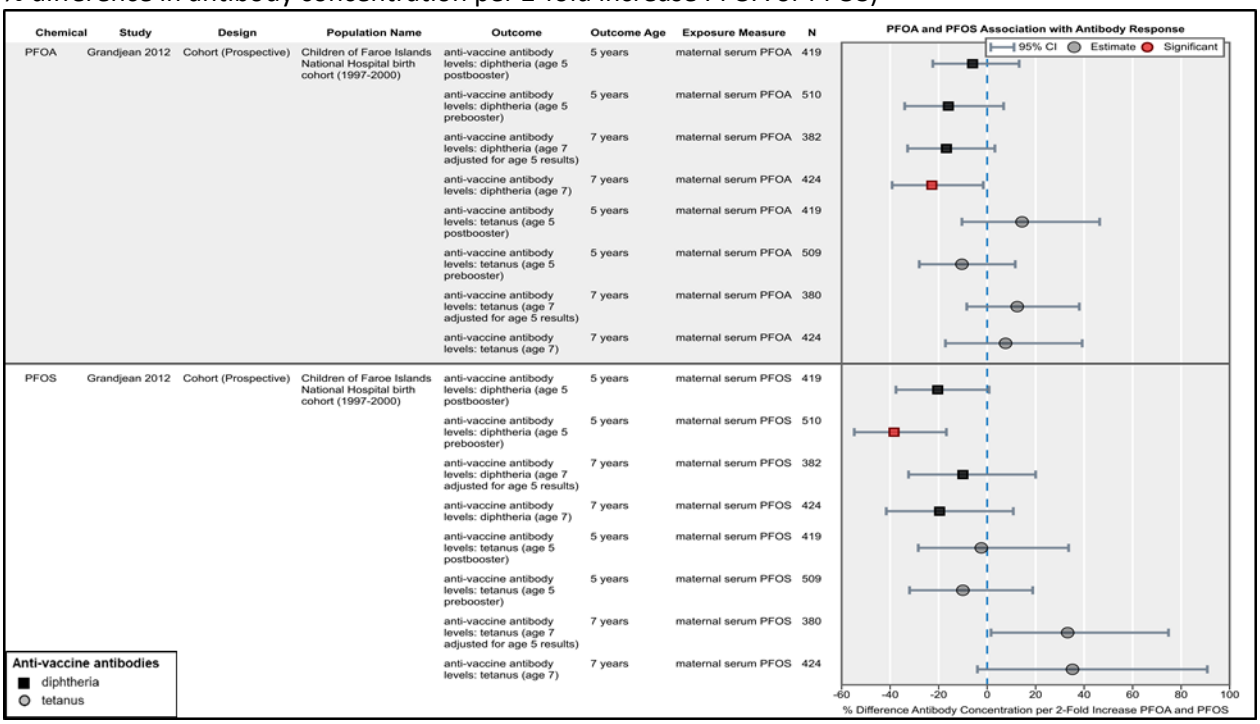
Antibody Response

Figure D1. Antibody response in children relative to maternal PFOA/PFOS levels (presented as beta)



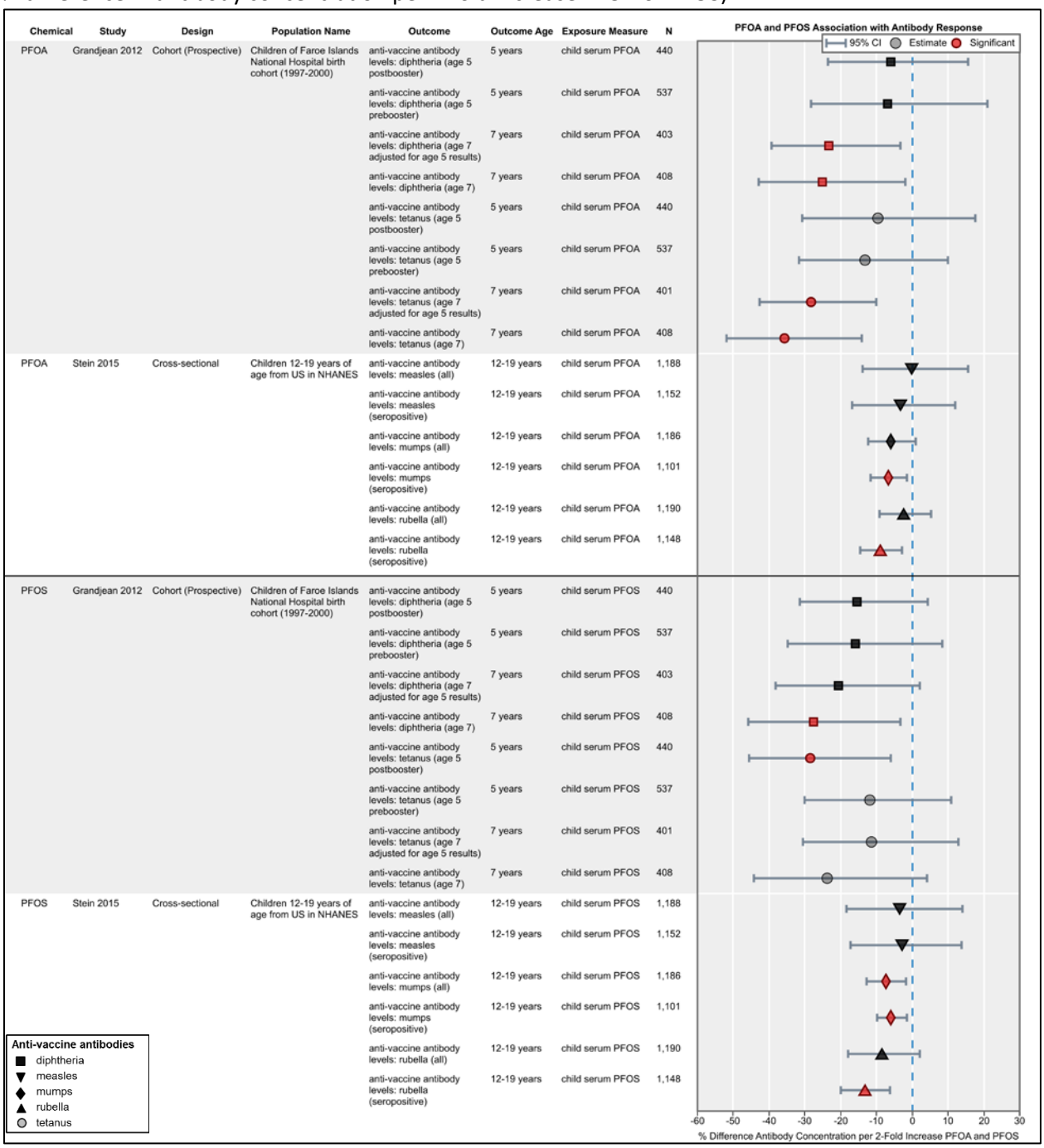
(Interactive figure and additional study details in HAWC *Figure D1 - Ab in children - beta*)

Figure D2. Antibody response in children relative to maternal PFOA/PFOS levels (presented as % difference in antibody concentration per 2-fold increase PFOA or PFOS)



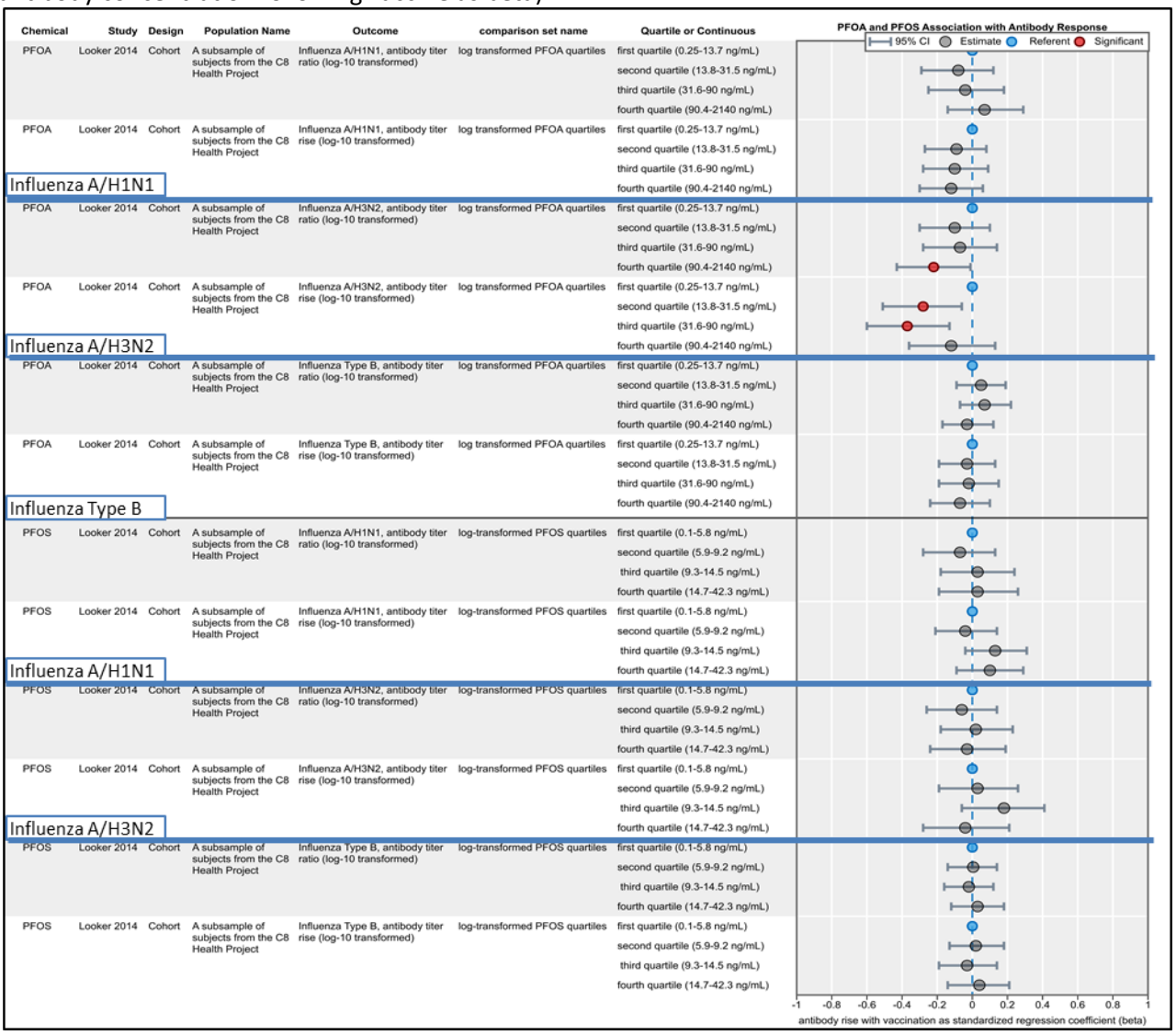
(Interactive figure and additional study details in HAWC *Figure D2 - Ab in children per 2-fold increase*)

Figure D3. Antibody response in children relative to PFOA/PFOS levels in children (presented as % difference in antibody concentration per 2-fold increase PFOA or PFOS)



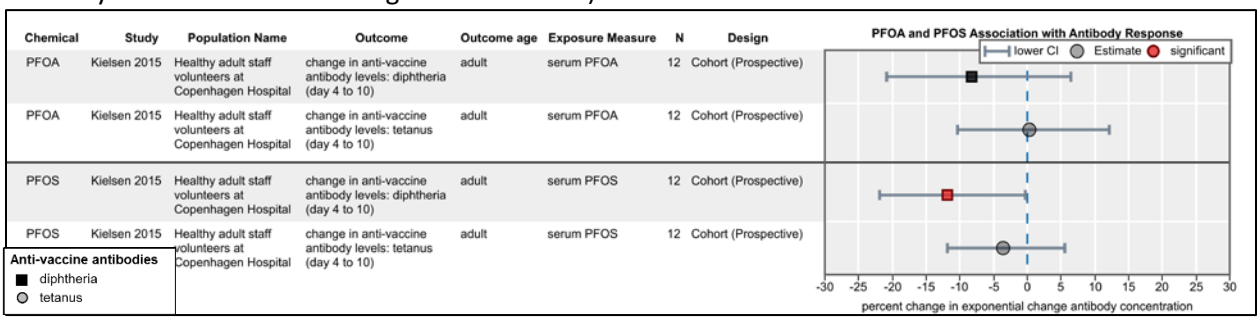
(Interactive figure and additional study details in HAWC *Figure D3 - Ab in children per 2-fold increase*)

Figure D4. Antibody response in adults relative to PFOA/PFOS levels in adults (presented as rise in antibody concentration following vaccine as beta)



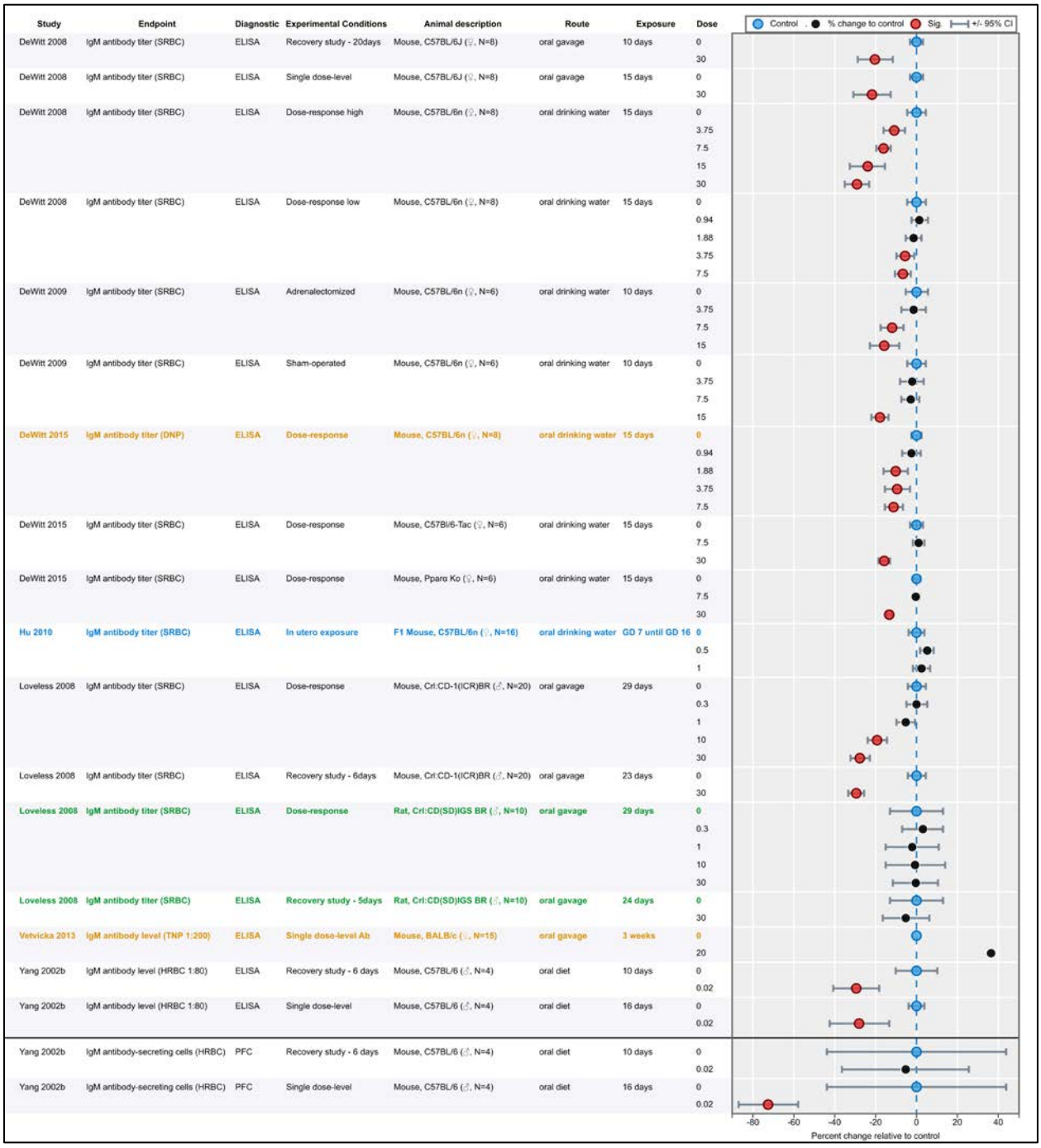
(Interactive figure and additional study details in HAWC *Figure D4 - Ab in adults - change as beta*)

Figure D5. Antibody response in adults relative to PFOA/PFOS levels in adults (presented as rise in antibody concentration following vaccine as beta)



(Interactive figure and additional study details in HAWC *Figure D5 - Ab in adults - change per 2-fold*)

Figure D6. Antigen-specific IgM antibody response in experimental animals following PFOA exposure



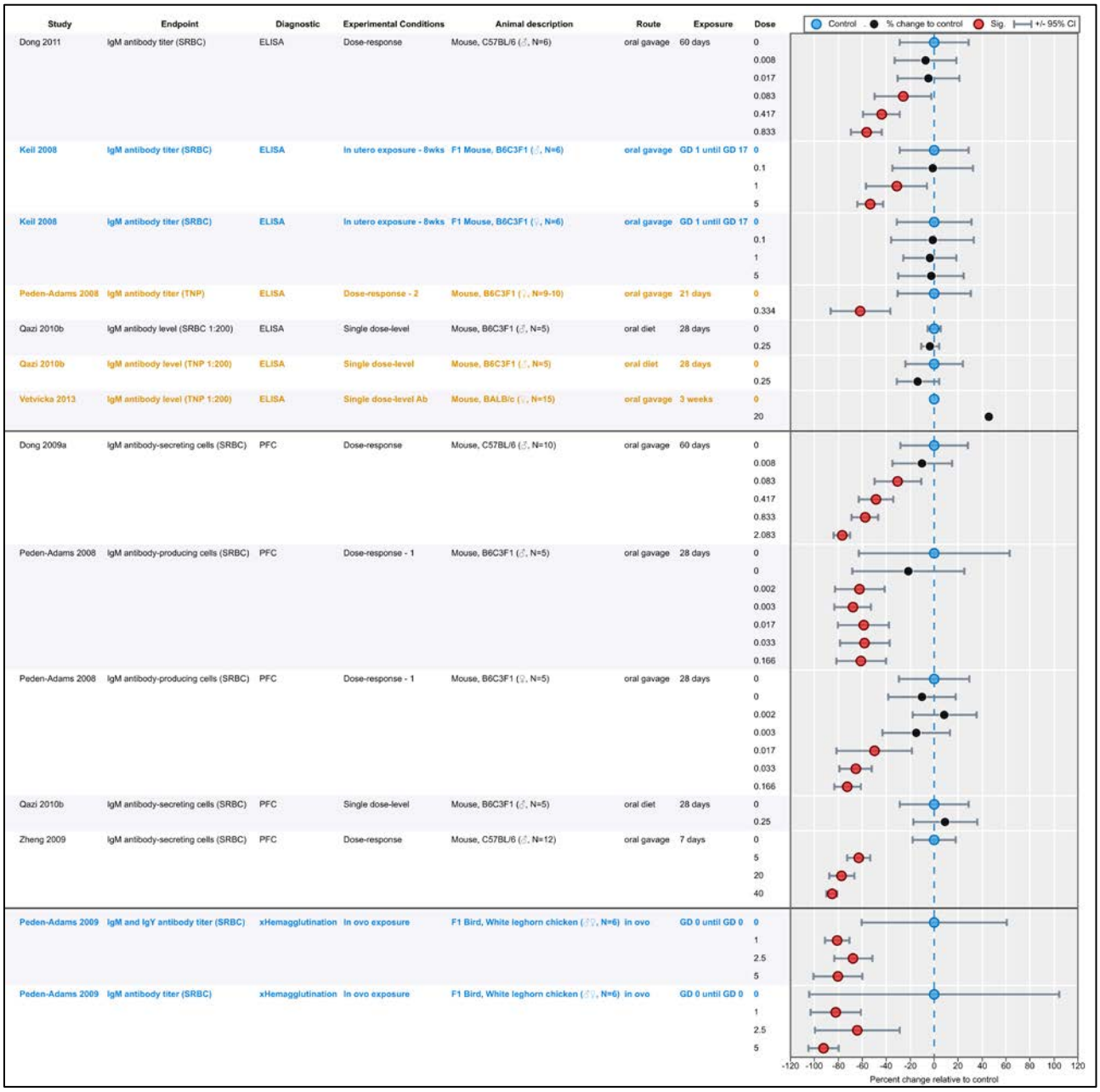
Study	Endpoint	Diagnostic	Experimental Conditions	Animal description	Route	Exposure	Dose
DeWitt 2008	IgM antibody titer (SRBC)	ELISA	Recovery study - 20days	Mouse, C57BL/6J (♀, N=8)	oral gavage	10 days	0
							30
DeWitt 2008	IgM antibody titer (SRBC)	ELISA	Single dose-level	Mouse, C57BL/6J (♀, N=8)	oral gavage	15 days	0
							30
DeWitt 2008	IgM antibody titer (SRBC)	ELISA	Dose-response high	Mouse, C57BL/6n (♀, N=8)	oral drinking water	15 days	0
							3.75
							7.5
							15
							30
DeWitt 2008	IgM antibody titer (SRBC)	ELISA	Dose-response low	Mouse, C57BL/6n (♀, N=8)	oral drinking water	15 days	0
							0.94
							1.88
							3.75
							7.5
DeWitt 2009	IgM antibody titer (SRBC)	ELISA	Adrenalectomized	Mouse, C57BL/6n (♀, N=6)	oral drinking water	10 days	0
							3.75
							7.5
							15
DeWitt 2009	IgM antibody titer (SRBC)	ELISA	Sham-operated	Mouse, C57BL/6n (♀, N=6)	oral drinking water	10 days	0
							3.75
							7.5
							15
DeWitt 2015	IgM antibody titer (DNP)	ELISA	Dose-response	Mouse, C57BL/6n (♀, N=8)	oral drinking water	15 days	0
							0.94
							1.88
							3.75
							7.5
DeWitt 2015	IgM antibody titer (SRBC)	ELISA	Dose-response	Mouse, C57Bl/6-Tac (♀, N=6)	oral drinking water	15 days	0
							7.5
							30
DeWitt 2015	IgM antibody titer (SRBC)	ELISA	Dose-response	Mouse, Ppara Ko (♀, N=6)	oral drinking water	15 days	0
							7.5
							30
Hu 2010	IgM antibody titer (SRBC)	ELISA	In utero exposure	F1 Mouse, C57BL/6n (♀, N=16)	oral drinking water	GD 7 until GD 16	0
							0.5
							1
Loveless 2008	IgM antibody titer (SRBC)	ELISA	Dose-response	Mouse, Crl:CD-1(ICR)BR (♂, N=20)	oral gavage	29 days	0
							0.3
							1
							10
							30
Loveless 2008	IgM antibody titer (SRBC)	ELISA	Recovery study - 6days	Mouse, Crl:CD-1(ICR)BR (♂, N=20)	oral gavage	23 days	0
							30
Loveless 2008	IgM antibody titer (SRBC)	ELISA	Dose-response	Rat, Crl:CD(SD)IGS BR (♂, N=10)	oral gavage	29 days	0
							0.3
							1
							10
							30
Loveless 2008	IgM antibody titer (SRBC)	ELISA	Recovery study - 5days	Rat, Crl:CD(SD)IGS BR (♂, N=10)	oral gavage	24 days	0
							30
Vetvicka 2013	IgM antibody level (TNP 1:200)	ELISA	Single dose-level Ab	Mouse, BALB/c (♀, N=15)	oral gavage	3 weeks	0
							20
Yang 2002b	IgM antibody level (HRBC 1:80)	ELISA	Recovery study - 6 days	Mouse, C57BL/6 (♂, N=4)	oral diet	10 days	0
							0.02
Yang 2002b	IgM antibody level (HRBC 1:80)	ELISA	Single dose-level	Mouse, C57BL/6 (♂, N=4)	oral diet	16 days	0
							0.02
Yang 2002b	IgM antibody-secreting cells (HRBC)	PFC	Recovery study - 6 days	Mouse, C57BL/6 (♂, N=4)	oral diet	10 days	0
							0.02
Yang 2002b	IgM antibody-secreting cells (HRBC)	PFC	Single dose-level	Mouse, C57BL/6 (♂, N=4)	oral diet	16 days	0
							0.02

(Interactive figure and additional study details in HAWC *Figure D6 - PFOA Ab in animals IgM*)

Figure D7. Antigen-specific IgG antibody response in experimental animals following PFOA exposure

(Interactive figure and additional study details in HAWC *Figure D7 - PFOA Ab in animals IgG*)

Figure D8. Antigen-specific IgM antibody response in experimental animals following PFOS exposure



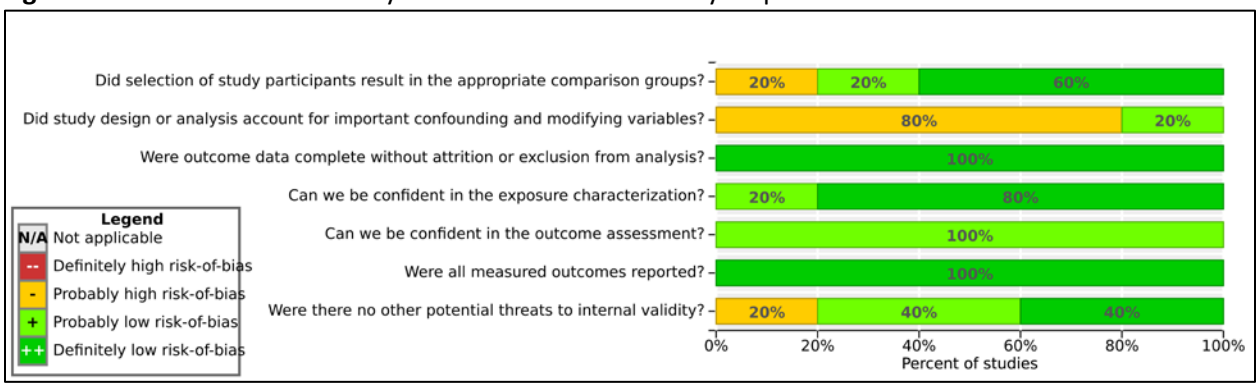
(Interactive figure and additional study details in HAWC see *Figure D8 - PFOS Ab in animals IgM*)

Figure D9. Antigen-specific IgG antibody response in experimental animals following PFOS exposure

(Interactive figure and additional study details in HAWC *Figure D9 - PFOS Ab in animals IgG*)

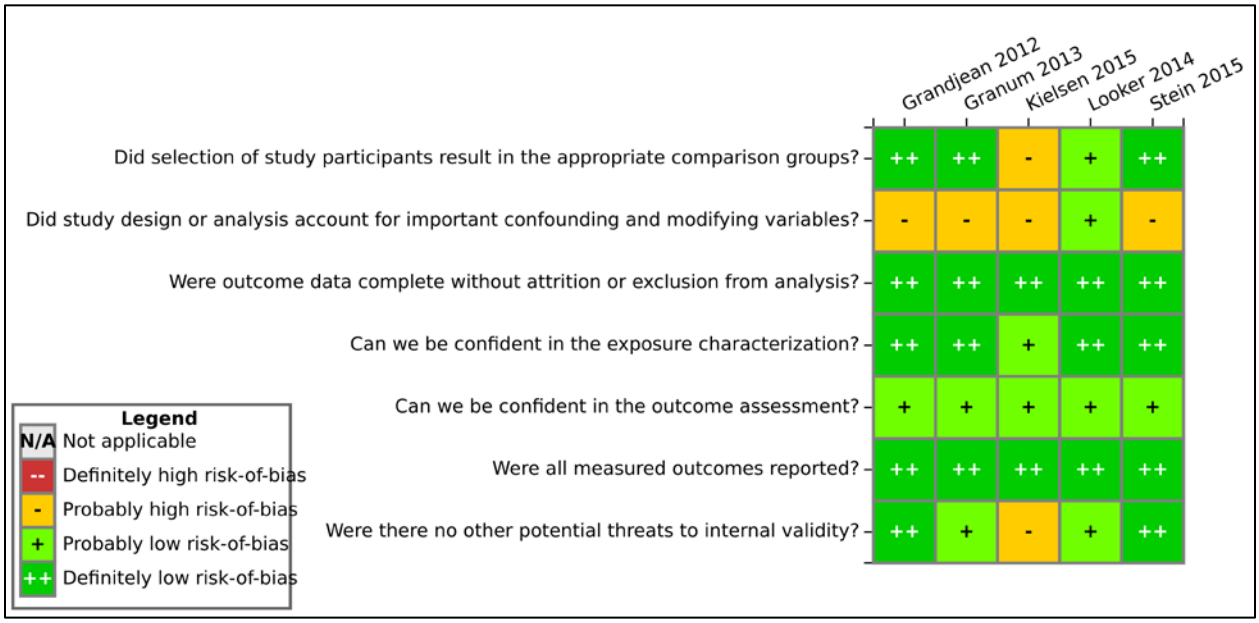
Risk of Bias Ratings for Studies of the Antibody Response

Figure D10. Risk of bias summary for studies of the antibody response in humans



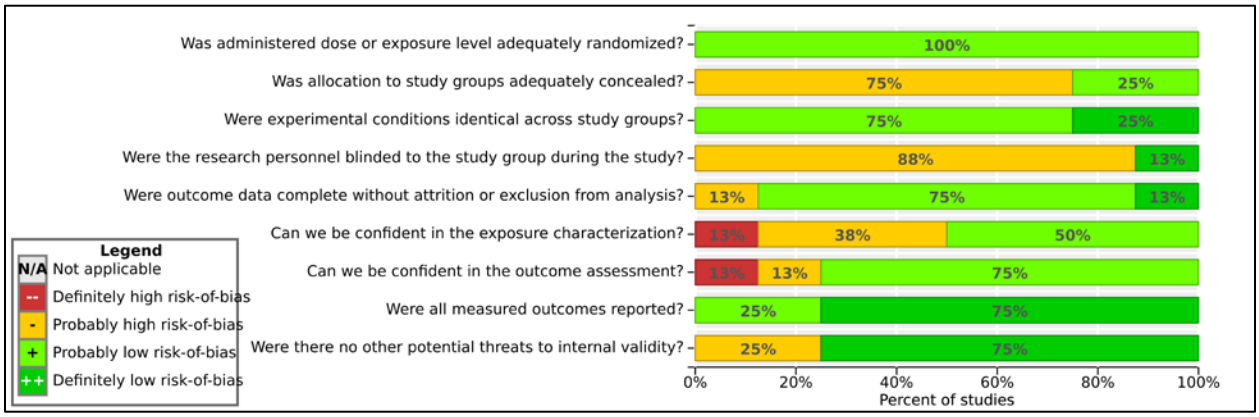
(Risk of bias ratings and study details in HAWC *Figure D10 - Human Antibody RoB*)

Figure D11. Risk of bias heatmap for studies of the antibody response in humans



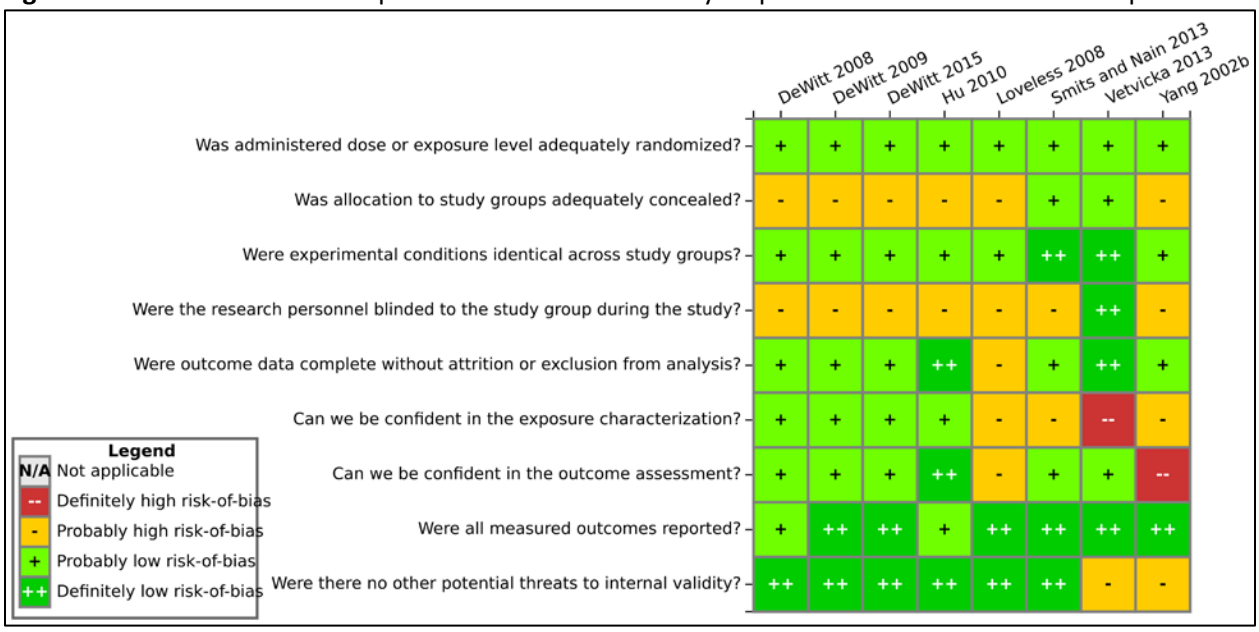
(Individual study details and risk of bias ratings in HAWC *Figure D11 - Human Antibody RoB Heatmap*)

Figure D12. Risk of bias summary for studies of the antibody response in animals after PFOA exposure



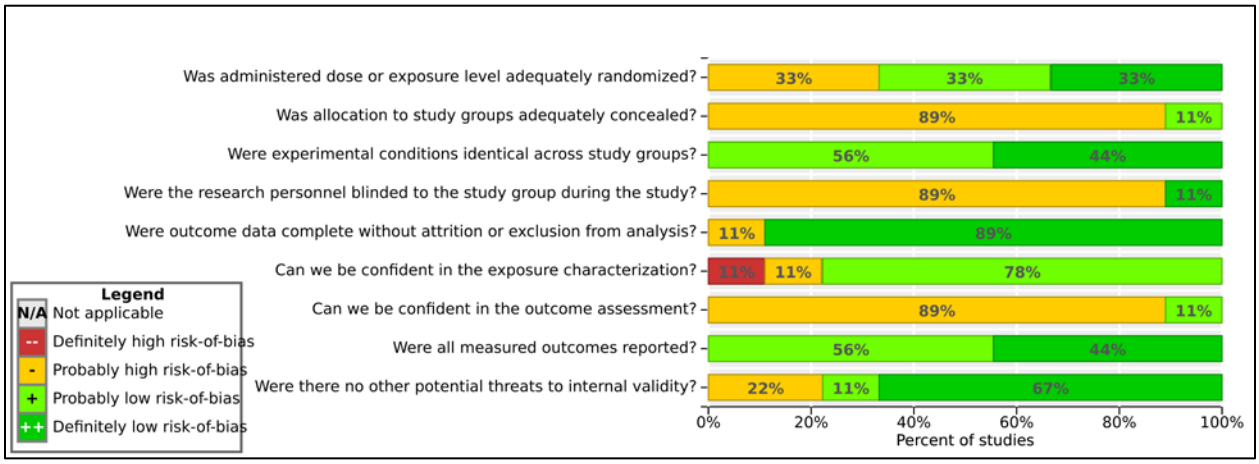
(Risk of bias ratings and study details in HAWC *Figure D12 - PFOA Animal Antibody RoB*)

Figure D13. Risk of bias heatmap for studies of the antibody response in animals after PFOA exposure



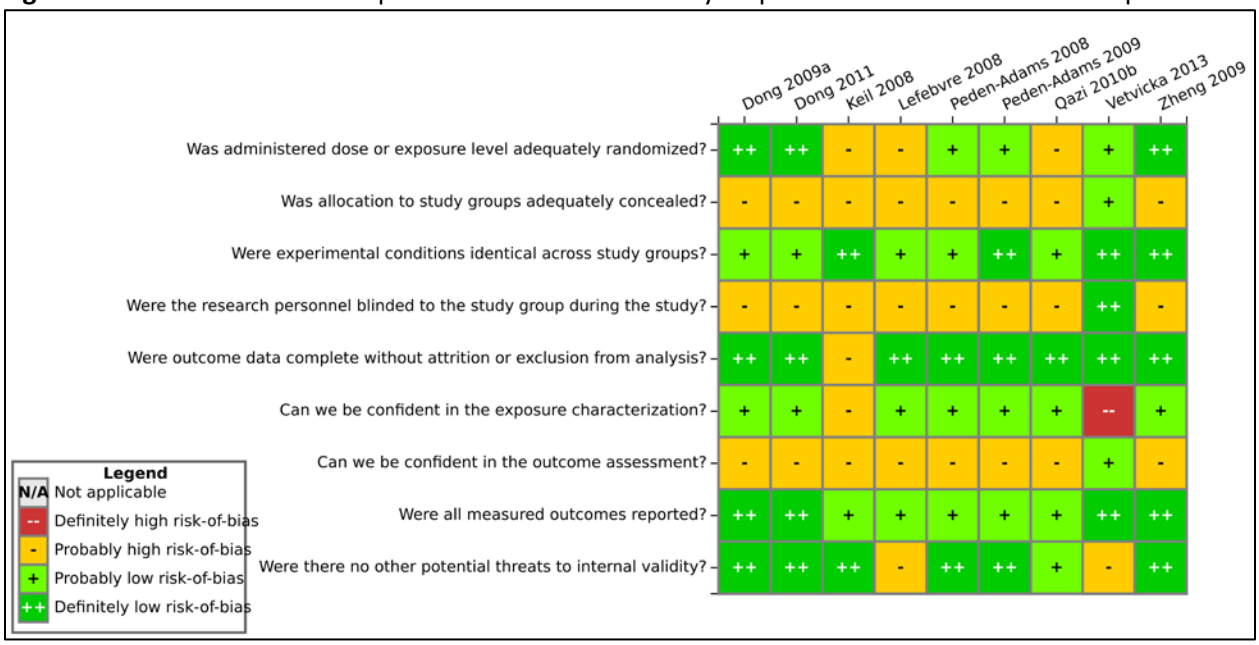
(Individual study details and risk of bias ratings in HAWC *Figure D13 - PFOA Animal Ab RoB Heatmap*)

Figure D14. Risk of bias summary for studies of the antibody response in animals after PFOS exposure



(Risk of bias ratings and study details in HAWC *Figure D14 - PFOS Animal Antibody RoB*)

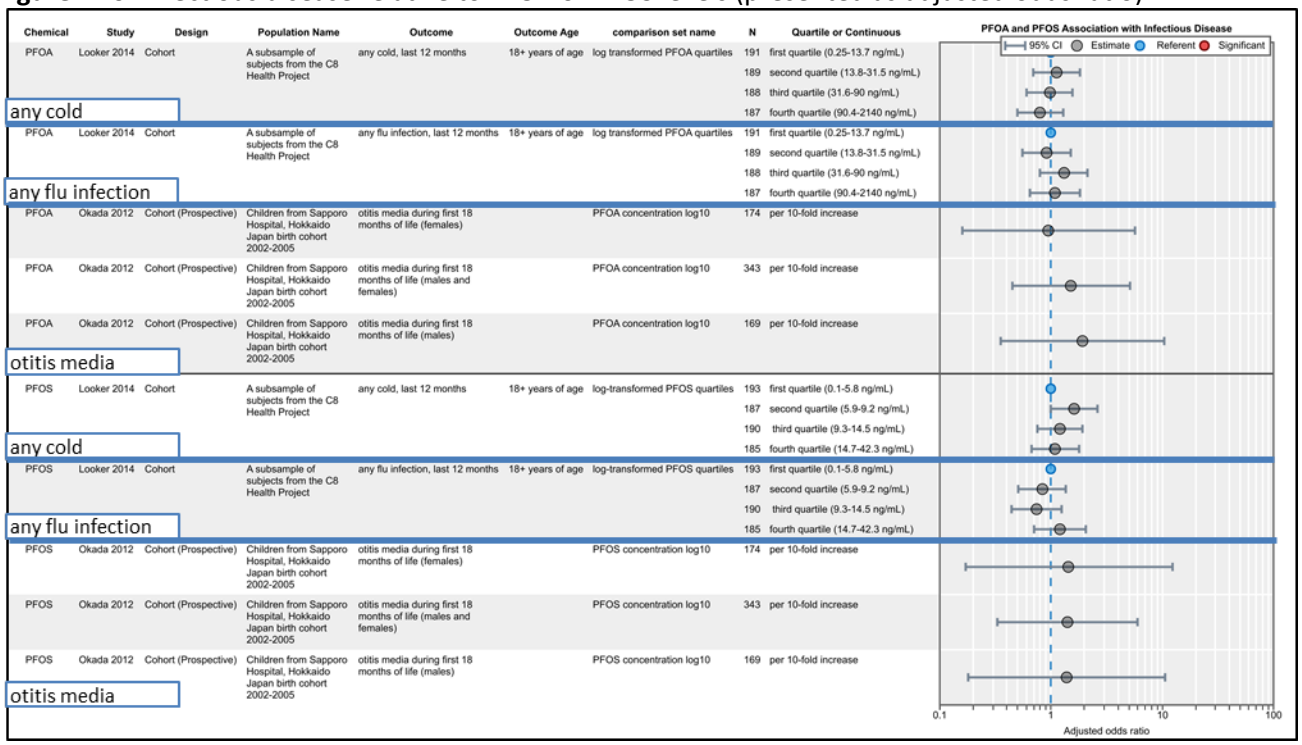
Figure D15. Risk of bias heatmap for studies of the antibody response in animals after PFOS exposure



(Individual study details and risk of bias ratings in HAWC *Figure D15 - PFOS Animal Ab RoB Heatmap*)

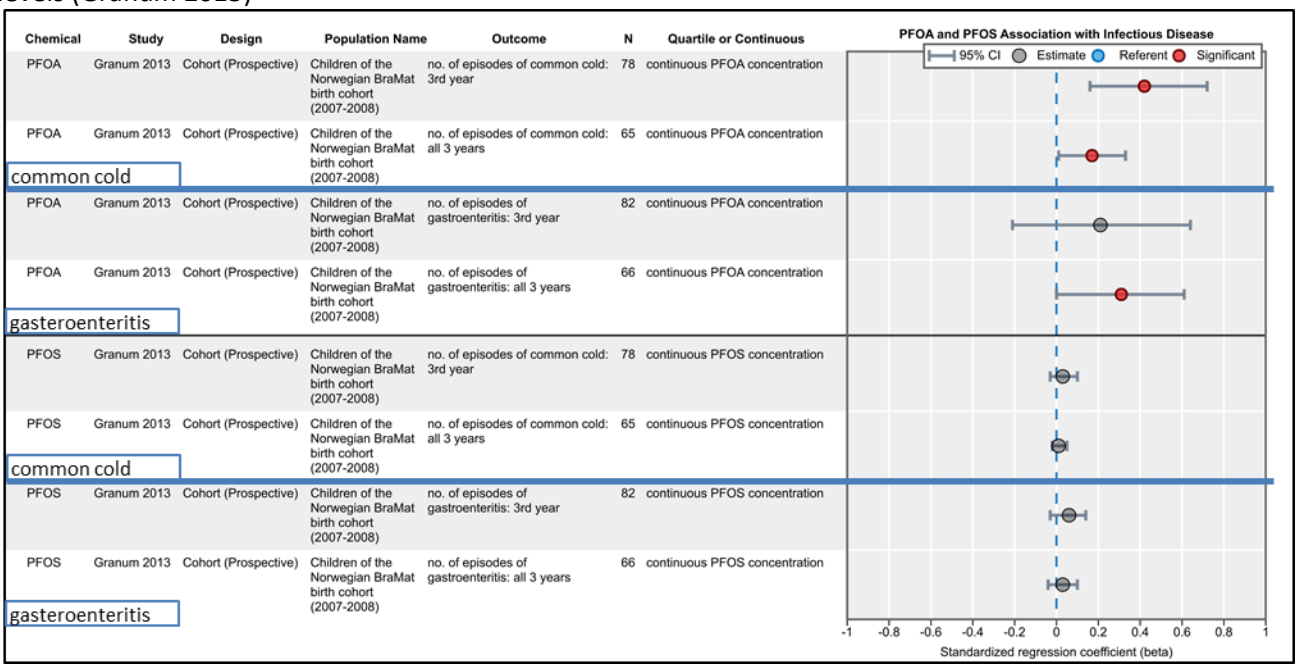
Disease Resistance/Infectious Disease Outcomes

Figure D16. Infectious disease relative to PFOA or PFOS levels (presented as adjusted odds ratio).



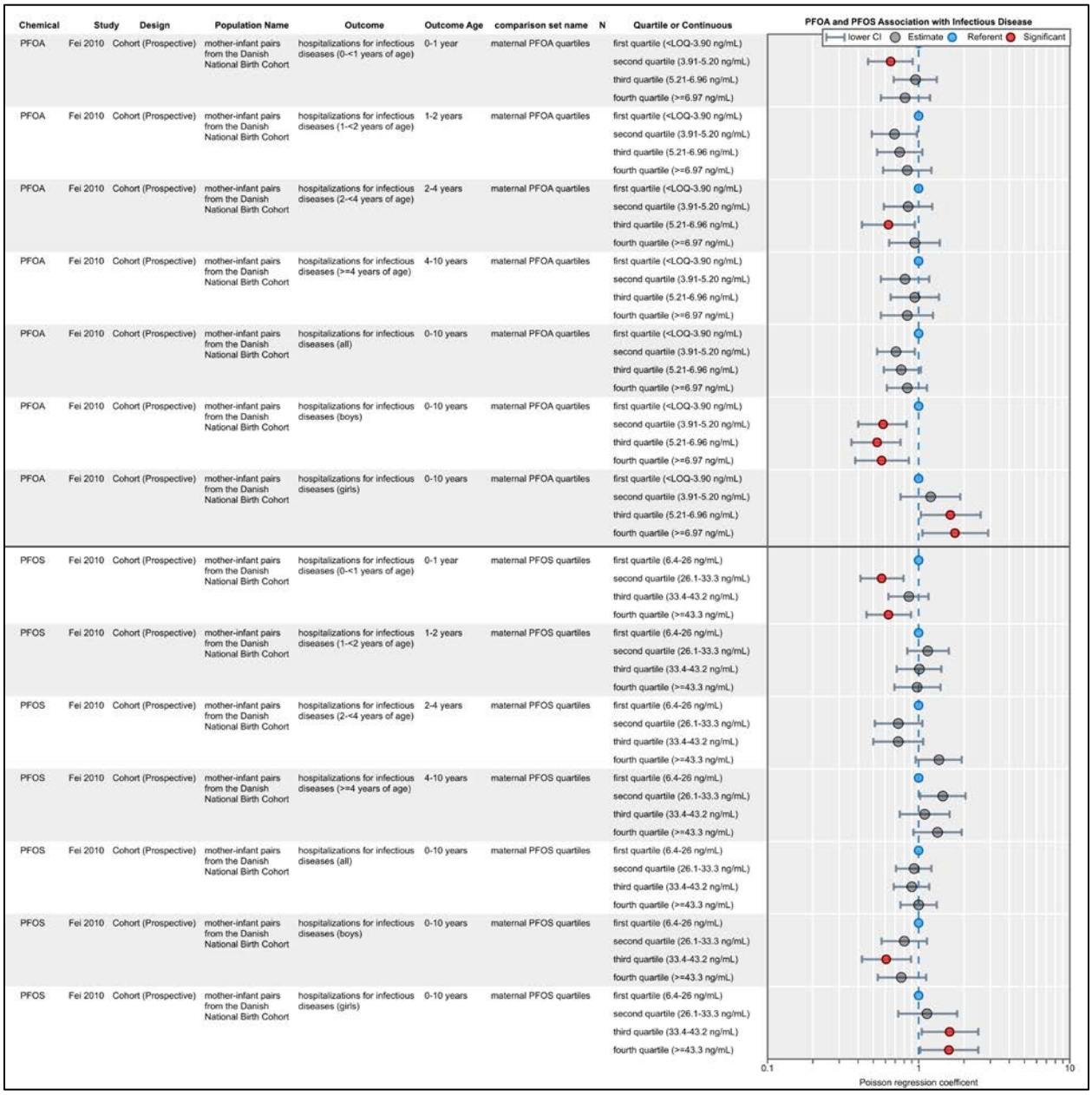
(Interactive figure and additional study details in HAWC *Figure D16 - Infectious disease general*)

Figure D17. Infectious disease in children by number of episodes relative to maternal PFOA or PFOS levels (Granum 2013)



(Interactive figure and additional study details in HAWC *Figure D17 - Infectious disease Granum*)

Figure D18. Hospitalizations for infectious diseases relative to PFOA or PFOS levels (Fei 2010)



Chemical	Study	Design	Population Name	Outcome	Outcome Age	comparison set name	N	Quartile or Continuous
PFOA	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (0-<1 years of age)	0-1 year	maternal PFOA quartiles		first quartile (<LOQ-3.90 ng/mL)
								second quartile (3.91-5.20 ng/mL)
								third quartile (5.21-6.96 ng/mL)
								fourth quartile (>=6.97 ng/mL)
PFOA	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (1-<2 years of age)	1-2 years	maternal PFOA quartiles		first quartile (<LOQ-3.90 ng/mL)
								second quartile (3.91-5.20 ng/mL)
								third quartile (5.21-6.96 ng/mL)
								fourth quartile (>=6.97 ng/mL)
PFOA	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (2-<4 years of age)	2-4 years	maternal PFOA quartiles		first quartile (<LOQ-3.90 ng/mL)
								second quartile (3.91-5.20 ng/mL)
								third quartile (5.21-6.96 ng/mL)
								fourth quartile (>=6.97 ng/mL)
PFOA	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (>=4 years of age)	4-10 years	maternal PFOA quartiles		first quartile (<LOQ-3.90 ng/mL)
								second quartile (3.91-5.20 ng/mL)
								third quartile (5.21-6.96 ng/mL)
								fourth quartile (>=6.97 ng/mL)
PFOA	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (all)	0-10 years	maternal PFOA quartiles		first quartile (<LOQ-3.90 ng/mL)
								second quartile (3.91-5.20 ng/mL)
								third quartile (5.21-6.96 ng/mL)
								fourth quartile (>=6.97 ng/mL)
PFOA	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (boys)	0-10 years	maternal PFOA quartiles		first quartile (<LOQ-3.90 ng/mL)
								second quartile (3.91-5.20 ng/mL)
								third quartile (5.21-6.96 ng/mL)
								fourth quartile (>=6.97 ng/mL)
PFOA	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (girls)	0-10 years	maternal PFOA quartiles		first quartile (<LOQ-3.90 ng/mL)
								second quartile (3.91-5.20 ng/mL)
								third quartile (5.21-6.96 ng/mL)
								fourth quartile (>=6.97 ng/mL)
PFOS	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (0-<1 years of age)	0-1 year	maternal PFOS quartiles		first quartile (6.4-26 ng/mL)
								second quartile (26.1-33.3 ng/mL)
								third quartile (33.4-43.2 ng/mL)
								fourth quartile (>=43.3 ng/mL)
PFOS	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (1-<2 years of age)	1-2 years	maternal PFOS quartiles		first quartile (6.4-26 ng/mL)
								second quartile (26.1-33.3 ng/mL)
								third quartile (33.4-43.2 ng/mL)
								fourth quartile (>=43.3 ng/mL)
PFOS	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (2-<4 years of age)	2-4 years	maternal PFOS quartiles		first quartile (6.4-26 ng/mL)
								second quartile (26.1-33.3 ng/mL)
								third quartile (33.4-43.2 ng/mL)
								fourth quartile (>=43.3 ng/mL)
PFOS	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (>=4 years of age)	4-10 years	maternal PFOS quartiles		first quartile (6.4-26 ng/mL)
								second quartile (26.1-33.3 ng/mL)
								third quartile (33.4-43.2 ng/mL)
								fourth quartile (>=43.3 ng/mL)
PFOS	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (all)	0-10 years	maternal PFOS quartiles		first quartile (6.4-26 ng/mL)
								second quartile (26.1-33.3 ng/mL)
								third quartile (33.4-43.2 ng/mL)
								fourth quartile (>=43.3 ng/mL)
PFOS	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (boys)	0-10 years	maternal PFOS quartiles		first quartile (6.4-26 ng/mL)
								second quartile (26.1-33.3 ng/mL)
								third quartile (33.4-43.2 ng/mL)
								fourth quartile (>=43.3 ng/mL)
PFOS	Fei 2010	Cohort (Prospective)	mother-infant pairs from the Danish National Birth Cohort	hospitalizations for infectious diseases (girls)	0-10 years	maternal PFOS quartiles		first quartile (6.4-26 ng/mL)
								second quartile (26.1-33.3 ng/mL)
								third quartile (33.4-43.2 ng/mL)
								fourth quartile (>=43.3 ng/mL)

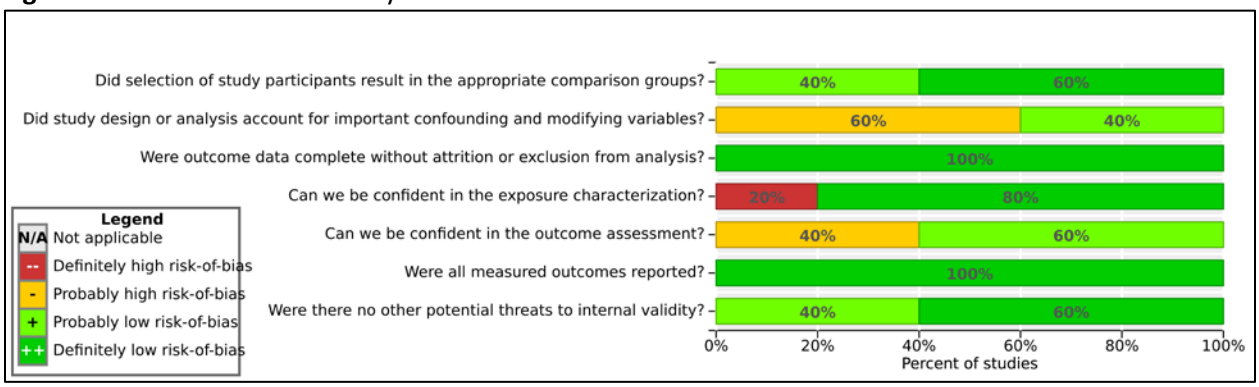
(Interactive figure and additional study details in HAWC *Figure D18 - Hospitalizations for Infectious disease Fei 2010*)

Figure D19. Disease resistance in experimental animals following PFOA or PFOS exposure

(Interactive figure and additional study details in HAWC *Figure D19 - disease resistance in animals*)

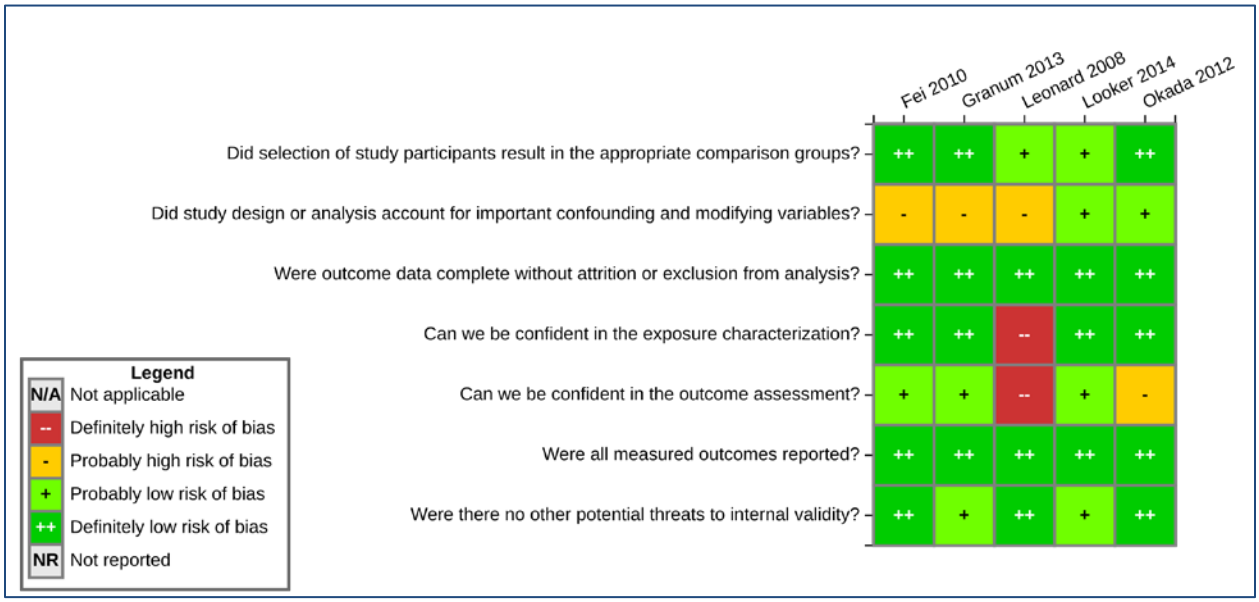
Risk of Bias Ratings for Studies of the Infectious Disease and Disease Response

Figure D20. Risk of bias summary for studies of the infectious disease in humans



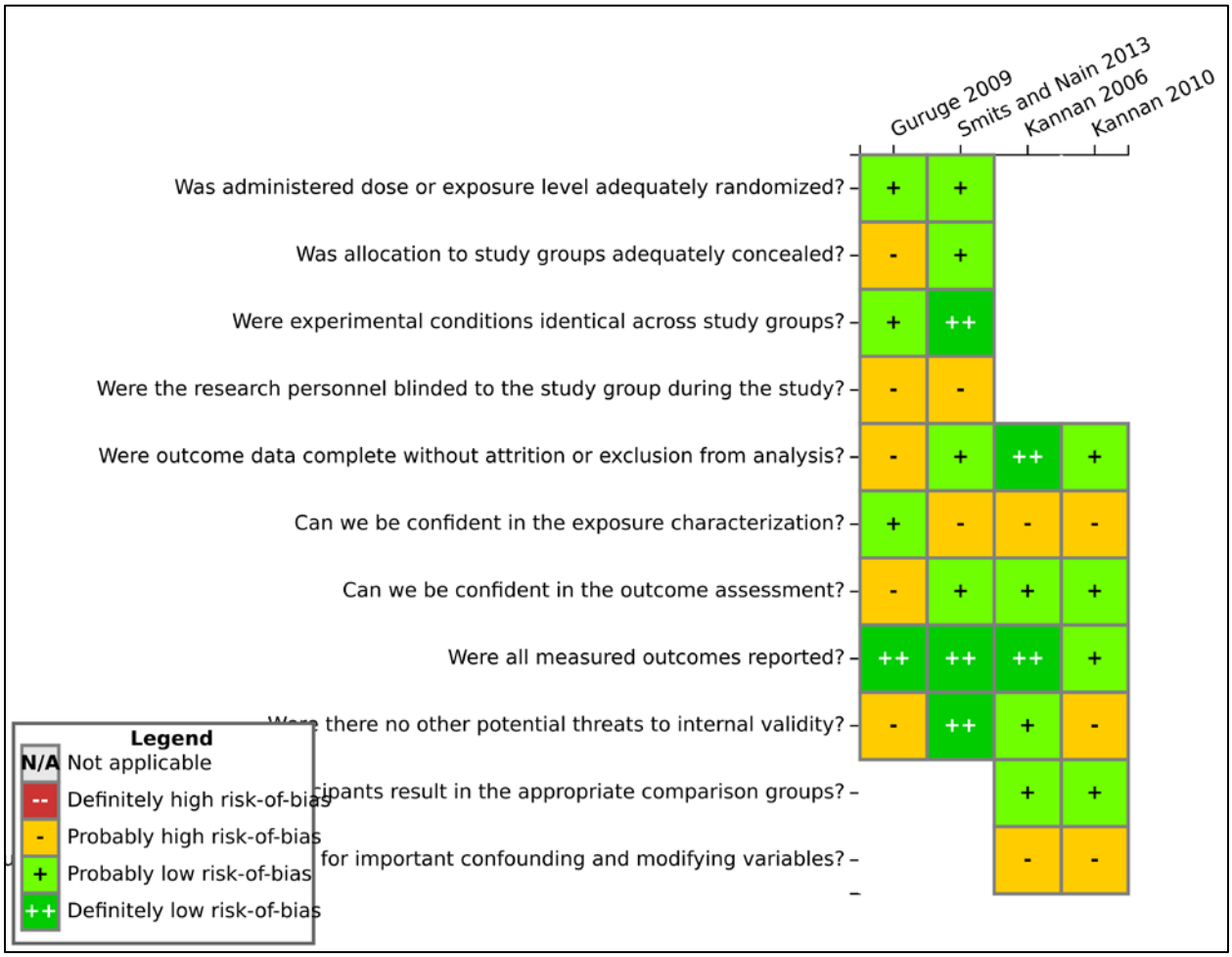
(Risk of bias ratings and study details in HAWC *Figure D20 - Human Infectious Disease RoB*)

Figure D21. Risk of bias heatmap for studies of the infectious disease in humans



(Individual study details and risk of bias ratings in HAWC *Figure D21 - Human Infectious RoB Heatmap*)

Figure D22. Risk of bias heatmap for studies of disease resistance in animals with PFOA or PFOS



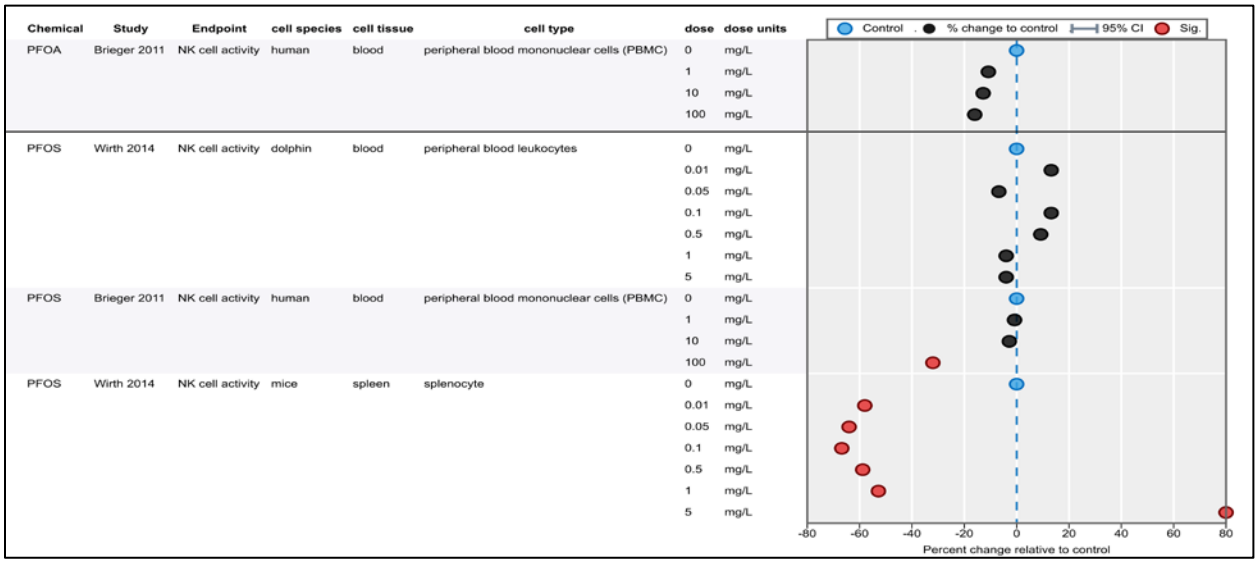
(Individual study details and risk of bias ratings in HAWC *Figure D22 - Animal Disease Resistance RoB Heatmap*). Note that Smits and Nain (2013) and Guruge (2009) are experimental exposures studies and therefore were evaluated with risk of bias questions appropriate to evaluate experimental studies. The Kannan (2006) and Kannan (2010) studies are wildlife studies and therefore were evaluated with risk of bias questions appropriate to evaluate observational studies.

Natural Killer (NK) Cell Activity

Figure D23. NK cell activity in experimental animals following PFOA or PFOS exposure

(Interactive figure and additional study details in HAWC *Figure D23 - NK cell activity in Animals*)

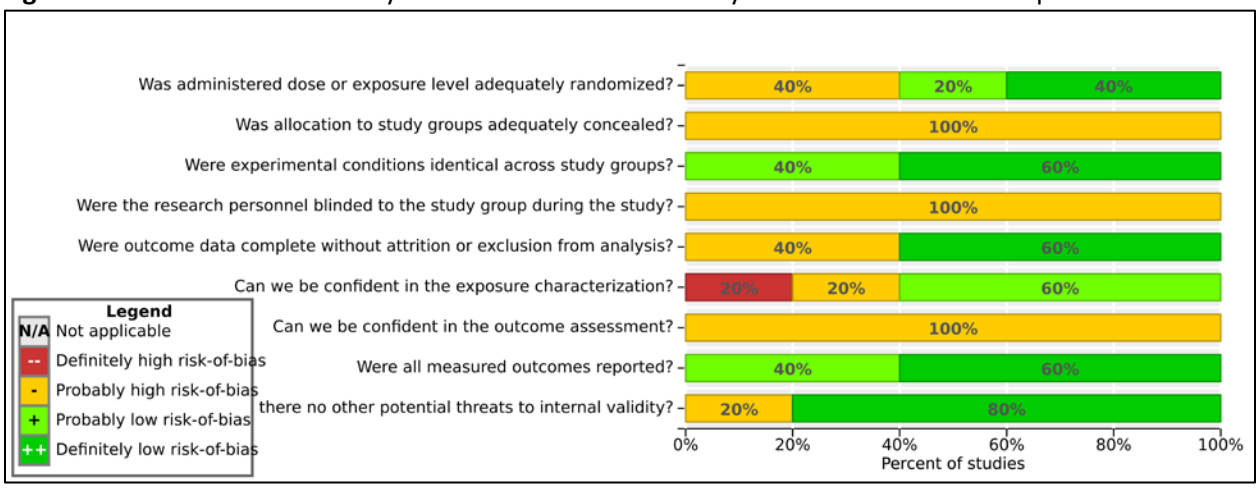
Figure D24. NK cell activity in human and animal cells following *in vitro* PFOA or PFOS exposure



(Interactive figure and additional study details in HAWC *Figure D24 - NK cell activity in vitro*)

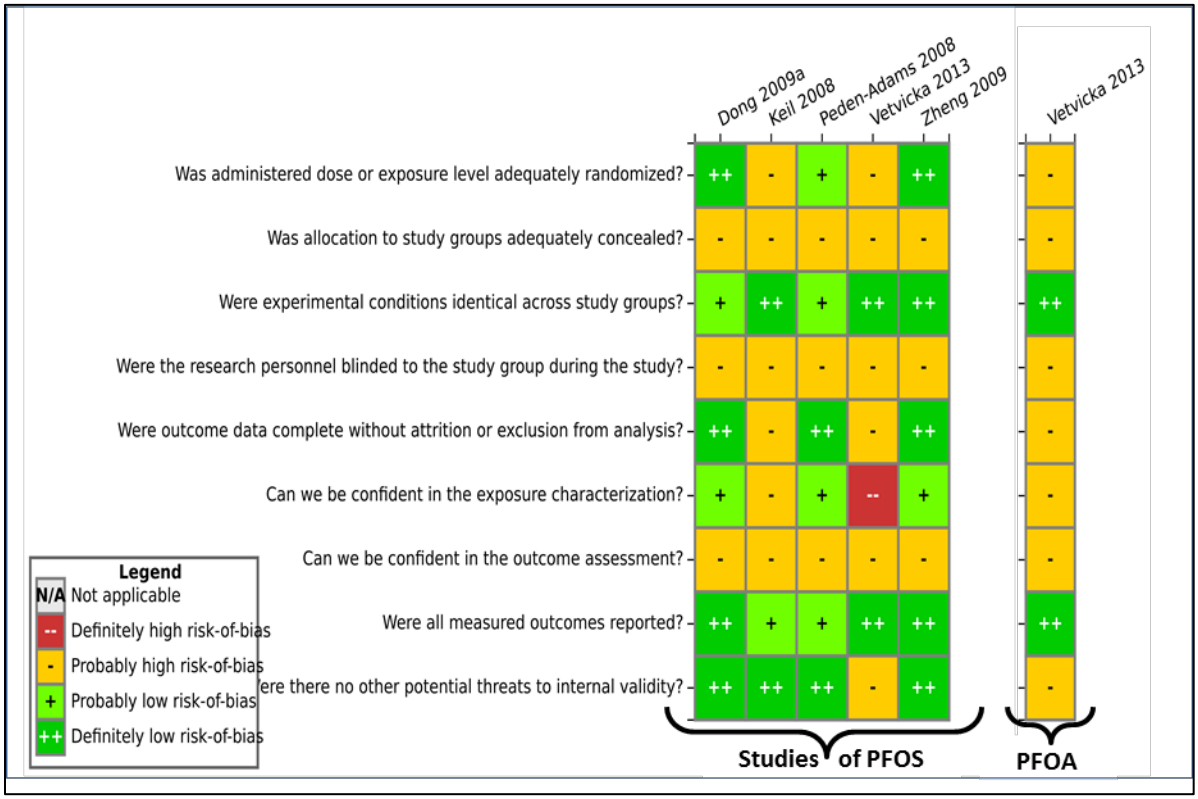
Risk of bias Ratings for Studies of NK Cell Activity

Figure D25. Risk of bias summary for studies of NK cell activity in animals after PFOS exposure



(Risk of bias ratings and study details in HAWC *Figure D25 - PFOS Animal NK RoB Summary*)

Figure D26. Risk of bias heatmap for studies of NK cell activity in animals after PFOA of PFOS exposure

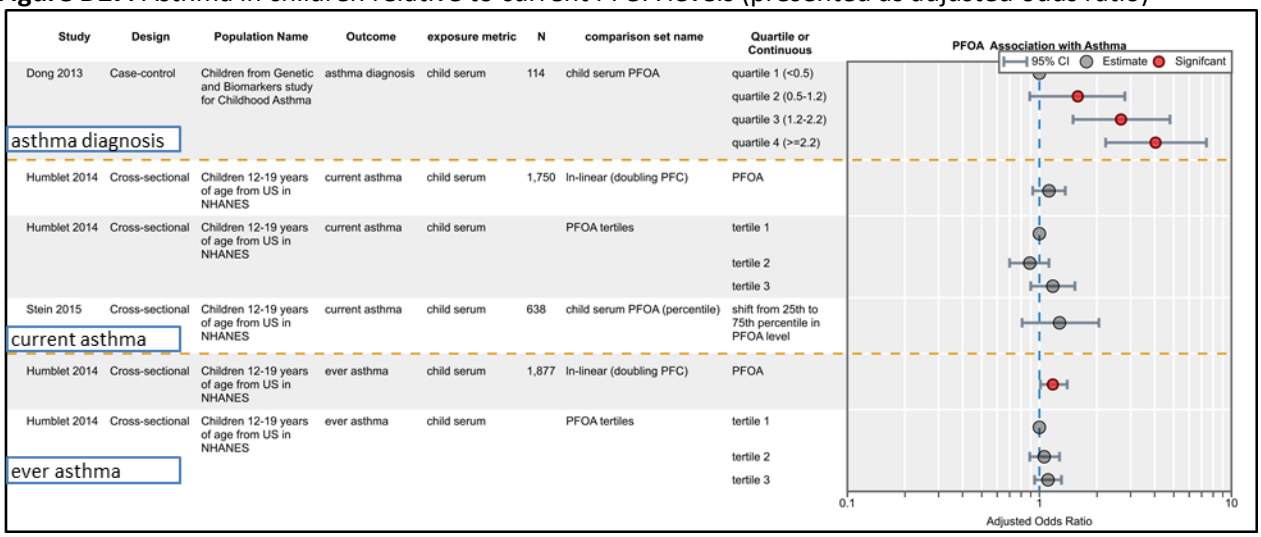


(Individual study details and risk of bias ratings in HAWC *Figure D26 - PFOA and PFOS Animal NK RoB Heatmap*)

Hypersensitivity-related Effects and Outcomes

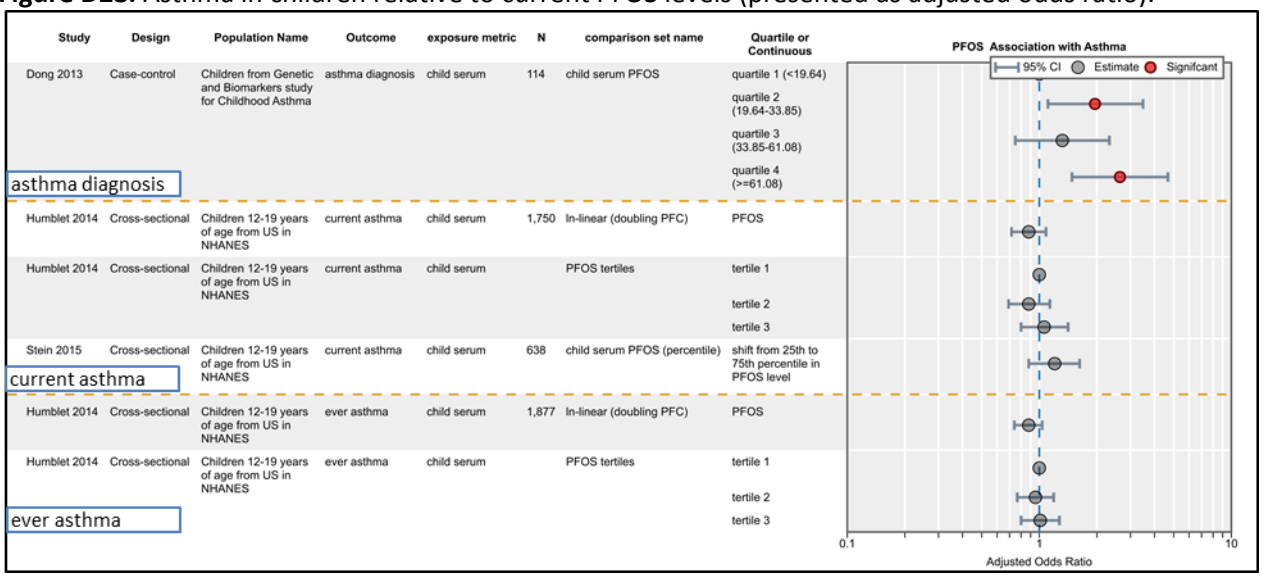
Hypersensitivity Data

Figure D27. Asthma in children relative to current PFOA levels (presented as adjusted odds ratio)



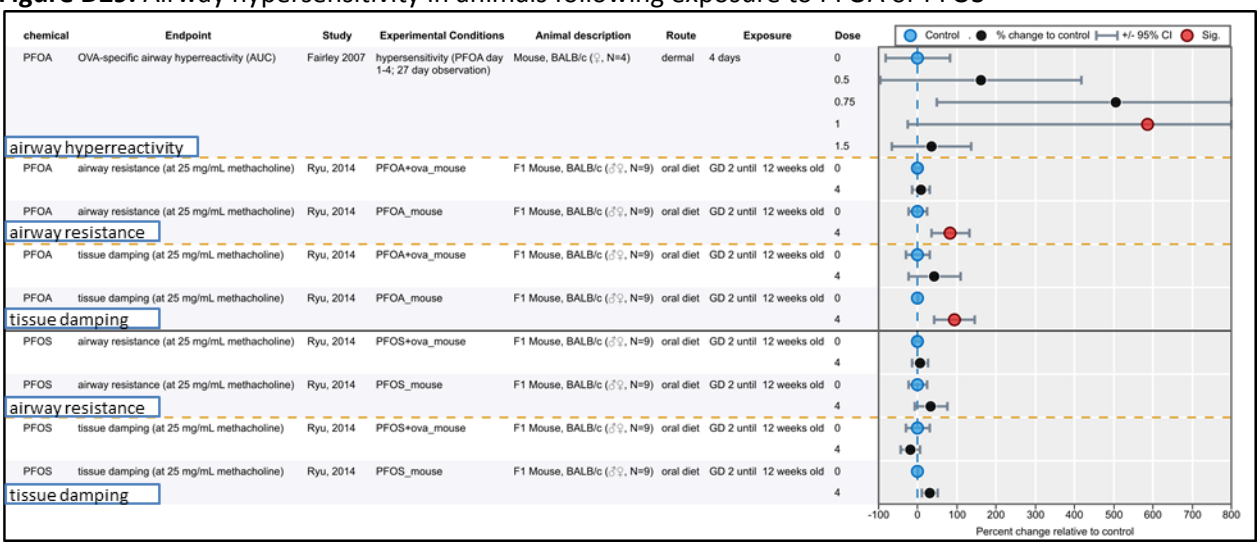
(Interactive figure and additional study details in HAWC *Figure D27 - PFOA Asthma in children*)

Figure D28. Asthma in children relative to current PFOS levels (presented as adjusted odds ratio).



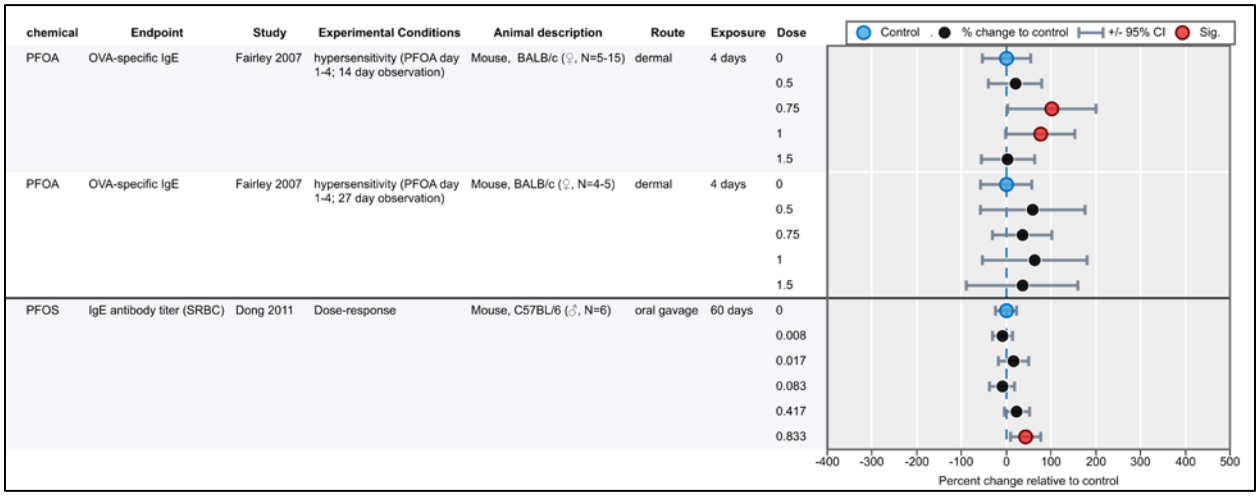
(Interactive figure and additional study details in HAWC *Figure D28 - PFOS Asthma in children*)

Figure D29. Airway hypersensitivity in animals following exposure to PFOA or PFOS



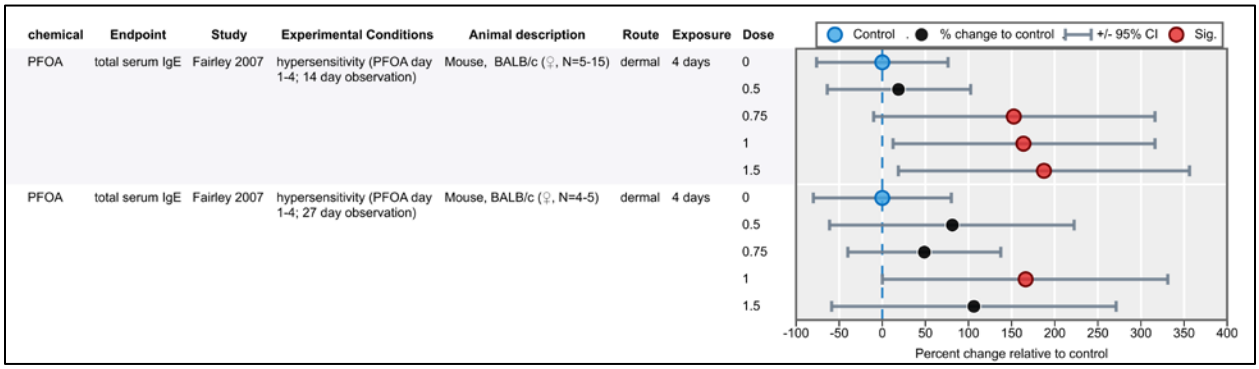
(Interactive figure and additional study details in HAWC *Figure D29 - Airway hypersensitivity in animals*)

Figure D30. Antigen-specific IgE in animals following exposure to PFOA or PFOS



(Interactive figure and additional study details in HAWC *Figure D30 - Antigen-specific IgE in animals*)

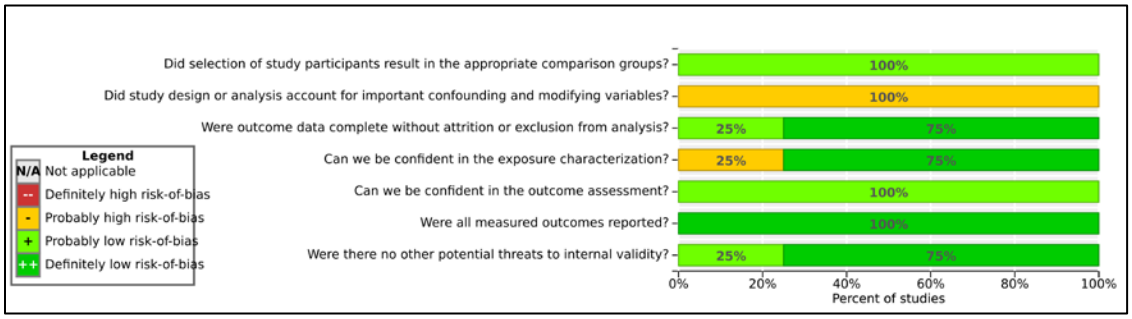
Figure D31. Total IgE in animals following exposure to PFOA



(Interactive figure and additional study details in HAWC *Figure D31 - Total IgE in Animals*)

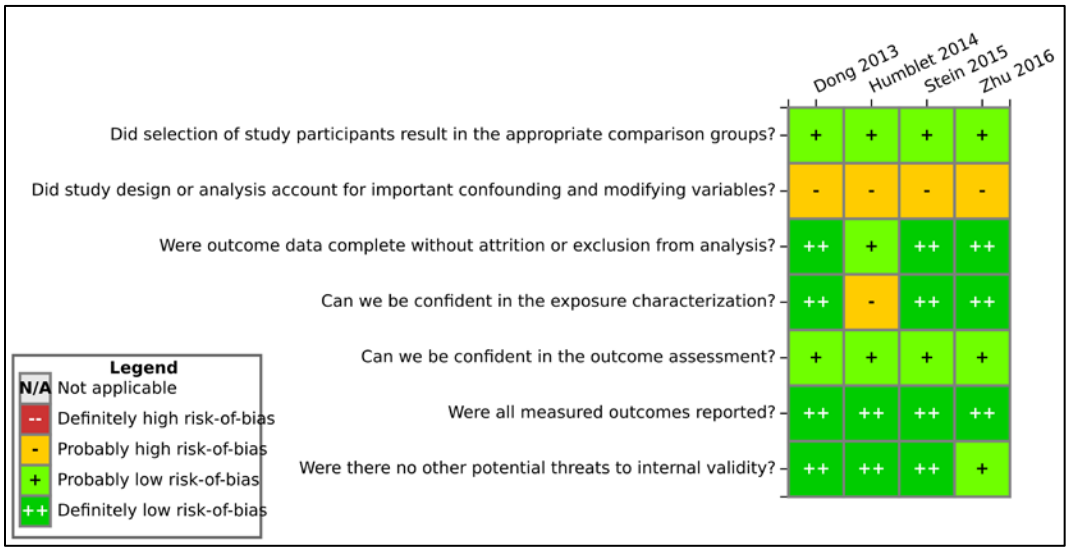
Risk of Bias Ratings for Studies of Hypersensitivity

Figure D32. Risk of bias summary for studies of asthma in children with current PFOA or PFOS levels



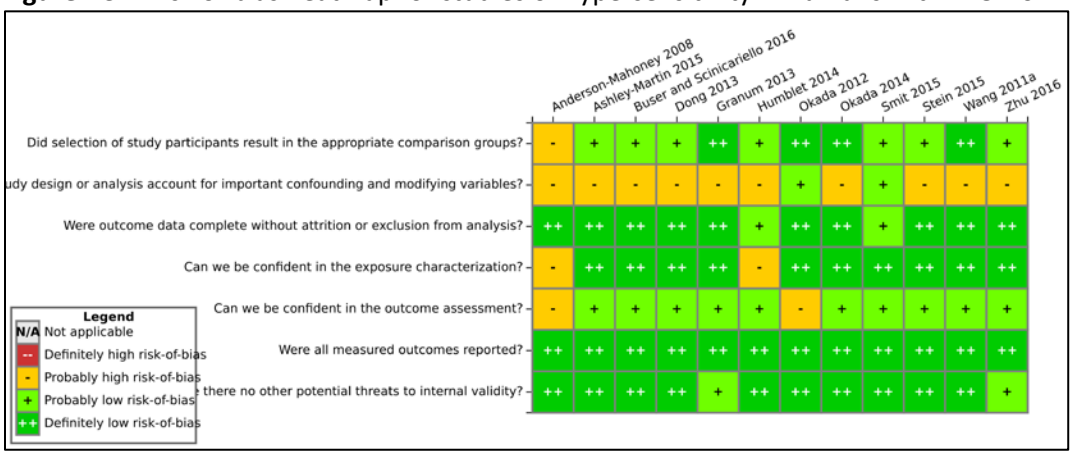
(Risk of bias ratings and study details in HAWC and *Figure D32 - Asthma in children RoB*)

Figure D33. Risk of bias heatmap for studies of asthma in children with current PFOA or PFOS level



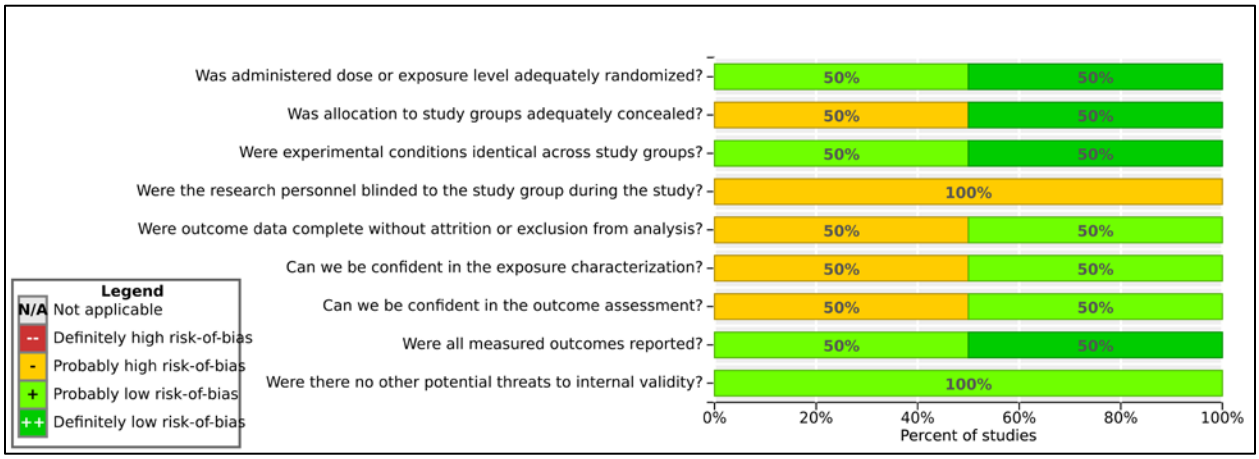
(Individual study details and risk of bias ratings in HAWC *Figure D33 - Asthma in children RoB Heatmap*)

Figure D34. Risk of bias heatmap for studies of hypersensitivity in humans with PFOA or PFOS level



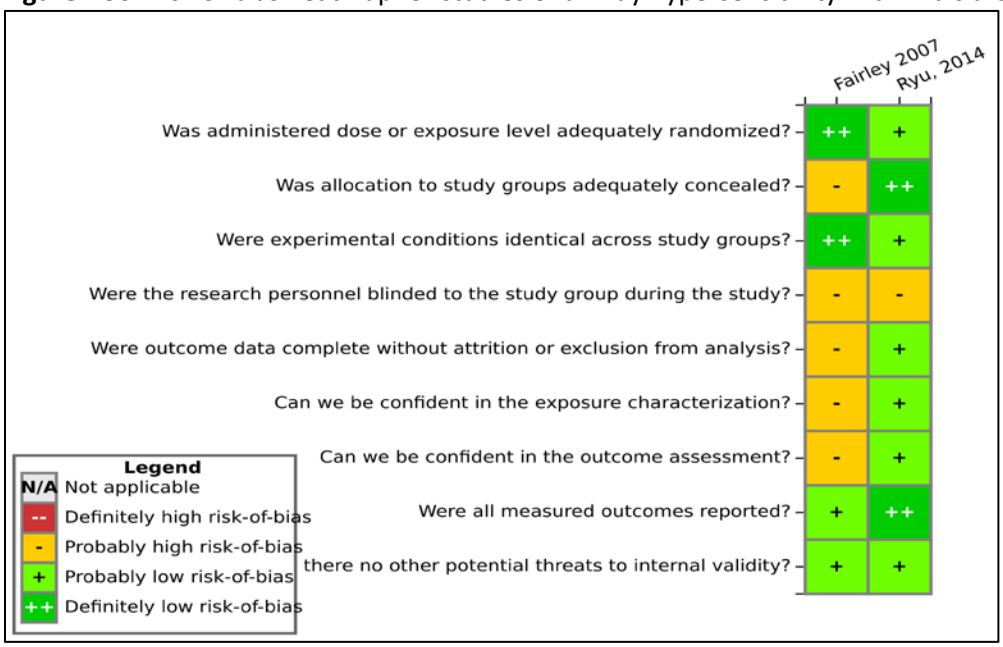
(Individual study details and risk of bias ratings in HAWC *Figure D34 - Hypersensitivity in humans RoB Heatmap*)

Figure D35. Risk of bias summary for studies of airway hypersensitivity in animals after PFOA exposure



(Risk of bias ratings and study details in HAWC *Figure D35 - Animal Airway Hypersensitivity RoB*)

Figure D36. Risk of bias heatmap for studies of airway hypersensitivity in animals after PFOA exposure

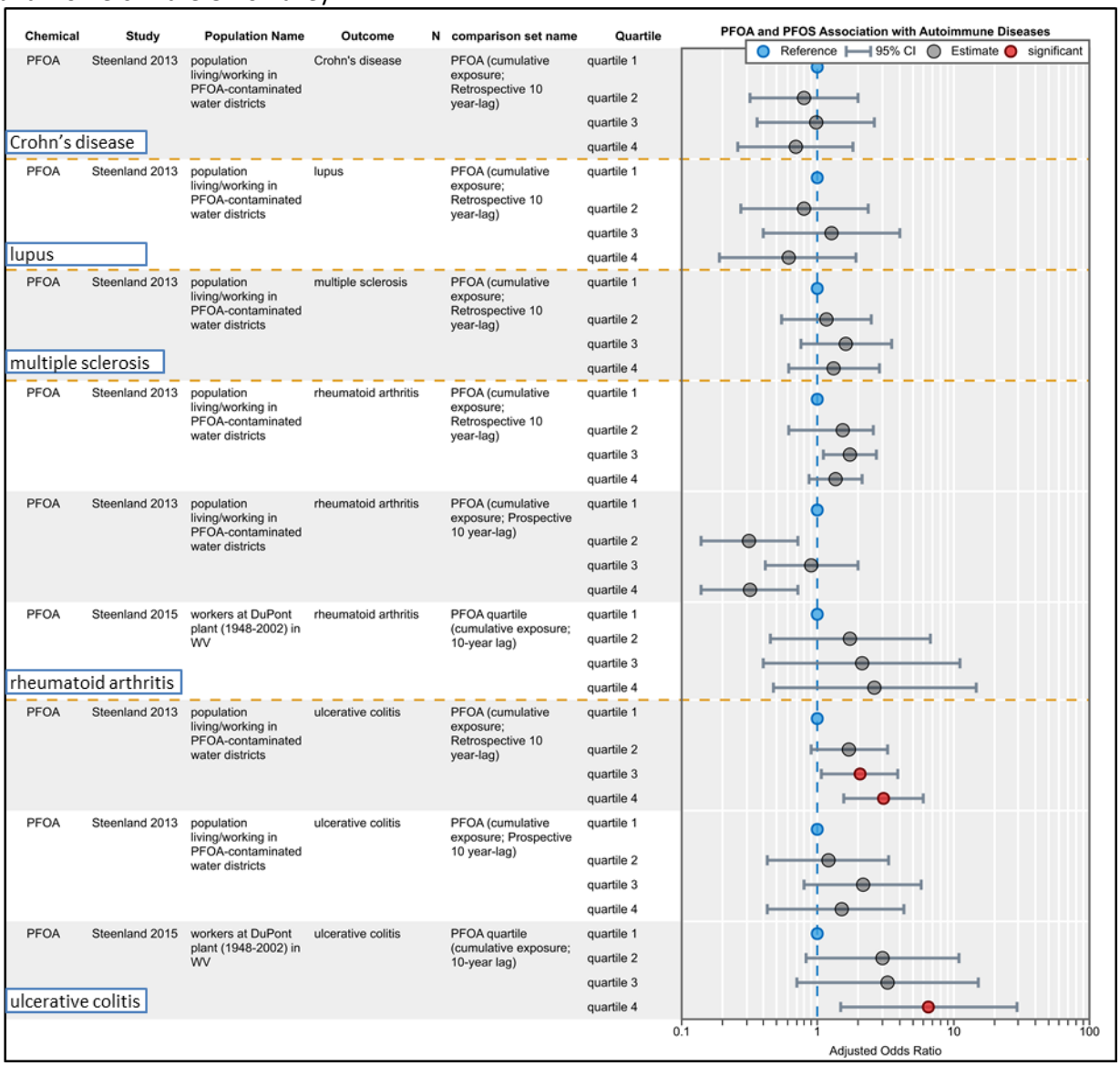


(Individual study details and risk of bias ratings in HAWC *Figure D36 - Animal Airway Hypersensitivity RoB Heatmap*)

Autoimmunity-related Effects and Outcomes

Autoimmunity Data

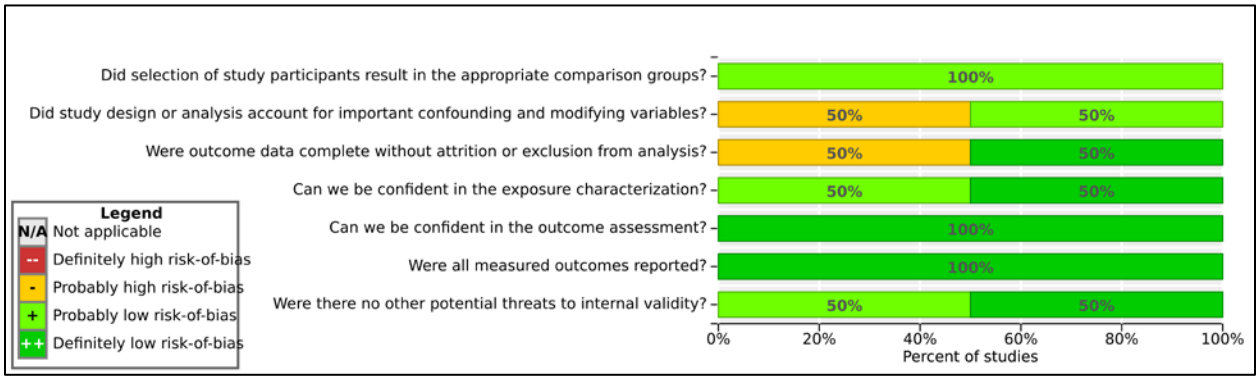
Figure D37. The incidence of autoimmune diseases relative to cumulative PFOA exposure in residents and workers in the Ohio Valley



(Interactive figure and additional study details in HAWC *Figure D37 - Autoimmunity*)

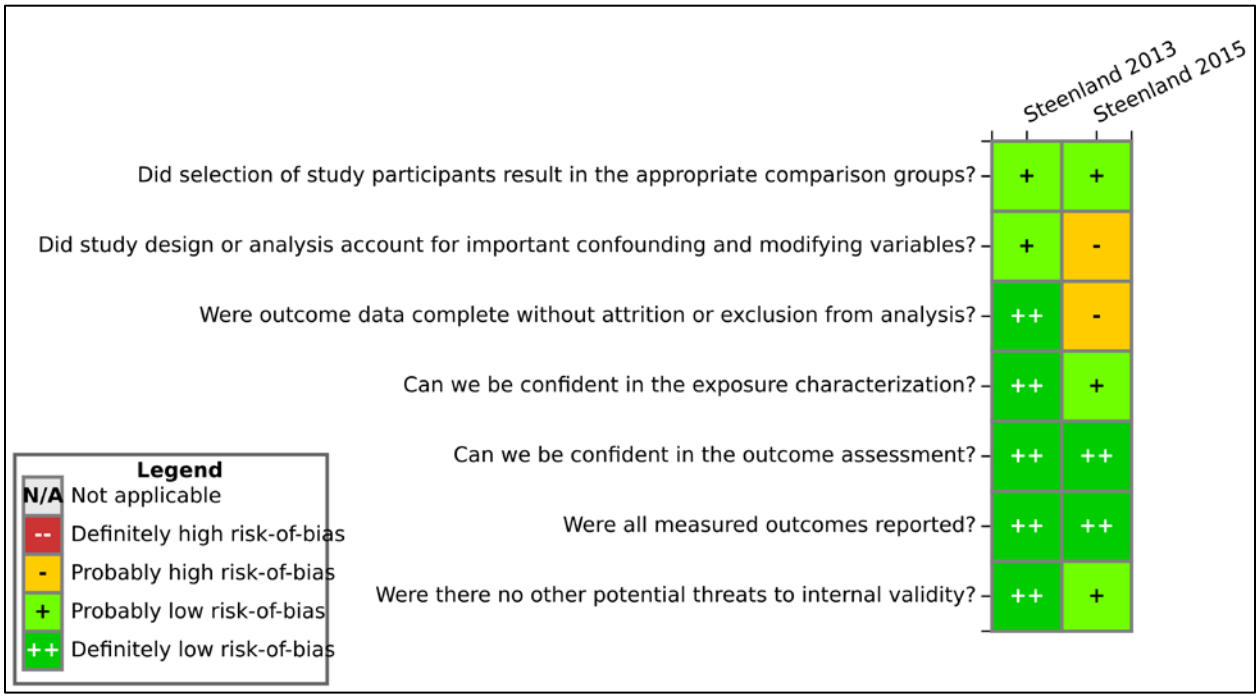
Risk of Bias Ratings for Studies of Autoimmunity

Figure D38. Risk of bias summary for studies of autoimmunity in humans with PFOA exposure levels



(Risk of bias ratings and study details in HAWC *Figure D38 - Human Autoimmunity RoB*)

Figure D39. Risk of bias heatmap for studies of autoimmunity in humans with PFOA exposure levels



(Individual study details and risk of bias ratings in HAWC *Figure D39 - Human Autoimmunity RoB*)

ABOUT THIS REVIEW

Sources of Support

National Institute of Environmental Health Sciences/Division of the National Toxicology Program

Contributors

Evaluation Team

The evaluation team is composed of federal staff and contractor staff support.

Name	Affiliation
Andrew Rooney, PhD	NIEHS/DNTP, Project Lead
Chad Blystone, PhD	NIEHS/DNTP
Abee Boyles, PhD	NIEHS/DNTP
John Cowden, PhD	US EPA, ORD/NCEA
Dori Germolec, PhD	NIEHS/DNTP
Stephanie Holmgren	NIEHS/OD
Kembra Howdeshell, PhD	NIEHS/DNTP
Christine Parks, PhD	Kelly Services, Inc.†
Katherine Pelch, PhD	NIEHS/DNTP
Andrew Shapiro, MS	NIEHS/DNTP
Kristina Thayer, PhD	NIEHS/DNTP
Kyla Taylor, PhD	NIEHS/DNTP
Vickie Walker	NIEHS/DNTP
Elizabeth Maull, PhD	NIEHS/DNTP [Contract support]
Josh Addington	Kelly Services, Inc.*
Robyn Blain	ICF International**
Pam Hartman	ICF International **
Pam Ross	ICF International **
Bryan Luukinen	ICF International **
Addie Harris	ICF International **
Susan Goldhaber	ICF International **
Thy Nguyen	ICF International **
Kaedra Jones	ICF International **
Ali Goldstone	ICF International **
Sorina Eftim	ICF International **
Jen Fownes	ICF International **

Note: the roles of individual contractors differed: †indicates review of data, results, and analyses; *indicates database and HAWC support; **indicates data extraction and assistance with risk of bias assessment

Peer Reviewers

The peer reviewers were outside experts selected for their experience with PFOA and PFOS, immunotoxicity, and systematic review procedures. Peer reviewers were screened for conflict of interest prior to their service and did not report any conflicts of interest. Service as a peer reviewer does not necessarily indicate that the reviewer endorses the final document.

Protocol Reviewers

Name	Affiliation
Jamie Dewitt, PhD	East Carolina University, Department of Pharmacology and Toxicology
Christopher Lau, PhD	US EPA, ORD/NHEERL
Tony Fletcher, PhD	London School of Hygiene and Tropical Medicine, Department of Social and Environmental Health Research
Roberta Scherer, PhD	Johns Hopkins University Bloomberg School of Public Health

no conflicts of interest declared

Draft Report Reviewers

Name	Affiliation
Jamie Dewitt, PhD	East Carolina University, Department of Pharmacology and Toxicology
Robert Luebke, PhD	US EPA, ORD/NHEERL

no conflicts of interest declared

Protocol History and Revisions

Date	Activity or revision
March 26, 2013:	**Draft case study protocol reviewed:** sent to peer reviewers for comment/review
April 9, 2013:	**Case study protocol posted on OHAT website:** posted as a case study used to test the OHAT Approach to Systematic Review and Evidence Integration
March 16, 2015:	**Draft evaluation protocol reviewed:** sent to peer reviewers for comment/ review (note: Tony Fletcher did not review the evaluation protocol because he was not available within a one month time frame)
June 16, 2015:	**Evaluation protocol posted on OHAT website:** Principal differences from case study protocol were: (1) updated/revised risk-of-bias tool; (2) addition of a risk-of-bias approach for in vitro studies; (3) no tiering process used to potentially exclude low quality studies

APPENDICES

Appendix 1. Literature Search Strategy

The strategy for this search is broad for the consideration of immune-related endpoints and comprehensive for PFOA or PFOS as an exposure or treatment in order to ensure inclusion of relevant papers. The search terms for PubMed are provided below. The specific search strategies for other databases are available in the protocol (http://ntp.niehs.nih.gov/go/749926).

Database	Search Terms
PUBMED	perfluoroalkyl*[tiab] OR perfluorocaprylic[tiab] OR perfluorocarbon*[tiab] OR perfluorocarboxyl*[tiab] OR perfluorochemical*[tiab] OR (perfluorinated[tiab] AND (C8[tiab] OR carboxylic[tiab] OR chemical*[tiab] OR compound*[tiab] OR octanoic[tiab])) OR PFAA*[tiab] OR "fluorinated polymer"[tiab] OR "fluorinated polymers"[tiab] OR (fluorinated[tiab] AND (polymer[tiab] OR polymers[tiab])) OR (fluorocarbon[tiab] AND (polymer[tiab] OR polymers[tiab])) OR Fluoropolymer*[tiab] OR (fluorinated[tiab] AND telomer*[tiab]) OR fluorotelomer*[tiab] OR fluoro-telomer*[tiab] OR fluorosurfactant*[tiab] OR "FC 143"[tiab] OR FC143[tiab] OR 335-67-1 [rn] OR Pentadecafluoroctanoate*[tiab] OR Pentadecafluorooctanoate*[tiab] OR pentadecafluoroctanoic[tiab] OR pentadecafluorooctanoic[tiab] OR "pentadecafluoro-1-octanoic"[tiab] OR "pentadecafluoro-n-octanoic"[tiab] OR "perfluoro-1-heptanecarboxylic"[tiab] OR perfluorocaprylic[tiab] OR perfluoroheptanecarboxylic[tiab] OR perfluoroctanoate[tiab] OR perfluorooctanoate[tiab] OR "perfluoro octanoate"[tiab] OR "perfluorooctanoic acid"[nm] OR perfluoroctanoic[tiab] OR perfluorooctanoic[tiab] OR "perfluoro octanoic"[tiab] OR "perfluoro-n-octanoic"[tiab] OR "perfluorooctanoyl chloride"[tiab] OR PFOA[tiab] OR APFO[tiab] OR 1763-23-1[rn] OR 307-35-7[rn] OR "1-octanesulfonic acid"[tiab] OR "1-perfluorooctanesulfonic"[tiab] OR "1-perfluoroctanesulfonic"[tiab] OR "heptadecafluoro-1-octanesulfonic"[tiab] OR "heptadecafluoro-1-octane sulfonic"[tiab] OR "heptadecafluorooctanesulfonic"[tiab] OR "heptadecafluorooctane sulfonic"[tiab] OR "heptadecafluoroctane sulfonic"[tiab] OR "perfluoroalkyl sulphonate"[tiab] OR perfluoroctanesulfonate[tiab] OR perfluorooctanesulfonate[tiab] OR "perfluoroctane sulfonate"[tiab] OR "perfluorooctane sulfonate"[tiab] OR "perfluoro-n-octanesulfonic"[tiab] OR perfluoroctanesulfonic[tiab] OR perfluorooctanesulfonic[tiab] OR "perfluorooctane sulfonic acid"[nm] OR "perfluoroctane sulfonic"[tiab] OR "perfluorooctane sulfonic"[tiab] OR perfluoroctanesulphonic[tiab] OR perfluorooctanesulphonic[tiab] OR "perfluoroctane sulphonic"[tiab] OR "perfluorooctane sulphonic"[tiab] OR perfluoroctylsulfonic[tiab] OR PFOS [tiab] AND immunology[sh] OR immune[tiab] OR immunocomp*[tiab] OR immunogen*[tiab] OR immunolog*[tiab] OR immunotox*[tiab] OR immunotoxins[mh] OR immunity[tiab] OR autoimmun*[tiab] OR "host resistance"[tiab] OR immunocompetence[mh] OR "immune system"[mh] OR spleen[tiab] OR splenic[tiab] OR splenocyt*[tiab] OR thymus[tiab] OR thymic[tiab] OR thymocyt*[tiab] OR leukocyt*[tiab] OR granulocyt*[tiab] OR basophil*[tiab] OR eosinophil*[tiab] OR neutrophil*[tiab] OR lymph[tiab] OR lymphoid*[tiab] OR lymphocyt*[tiab] OR "b-lymphocyte"[tiab] OR "b-lymphocytes"[tiab] OR "t-lymphocyte"[tiab] OR "t-lymphocytes"[tiab] OR "killer cell"[tiab] OR "killer cells"[tiab] OR "NK cell"[tiab] OR "NK-cell"[tiab] OR "NK-cells"[tiab] OR macrophag*[tiab] OR "mast cell"[tiab] OR "mast cells"[tiab] OR monocyt*[tiab] OR phagocyt*[tiab] OR dendrit*[tiab] OR "t-cell"[tiab] OR "t cell"[tiab] OR "t cells"[tiab] OR "t-cells"[tiab] OR "T helper"[tiab] OR "T-helper"[tiab] OR "b-cell"[tiab] OR "b cell"[tiab] OR "b cells"[tiab] OR "b-cells"[tiab] OR antibod*[tiab] OR histamine*[tiab] OR histocompatib*[tiab] OR

Database	Search Terms (continued)
	immunoglobulins[mh] OR immunoglobulin*[tiab] OR "immunoglobulin A"[tiab] OR IgA[tiab] OR "immunoglobulin D"[tiab] OR IgD[tiab] OR "immunoglobulin E"[tiab] OR IgE[tiab] OR "immunoglobulin G"[tiab] OR IgG[tiab] OR "immunoglobulin M"[tiab] OR IgM[tiab] OR "antigens, CD"[mh] OR CD3 [tiab] OR CD4 [tiab] OR CD8 [tiab] OR CD25 [tiab] OR CD27 [tiab] OR CD28 [tiab] OR CD29 [tiab] OR CD45*[tiab] OR cytokines[mh] OR cytokine*[tiab] OR chemokine*[tiab] OR inteferon*[tiab] OR interleukin*[tiab] OR "IL-6"[tiab] OR "IL-8"[tiab] OR lymphokine*[tiab] OR monokine*[tiab] OR ("tumor necrosis"[tiab] AND (factor[tiab] OR factors[tiab])) OR "TNF alpha"[tiab] OR "TNFalpha"[tiab] OR "immune system diseases"[mh] OR autoimmun*[tiab] OR addison[tiab] OR rheumatoid[tiab] OR glomerulonephritis[tiab] OR diabetes[tiab] OR graves[tiab] OR lupus[tiab] OR thyroiditis[tiab] OR hypersensitiv*[tiab] OR sensitization OR hyperresponsiv*[tiab] OR allergy[mh] OR allerg*[tiab] OR atopy[tiab] OR atopic[tiab] OR dermatitis[tiab] OR eczema[tiab] OR otitis[tiab] OR “ear infection”[tiab] OR “ear inflammation”[tiab] OR Respiratory tract infections[mh] OR (respiratory[tiab] AND infection*[tiab]) OR asthma[tiab] OR bronchitis[tiab] OR pneumonia[tiab] OR bronchiolitis[tiab] OR rhinitis[tiab] OR sinusitis[tiab] OR wheez*[tiab] OR crackle*[tiab] OR cough[mh] OR cough*[tiab] OR dyspnea[tiab] OR gastroenteritis[tiab] OR inflammation[mh] OR inflammat*[tiab] OR pro-inflammat*[tiab] OR anti-inflamm*[tiab] OR "inflammation mediators"[mh] OR autacoid*[tiab] OR eicosanoid*[tiab] OR prostaglandin*[tiab] OR immunomodulation[mh] OR immunomodul*[tiab] OR immunotherap*[tiab] OR vaccin*[tiab] OR immuniz*[tiab] OR immunosuppress*[tiab] OR desensitiz*[tiab] OR immunoproteins[mh] OR immunoprotein*[tiab] OR "c-reactive protein"[tiab] OR CRP[tiab] OR "complement component" [tiab] OR (complement[tiab] AND (C1 OR C2 OR C3 OR C4 OR C5 OR C6 OR C7 OR C8 OR C9))

Appendix 2. List of Included Studies

Studies in Humans

Amano K, Leung PSC, Rieger R, Quan C, Wang X, Marik J, Suen YF, Kurth MJ, Nantz MH, Ansari AA, Lam KS, Zeniya M, Matsuura E, Coppel RL, Gershwin ME. 2005. Chemical xenobiotics and mitochondrial autoantigens in primary biliary cirrhosis: Identification of antibodies against a common environmental, cosmetic, and food additive, 2-octynoic acid. *J Immunol* 174(9): 5874-5883.

Anderson-Mahoney P, Kotlerman J, Takhar H, Gray D, Dahlgren J. 2008. Self-reported health effects among community residents exposed to perfluorooctanoate. *New Solut* 18(2): 129-143.

Ashley-Martin J, Dodds L, Levy AR, Platt RW, Marshall JS, Arbuckle TE. 2015. Prenatal exposure to phthalates, bisphenol A and perfluoroalkyl substances and cord blood levels of IgE, TSLP and IL-33. *Environ Res* 140: 360-368.

Buser MC, Scinicariello F. 2016. Perfluoroalkyl substances and food allergies in adolescents. *Environ Int* 88: 74-79.

Costa G, Sartori S, Consonni D. 2009. Thirty years of medical surveillance in perfluooctanoic acid production workers. *J Occup Environ Med* 51(3): 364-372.

Dong GH, Tung KY, Tsai CH, Liu MM, Wang D, Liu W, Jin YH, Hsieh WS, Lee YL, Chen PC. 2013. Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. *Environ Health Perspect* 121(4): 507-513, 513e501-508.

Emmett EA, Zhang H, Shofer FS, Freeman D, Rodway NV, Desai C, Shaw LM. 2006. Community exposure to perfluorooctanoate: Relationships between serum levels and certain health parameters. *J Occup Environ Med* 48(8): 771-779.

Fei C, McLaughlin JK, Lipworth L, Olsen J. 2010. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. *Environ Res* 110(8): 773-777.

Genser B, Teles CA, Barreto ML, Fischer JE. 2015. Within- and between-group regression for improving the robustness of causal claims in cross-sectional analysis. *Environ Health* 14: 60.

Grandjean P, Andersen EW, Budtz-Jorgensen E, Nielsen F, Molbak K, Weihe P, Heilmann C. 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *J Am Med Assoc* 307(4): 391-397.

Granum B, Haug LS, Namork E, Stolevik SB, Thomsen C, Aaberge IS, van Loveren H, Lovik M, Nygaard UC. 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotox* 10(4): 373-379.

Humblet O, Diaz-Ramirez LG, Balmes JR, Pinney SM, Hiatt RA. 2014. Perfluoroalkyl Chemicals and Asthma among Children 12-19 Years of Age: NHANES (1999-2008). *Environ Health Perspect* 122(10): 1129-1133.

Innes KE, Ducatman AM, Luster MI, Shankar A. 2011. Association of osteoarthritis with serum levels of the environmental contaminants perfluorooctanoate and perfluorooctane sulfonate in a large Appalachian population. *Am J Epidemiol* 174(4): 440-450.

Jiang W, Zhang Y, Zhu L, Deng J. 2014. Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women. *Environ Int* 64: 40-47.

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

Leonard RC, Kreckmann KH, Sakr CJ, Symons JM. 2008. Retrospective cohort mortality study of workers in a polymer production plant including a reference population of regional workers. *Ann Epidemiol* 18(1): 15-22.

Lin CY, Wen LL, Lin LY, Wen TW, Lien GW, Chen CY, Hsu SH, Chien KL, Sung FC, Chen PC, Su TC. 2011. Associations between levels of serum perfluorinated chemicals and adiponectin in a young hypertension cohort in Taiwan. *Environ Sci Technol* 45(24): 10691-10698.

Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, Fletcher T. 2014. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctane sulfonate. *Toxicol Sci* 138(1): 76-88.

Mogensen UB, Grandjean P, Heilmann C, Nielsen F, Weihe P, Budtz-Jorgensen E. 2015. Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. *Environ Health* 14: 47.

Okada E, Sasaki S, Saijo Y, Washino N, Miyashita C, Kobayashi S, Konishi K, Ito YM, Ito R, Nakata A, Iwasaki Y, Saito K, Nakazawa H, Kishi R. 2012. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. *Environ Res* 112: 118-125.

Okada E, Sasaki S, Kashino I, Matsuura H, Miyashita C, Kobayashi S, Itoh K, Ikeno T, Tamakoshi A, Kishi R. 2014. Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. *Environ Int* 65: 127-134.

Osuna CE, Grandjean P, Weihe P, El-Fawal HA. 2014. Autoantibodies Associated with Prenatal and Childhood Exposure to Environmental Chemicals in Faroese Children. *Toxicol Sci*. 142(1): 158-66.

Pennings JL, Jennen DG, Nygaard UC, Namork E, Haug LS, van Loveren H, Granum B. 2016. Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. *J Immunotoxicol* 13(2): 173-180.

Predieri B, Iughetti L. 2015. High Levels of Perfluorooctane Sulfonate in Children at the Onset of Diabetes. 2015: 234358.

Smit LA, Lenters V, Hoyer BB, Lindh CH, Pedersen HS, Liermontova I, Jonsson BA, Piersma AH, Bonde JP, Toft G, Vermeulen R, Heederik D. 2015. Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. *Allergy* 70(6): 653-660.

Steenland K, Zhao L, Winquist A, Parks C. 2013. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the mid-Ohio valley. *Environ Health Perspect* 121(8): 900-905.

Steenland K, Zhao L, Winquist A. 2015. A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). *Occup Environ Med* 72(5): 373-380.

Stein CR, McGovern KJ, Pajak AM, Maglione PJ, Wolff MS. 2016. Perfluoroalkyl and Polyfluoroalkyl Substances and Indicators of Immune Function in Children Aged 12 - 19 years: National Health and Nutrition Examination Survey. *Pediatr Res* 79(2): 348-357.

Uhl SA, James-Todd T, Bell ML. 2013. Association of osteoarthritis with perfluorooctanoate and perfluorooctane sulfonate in NHANES 2003-2008. *Environ Health Perspect* 121(4): 447-452, 452e441-443.

Wang IJ, Hsieh WS, Chen CY, Fletcher T, Lien GW, Chiang HL, Chiang CF, Wu TN, Chen PC. 2011. The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. *Environ Res* 111(6): 785-791.

Weber KE, Fischl AF, Murray PJ, Conway BN. 2014. Effect of BMI on cardiovascular and metabolic syndrome risk factors in an Appalachian pediatric population. *Diabetes Metab Syndr Obes* 7: 445-453.

White SS, Fenton SE, Hines EP. 2011. Endocrine disrupting properties of perfluorooctanoic acid. *J Steroid Biochem Mol Biol* 127(1-2): 16-26.

Zhu Y, Qin XD, Zeng XW, Paul G, Morawska L, Su MW, Tsai CH, Wang SQ, Lee YL, Dong GH. 2016. Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: By gender and asthma status. *Sci Total Environ* 559: 166-173.

Studies in Non-human Animals

Adinehzadeh M, Reo NV. 1998. Effects of peroxisome proliferators on rat liver phospholipids: sphingomyelin degradation may be involved in hepatotoxic mechanism of perfluorodecanoic acid. *Chem Res Toxicol* 11(5): 428-440.

Alsarra IA, Brockmann WG, Cunningham ML, Badr MZ. 2006. Hepatocellular proliferation in response to agonists of peroxisome proliferator-activated receptor alpha: a role for Kupffer cells? *J Carcinog* 5: 26.

Arukwe A, Cangialosi MV, Letcher RJ, Rocha E, Mortensen AS. 2013. Changes in morphometry and association between whole-body fatty acids and steroid hormone profiles in relation to bioaccumulation patterns in salmon larvae exposed to perfluorooctane sulfonic or perfluorooctane carboxylic acids. *Aquat Toxicol* 130-131: 219-230.

Bilbao E, Raingeard D, de Cerio OD, Ortiz-Zarragoitia M, Ruiz P, Izagirre U, Orbea A, Marigomez I, Cajaraville MP, Cancio I. 2010. Effects of exposure to Prestige-like heavy fuel oil and to perfluorooctane sulfonate on conventional biomarkers and target gene transcription in the thicklip grey mullet Chelon labrosus. *Aquat Toxicol* 98(3): 282-296.

Botelho SC, Saghafian M, Pavlova S, Hassan M, DePierre JW, Abedi-Valugerdi M. 2015. Complement activation is involved in the hepatic injury caused by high-dose exposure of mice to perfluorooctanoic acid. *Chemosphere* 129: 225-231.

Butenhoff J, Costa G, Elcombe C, Farrar D, Hansen K, Iwai H, Jung R, Kennedy G, Jr., Lieder P, Olsen G, Thomford P. 2002. Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. *Toxicol Sci* 69(1): 244-257.

Butenhoff JL, Kennedy Jr GL, Frame SR, O'Connor JC, York RG. 2004. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. *Toxicology* 196(1–2): 95-116.

Butenhoff JL, Bjork JA, Chang SC, Ehresman DJ, Parker GA, Das K, Lau C, Lieder PH, van Otterdijk FM, Wallace KB. 2012. Toxicological evaluation of ammonium perfluorobutyrate in rats: Twenty-eight-day and ninety-day oral gavage studies. *Reprod Toxicol* 33(4): 513-530.

Cui L, Zhou QF, Liao CY, Fu JJ, Jiang GB. 2009. Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. *Arch Environ Contam Toxicol* 56(2): 338-349.

Dewitt JC, Copeland CB, Strynar MJ, Luebke RW. 2008. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. *Environ Health Perspect* 116(5): 644-650.

DeWitt JC, Copeland CB, Luebke RW. 2009. Suppression of humoral immunity by perfluorooctanoic acid is independent of elevated serum corticosterone concentration in mice. *Toxicol Sci* 109(1): 106-112.

DeWitt JC, Williams WC, Creech NJ, Luebke RW. 2016. Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPARalpha and T- and B-cell targeting. *J Immunotoxicol* 13(1): 38-45.

Dong G, Zheng L, Jin Y, Lu X, Liu J, He Q. 2009. Effect of oral exposure to Perfluorooctane Sulfonate (PFOS) on spleen T cell subsets CD4+/CD8+ of C57BL/6 mice. *Huanjing Kexue Xuebao / Acta Scientiae Circumstantiae* 29(1): 181-185.

Dong GH, Zhang YH, Zheng L, Liu W, Jin YH, He QC. 2009. Chronic effects of perfluorooctane sulfonate exposure on immunotoxicity in adult male C57BL/6 mice. *Arch Toxicol* 83(9): 805-815.

Dong GH, Liu MM, Wang D, Zheng L, Liang ZF, Jin YH. 2011. Sub-chronic effect of perfluorooctane sulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. *Arch Toxicol* 85(10): 1235-1244.

Dong GH, Wang J, Zhang YH, Liu MM, Wang D, Zheng L, Jin YH. 2012. Induction of p53-mediated apoptosis in splenocytes and thymocytes of C57BL/6 mice exposed to perfluorooctane sulfonate (PFOS). *Toxicol Appl Pharmacol* 264(2): 292-299.

Dong GH, Zhang YH, Zheng L, Liang ZF, Jin YH, He QC. 2012. Subchronic effects of perfluorooctane sulfonate exposure on inflammation in adult male C57BL/6 mice. *Environ Toxicol* 27(5): 285-296.

Fair PA, Driscoll E, Mollenhauer MA, Bradshaw SG, Yun SH, Kannan K, Bossart GD, Keil DE, Peden-Adams MM. 2011. Effects of environmentally-relevant levels of perfluorooctane sulfonate on clinical parameters and immunological functions in B6C3F1 mice. *J Immunotoxicol* 8(1): 17-29.

Fair PA, Romano T, Schaefer AM, Reif JS, Bossart GD, Houde M, Muir D, Adams J, Rice C, Hulsey TC, Peden-Adams M. 2013. Associations between perfluoroalkyl compounds and immune and clinical chemistry parameters in highly exposed bottlenose dolphins (Tursiops truncatus). *Environ Toxicol Chem* 32(4): 736-746.

Fairley KJ, Purdy R, Kearns S, Anderson SE, Meade B. 2007. Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. *Toxicol Sci* 97(2): 375-383.

Fang C, Huang Q, Ye T, Chen Y, Liu L, Kang M, Lin Y, Shen H, Dong S. 2013. Embryonic exposure to PFOS induces immunosuppression in the fish larvae of marine medaka. *Ecotoxicol Environ Saf* 92: 104-111.

Griffith FD, Long JE. 1980. Animal toxicity studies with ammonium perfluorooctanoate. *Am Ind Hyg Assoc J* 41(8): 576-583.

Guruge KS, Hikono H, Shimada N, Murakami K, Hasegawa J, Yeung LW, Yamanaka N, Yamashita N. 2009. Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F1 mice. *J Toxicol Sci* 34(6): 687-691.

Hines EP, White SS, Stanko JP, Gibbs-Flournoy EA, Lau C, Fenton SE. 2009. Phenotypic dichotomy following developmental exposure to perfluorooctanoic acid (PFOA) in female CD-1 mice: Low doses induce elevated serum leptin and insulin, and overweight in mid-life. *Mol Cell Endocrinol* 304(1-2): 97-105.

Houde M, Giraudo M, Douville M, Bougas B, Couture P, De Silva AO, Spencer C, Lair S, Verreault J, Bernatchez L, Gagnon C. 2014. A multi-level biological approach to evaluate impacts of a major municipal effluent in wild St. Lawrence River yellow perch (*Perca flavescens*). *Sci Total Environ* 497-498: 307-318.

Hu Q, Strynar MJ, DeWitt JC. 2010. Are developmentally exposed C57BL/6 mice insensitive to suppression of TDAR by PFOA? *J Immunotoxicol* 7(4): 344-349.

Hu Q, Franklin JN, Bryan I, Morris E, Wood A, DeWitt JC. 2012. Does developmental exposure to perflurooctanoic acid (PFOA) induce immunopathologies commonly observed in neurodevelopmental disorders? *Neurotox* 33(6): 1491-1498.

Huang Q, Chen Y, Chi Y, Lin Y, Zhang H, Fang C, Dong S. 2015. Immunotoxic effects of perfluorooctane sulfonate and di(2-ethylhexyl) phthalate on the marine fish *Oryzias melastigma*. *Fish & shellfish immunology* 44(1): 302-306.

IRDC (International Research and Development Corporation). 1978. *Ninety day subacute rat toxicity study. Sponsor: 3M*. U.S. Environmental Protection Agency Administrative Record 226-0441: 53.

Iwai H, Yamashita K. 2006. A fourteen-day repeated dose oral toxicity study of APFO in rats. *Drug Chem Toxicol* 29(3): 323-332.

Jacobson T, Holmstrom K, Yang G, Ford AT, Berger U, Sundelin B. 2010. Perfluorooctane sulfonate accumulation and parasite infestation in a field population of the amphipod *Monoporeia affinis* after microcosm exposure. *Aquat Toxicol* 98(1): 99-106.

Kannan K, Perrotta E, Thomas NJ. 2006. Association between perfluorinated compounds and pathological conditions in southern sea otters. *Environ Sci Technol* 40(16): 4943-4948.

Kannan K, Yun SH, Rudd RJ, Behr M. 2010. High concentrations of persistent organic pollutants including PCBs, DDT, PBDEs and PFOS in little brown bats with white-nose syndrome in New York, USA. *Chemosphere* 80(6): 613-618.

Keil DE, Mehlmann T, Butterworth L, Peden-Adams MM. 2008. Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. *Toxicol Sci* 103(1): 77-85.

Lefebvre DE, Curran I, Armstrong C, Coady L, Parenteau M, Liston V, Barker M, Aziz S, Rutherford K, Bellon-Gagnon P, Shenton J, Mehta R, Bondy G. 2008. Immunomodulatory effects of dietary potassium perfluorooctane sulfonate (PFOS) exposure in adult Sprague-Dawley rats. *J Toxicol Environ Health A* 71(23): 1516-1525.

Liu W, Xu C, Sun X, Kuang H, Kuang X, Zou W, Yang B, Wu L, Liu F, Zou T, Zhang D. 2016. Grape seed proanthocyanidin extract protects against perfluorooctanoic acid-induced hepatotoxicity by attenuating inflammatory response, oxidative stress and apoptosis in mice. *Toxicology Research* 5(1): 224-234.

Loveless SE, Hoban D, Sykes G, Frame SR, Everds NE. 2008. Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate. *Toxicol Sci* 105(1): 86-96.

Lu Y, Luo B, Li J, Dai J. 2016. Perfluorooctanoic acid disrupts the blood-testis barrier and activates the TNFalpha/p38 MAPK signaling pathway in vivo and in vitro. *Arch Toxicol* 90(4): 971-983.

Lunardi D, Abelli L, Panti C, Marsili L, Fossi MC, Mancia A. 2016. Transcriptomic analysis of bottlenose dolphin (*Tursiops truncatus*) skin biopsies to assess the effects of emerging contaminants. *Mar Environ Res* 114: 74-79.

Lv QY, Wan B, Guo LH, Yang Y, Ren XM, Zhang H. 2015. In vivo immunotoxicity of perfluorooctane sulfonate in BALB/c mice: Identification of T-cell receptor and calcium-mediated signaling pathway disruption through gene expression profiling of the spleen. *Chem Biol Interact* 240: 84-93.

Minata M, Harada KH, Karrman A, Hitomi T, Hirosawa M, Murata M, Gonzalez FJ, Koizumi A. 2010. Role of peroxisome proliferator-activated receptor-alpha in hepatobiliary injury induced by ammonium perfluorooctanoate in mouse liver. *Ind Health* 48(1): 96-107.

Mollenhauer MA, Bradshaw SG, Fair PA, McGuinn WD, Peden-Adams MM. 2011. Effects of perfluorooctane sulfonate (PFOS) exposure on markers of inflammation in female B6C3F1 mice. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 46(2): 97-108.

Nakagawa T, Ramdhan DH, Tanaka N, Naito H, Tamada H, Ito Y, Li Y, Hayashi Y, Yamagishi N, Yanagiba Y, Aoyama T, Gonzalez FJ, Nakajima T. 2012. Modulation of ammonium perfluorooctanoate-induced hepatic damage by genetically different PPARalpha in mice. *Arch Toxicol* 86(1): 63-74.

Oh JH, Moon HB, Choe ES. 2013. Alterations in differentially expressed genes after repeated exposure to perfluorooctanoate and perfluorooctane sulfonate in liver of *Oryzias latipes*. *Arch Environ Contam Toxicol* 64(3): 475-483.

Peden-Adams MM, Keller JM, Eudaly JG, Berger J, Gilkeson GS, Keil DE. 2008. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicol Sci* 104(1): 144-154.

Peden-Adams MM, Stuckey JE, Gaworecki KM, Berger-Ritchie J, Bryant K, Jodice PG, Scott TR, Ferrario JB, Guan B, Vigo C, Boone JS, McGuinn WD, DeWitt JC, Keil DE. 2009. Developmental toxicity in white leghorn chickens following in ovo exposure to perfluorooctane sulfonate (PFOS). *Reprod Toxicol* 27(3-4): 307-318.

Qazi MR, Bogdanska J, Butenhoff JL, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2009. High-dose, short-term exposure of mice to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) affects the number of circulating neutrophils differently, but enhances the inflammatory responses of macrophages to lipopolysaccharide (LPS) in a similar fashion. *Toxicology* 262(3): 207-214.

Qazi MR, Xia Z, Bogdanska J, Chang SC, Ehresman DJ, Butenhoff JL, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2009. The atrophy and changes in the cellular compositions of the thymus and spleen observed in mice subjected to short-term exposure to perfluorooctanesulfonate are high-dose phenomena mediated in part by peroxisome proliferator-activated receptor-alpha (PPARalpha). *Toxicology* 260(1-3): 68-76.

Qazi MR, Abedi MR, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2010. Dietary exposure to perfluorooctanoate or perfluorooctane sulfonate induces hypertrophy in centrilobular hepatocytes and alters the hepatic immune status in mice. *Int Immunopharmacol* 10(11): 1420-1427.

Qazi MR, Nelson BD, Depierre JW, Abedi-Valugerdi M. 2010. 28-Day dietary exposure of mice to a low total dose (7 mg/kg) of perfluorooctanesulfonate (PFOS) alters neither the cellular compositions of the thymus and spleen nor humoral immune responses: does the route of administration play a pivotal role in PFOS-induced immunotoxicity? *Toxicology* 267(1-3): 132-139.

Qazi MR, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2012. High-dose dietary exposure of mice to perfluorooctanoate or perfluorooctane sulfonate exerts toxic effects on myeloid and B-lymphoid cells in the bone marrow and these effects are partially dependent on reduced food consumption. *Food Chem Toxicol* 50(9): 2955-2963.

Qazi MR, Hassan M, Nelson BD, Depierre JW, Abedi-Valugerdi M. 2013. Sub-acute, moderate-dose, but not short-term, low-dose dietary pre-exposure of mice to perfluorooctanoate aggravates concanavalin A-induced hepatitis. *Toxicol Lett* 219(1): 1-7.

Qazi MR, Hassan M, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2013. Both sub-acute, moderate-dose and short-term, low-dose dietary exposure of mice to perfluorooctane sulfonate exacerbates concanavalin A-induced hepatitis. *Toxicol Lett* 217(1): 67-74.

Riker Laboratories. 1987. *Two year oral (diet) toxicity/carcinogenicity study of FC-143 in rats*. U.S. Environmental Protection Agency Administrative Records 226-0437, 226-0438, 226-0439, and 226-440.

Rosen MB, Thibodeaux JR, Wood CR, Zehr RD, Schmid JE, Lau C. 2007. Gene expression profiling in the lung and liver of PFOA-exposed mouse fetuses. *Toxicology* 239(1-2): 15-33.

Rosen MB, Abbott BD, Wolf DC, Corton JC, Wood CR, Schmid JE, Das KP, Zehr RD, Blair ET, Lau C. 2008. Gene profiling in the livers of wild-type and PPARalpha-null mice exposed to perfluorooctanoic acid. *Toxicol Pathol* 36(4): 592-607.

Rosen MB, Lee JS, Ren H, Vallanat B, Liu J, Waalkes MP, Abbott BD, Lau C, Corton JC. 2008. Toxicogenomic dissection of the perfluorooctanoic acid transcript profile in mouse liver: Evidence for the involvement of nuclear receptors PPAR(alpha) and CAR. *Toxicol Sci* 103(1): 46-56.

Rosen MB, Schmid JR, Corton JC, Zehr RD, Das KP, Abbott BD, Lau C. 2010. Gene Expression Profiling in Wild-Type and PPARalpha-Null Mice Exposed to Perfluorooctane Sulfonate Reveals PPARalpha-Independent Effects. *PPAR Res* 2010.

Ryu MH, Jha A, Ojo OO, Mahood TH, Basu S, Detillieux KA, Nikoobakht N, Wong CS, Loewen M, Becker AB, Halayko AJ. 2014. Chronic exposure to perfluorinated compounds: impact on airway hyperresponsiveness and inflammation. *Am J Physiol Lung Cell Mol Physiol* 307(10): L765-74.

Seacat AM, Thomford PJ, Hansen KJ, Olsen GW, Case MT, Butenhoff JL. 2002. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. *Toxicol Sci* 68(1): 249-264.

Seacat AM, Thomford PJ, Hansen KJ, Clemen LA, Eldridge SR, Elcombe CR, Butenhoff JL. 2003. Sub-chronic dietary toxicity of potassium perfluorooctane sulfonate in rats. *Toxicology* 183(1-3): 117-131.

Singh TS, Lee S, Kim HH, Choi JK, Kim SH. 2012. Perfluorooctanoic acid induces mast cell-mediated allergic inflammation by the release of histamine and inflammatory mediators. *Toxicol Lett* 210(1): 64-70.

Sletten S, Bourgeon S, Bardsen BJ, Herzke D, Criscuolo F, Massemin S, Zahn S, Johnsen TV, Bustnes JO. 2016. Organohalogenated contaminants in white-tailed eagle (Haliaeetus albicilla) nestlings: An assessment of relationships to immunoglobulin levels, telomeres and oxidative stress. *Sci Total Environ* 539: 337-349.

Smits JE, Nain S. 2013. Immunomodulation and hormonal disruption without compromised disease resistance in perfluorooctanoic acid (PFOA) exposed Japanese quail. *Environ Pollut* 179: 13-18.

Son HY, Lee S, Tak EN, Cho HS, Shin HI, Kim SH, Yang JH. 2009. Perfluorooctanoic acid alters T lymphocyte phenotypes and cytokine expression in mice. *Environ Toxicol* 24(6): 580-588.

Tan X, Xie G, Sun X, Li Q, Zhong W, Qiao P, Sun X, Jia W, Zhou Z. 2013. High fat diet feeding exaggerates perfluorooctanoic acid-induced liver injury in mice via modulating multiple metabolic pathways. *PLoS ONE* 8(4): e61409.

Taylor BK, Dadia N, Yang CB, Krishnan S, Badr M. 2002. Peroxisome proliferator-activated receptor agonists inhibit inflammatory edema and hyperalgesia. *Inflammation* 26(3): 121-127.

Taylor BK, Kriedt C, Nagalingam S, Dadia N, Badr M. 2005. Central administration of perfluorooctanoic acid inhibits cutaneous inflammation. *Inflamm Res* 54(6): 235-242.

Thomford PJ. 2001. *4-Week capsule toxicity study with ammonium perfluorooctanoate (APFO) in Cynomolgus monkeys. Sponsor: APME Ad-Hoc APFO toxicology working group*. 159.

Thomford PJ. 2002. *4-week capsule toxicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in Cynomolgus monkeys. Sponsor: 3M*. 235.

Thomford PJ. 2002. *104-Week dietary chronic toxicity and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in rats. Prepared for: 3M*. 216.

Vetvicka V, Vetvickova J. 2013. Reversal of perfluorooctane sulfonate-induced immunotoxicity by a glucan-resveratrol-vitamin C combination. *Oriental Pharmacy and Experimental Medicine* 13(1): 77-84.

Wan YJ, Badr MZ. 2006. Inhibition of Carrageenan-Induced Cutaneous Inflammation by PPAR Agonists Is Dependent on Hepatocyte-Specific Retinoid X ReceptorAlpha. *PPAR Res* 2006: 96341.

Wang Y, Wang L, Liang Y, Qiu W, Zhang J, Zhou Q, Jiang G. 2011. Modulation of dietary fat on the toxicological effects in thymus and spleen in BALB/c mice exposed to perfluorooctane sulfonate. *Toxicol Lett* 204(2-3): 174-182.

Wang Y, Wang L, Li J, Liang Y, Ji H, Zhang J, Zhou Q, Jiang G. 2014. The mechanism of immunosuppression by perfluorooctanoic acid in BALB/c mice. *Toxicology Research* 3(3): 205-213.

Watanabe MX, Kunisue T, Tao L, Kannan K, Subramanian A, Tanabe S, Iwata H. 2010. Dioxin-like and perfluorinated compounds in pigs in an Indian open waste dumping site: toxicokinetics and effects on hepatic cytochrome P450 and blood plasma hormones. *Environ Toxicol Chem* 29(7): 1551-1560.

Wei Y, Liu Y, Wang J, Tao Y, Dai J. 2008. Toxicogenomic analysis of the hepatic effects of perfluorooctanoic acid on rare minnows (Gobiocypris rarus). *Toxicol Appl Pharmacol* 226(3): 285-297.

Xie Y, Yang Q, Nelson BD, DePierre JW. 2002. Characterization of the adipose tissue atrophy induced by peroxisome proliferators in mice. *Lipids* 37(2): 139-146.

Xing J, Wang G, Zhao J, Wang E, Yin B, Fang D, Zhao J, Zhang H, Chen YQ, Chen W. 2016. Toxicity assessment of perfluorooctane sulfonate using acute and subchronic male C57BL/6J mouse models. *Environ Pollut* 210: 388-396.

Yang Q, Xie Y, Depierre JW. 2000. Effects of peroxisome proliferators on the thymus and spleen of mice. *Clin Exp Immunol* 122(2): 219-226.

Yang Q, Xie Y, Eriksson AM, Nelson BD, DePierre JW. 2001. Further evidence for the involvement of inhibition of cell proliferation and development in thymic and splenic atrophy induced by the peroxisome proliferator perfluoroctanoic acid in mice. *Biochem Pharmacol* 62(8): 1133-1140.

Yang Q, Abedi-Valugerdi M, Xie Y, Zhao XY, Moller G, Nelson BD, DePierre JW. 2002. Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. *Int Immunopharmacol* 2(2-3): 389-397.

Yang Q, Xie Y, Alexson SE, Nelson BD, DePierre JW. 2002. Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. *Biochem Pharmacol* 63(10): 1893-1900.

Yang J, Li A, Yang Q, Li X. 2006. [Effects of peroxisome proliferators PFOA on immune system of mice]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 22(2): 157-160.

Yang JH. 2010. Perfluorooctanoic acid induces peroxisomal fatty acid oxidation and cytokine expression in the liver of male Japanese medaka (*Oryzias latipes*). *Chemosphere* 81(4): 548-552.

Yang B, Zou WY, Hu ZZ, Liu FM, Zhou L, Yang SL, Kuang HB, Wu L, Wei J, Wang JL, Zou T, Zhang DL. 2014. Involvement of Oxidative Stress and Inflammation in Liver Injury Caused by Perfluorooctanoic Acid Exposure in Mice. *Biomed Res. Int.*: 7.

Zeng HC, Zhang L, Li YY, Wang YJ, Xia W, Lin Y, Wei J, Xu SQ. 2011. Inflammation-like glial response in rat brain induced by prenatal PFOS exposure. *Neurotox* 32(1): 130-139.

Zhang YH, Wang J, Dong GH, Liu MM, Wang D, Zheng L, Jin YH. 2013. Mechanism of perfluorooctanesulfonate (PFOS)-induced apoptosis in the immunocyte. *J Immunotoxicol* 10(1): 49-58.

Zhang H, Fang W, Wang D, Gao N, Ding Y, Chen C. 2014. The role of interleukin family in perfluorooctanoic acid (PFOA)-induced immunotoxicity. *J Hazard Mater* 280: 552-560.

Zhang F, Wei J, Li Q, Jiang R, Yu N, Qin J, Chen L. 2015. Effects of perfluorooctane sulfonate on the immune responses and expression of immune-related genes in Chinese mitten-handed crab Eriocheir sinensis. *Comp Biochem Physiol C Toxicol Pharmacol* 172-173: 13-18.

Zheng L, Dong GH, Jin YH, He QC. 2009. Immunotoxic changes associated with a 7-day oral exposure to perfluorooctane sulfonate (PFOS) in adult male C57BL/6 mice. *Arch Toxicol* 83(7): 679-689.

Zheng L, Dong GH, Zhang YH, Liang ZF, Jin YH, He QC. 2011. Type 1 and Type 2 cytokines imbalance in adult male C57BL/6 mice following a 7-day oral exposure to perfluorooctane sulfonate (PFOS). *J Immunotoxicol* 8(1): 30-38.

Zou W, Liu W, Yang B, Wu L, Yang J, Zou T, Liu F, Xia L, Zhang D. 2015. Quercetin protects against perfluorooctanoic acid-induced liver injury by attenuating oxidative stress and inflammatory response in mice. *Int Immunopharmacol* 28(1): 129-135.

In Vitro Experimental Studies

Ahuja V, Eisenblatter M, Ignatius R, Stahlmann R. 2009. Ammonium perfluorooctanoate substantially alters phenotype and cytokine secretion of human monocyte-derived dendritic cells in vitro. *Immunopharm Immunot* 31(4): 641-646.

Botelho SC, Saghafian M, Pavlova S, Hassan M, DePierre JW, Abedi-Valugerdi M. 2015. Complement activation is involved in the hepatic injury caused by high-dose exposure of mice to perfluorooctanoic acid. *Chemosphere* 129: 225-231.

Brieger A, Bienefeld N, Hasan R, Goerlich R, Haase H. 2011. Impact of perfluorooctane sulfonate and perfluorooctanoic acid on human peripheral leukocytes. *Toxicol In Vitro* 25(4): 960-968.

Corsini E, Avogadro A, Galbiati V, dell'Agli M, Marinovich M, Galli CL, Germolec DR. 2011. In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). *Toxicol Appl Pharmacol* 250(2): 108-116.

Corsini E, Sangiovanni E, Avogadro A, Galbiati V, Viviani B, Marinovich M, Galli CL, Dell'Agli M, Germolec DR. 2012. In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). *Toxicol Appl Pharmacol* 258(2): 248-255.

Gimenez-Bastida JA, Surma M, Zielinski H. 2015. In vitro evaluation of the cytotoxicity and modulation of mechanisms associated with inflammation induced by perfluorooctane sulfonate and perfluorooctanoic acid in human colon myofibroblasts CCD-18Co. *Toxicol In Vitro* 29(7): 1683-1691.

Han Z, Liu Y, Wu D, Zhu Z, Lü C. 2012. Immunotoxicity and hepatotoxicity of PFOS and PFOA in tilapia (Oreochromis niloticus). *Chinese Journal of Geochemistry* 31(4): 424-430.

Lee SW, Son HY, Yoon WK, Jung JY, Park BK, Cho ES, Park SJ, Kim TH, Ryu SY. 2010. Effect of Atrazine, Perfluorooctanoic Acid and Zearalenone on IFN gamma, TNF alpha, and IL-5 mRNA Expression in Jurkat Cells. *Biomolecules & Therapeutics* 18(3): 286-293.

Levitt D, Liss A. 1986. Toxicity of perfluorinated fatty acids for human and murine B cell lines. *Toxicol Appl Pharmacol* 86(1): 1-11.

Levitt D, Liss A. 1987. Perfluorinated fatty acids alter merocyanine 540 dye binding to plasma membranes. *J Toxicol Environ Health* 20(3): 303-316.

Liao Y, Wang J, Huang QS, Fang C, Kiyama R, Shen H, Dong S. 2012. Evaluation of cellular response to perfluorooctane sulfonate in human umbilical vein endothelial cells. *Toxicol In Vitro* 26(3): 421-428.

Liao Y, Dong S, Kiyama R, Cai P, Liu L, Shen H. 2013. Flos lonicerae extracts and chlorogenic acid protect human umbilical vein endothelial cells from the toxic damage of perfluorooctane sulphonate. *Inflammation* 36(3): 767-779.

Lu Y, Luo B, Li J, Dai J. 2015. Perfluorooctanoic acid disrupts the blood-testis barrier and activates the TNFalpha/p38 MAPK signaling pathway in vivo and in vitro. *Arch Toxicol*.

Messina PV, Prieto G, Sabín J, Blanco E, Varela C, Dodero V, Ruso JM, Sarmiento F. 2007. A potentiometrie and spectroscopic study on the interaction between human immunoglobulin G and sodium perfluorooctanoate in aqueous solution. *Macromol Symp* 251: 103-111.

Messina PV, Prieto G, Salgado F, Varela C, Nogueira M, Dodero V, Ruso JM, Sarmiento F. 2007. The influence of sodium perfluorooctanoate on the conformational transitions of human immunoglobulin. *J Phys Chem B* 111(28): 8045-8052.

Midgett K, Peden-Adams MM, Gilkeson GS, Kamen DL. 2015. In vitro evaluation of the effects of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) on IL-2 production in human T-cells. *J Appl Toxicol* 35(5): 459-465.

Miyano Y, Tsukuda S, Sakimoto I, Takeuchi R, Shimura S, Takahashi N, Kusayanagi T, Takakusagi Y, Okado M, Matsumoto Y, Takakusagi K, Takeuchi T, Kamisuki S, Nakazaki A, Ohta K, Miura M, Kuramochi K, Mizushina Y, Kobayashi S, Sugawara F, Sakaguchi K. 2012. Exploration of the binding proteins of perfluorooctane sulfonate by a T7 phage display screen. *Bioorg Med Chem* 20(13): 3985-3990.

Singh TS, Lee S, Kim HH, Choi JK, Kim SH. 2012. Perfluorooctanoic acid induces mast cell-mediated allergic inflammation by the release of histamine and inflammatory mediators. *Toxicol Lett* 210(1): 64-70.

Wang C, Nie X, Zhang Y, Li T, Mao J, Liu X, Gu Y, Shi J, Xiao J, Wan C, Wu Q. 2015. Reactive oxygen species mediate nitric oxide production through ERK/JNK MAPK signaling in HAPI microglia after PFOS exposure. *Toxicol Appl Pharmacol* 288(2): 143-151.

Wei Y, Shi X, Zhang H, Wang J, Zhou B, Dai J. 2009. Combined effects of polyfluorinated and perfluorinated compounds on primary cultured hepatocytes from rare minnow (Gobiocypris rarus) using toxicogenomic analysis. *Aquat Toxicol* 95(1): 27-36.

Wens B, De Boever P, Verbeke M, Hollanders K, Schoeters G. 2013. Cultured human peripheral blood mononuclear cells alter their gene expression when challenged with endocrine-disrupting chemicals. *Toxicology* 303: 17-24.

Wirth JR, Peden-Adams MM, White ND, Bossart GD, Fair PA. 2014. In vitro PFOS exposure on immune endpoints in bottlenose dolphins (*Tursiops truncatus*) and mice. *J Appl Toxicol* 34(6): 658-666.

Yamaki K, Yoshino S. 2010. Enhancement of FcvarepsilonRI-mediated degranulation response in the rat basophilic leukemia cell line RBL2H3 by the fluorosurfactants perfluorooctanoic acid and perfluorooctane sulfonate. *Environ Toxicol Pharmacol* 29(2): 183-189.

Yang Q, Xie Y, Depierre JW. 2000. Effects of peroxisome proliferators on the thymus and spleen of mice. *Clin Exp Immunol* 122(2): 219-226.

Yang Q, Abedi-Valugerdi M, Xie Y, Zhao XY, Moller G, Nelson BD, DePierre JW. 2002. Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. *Int Immunopharmacol* 2(2-3): 389-397.

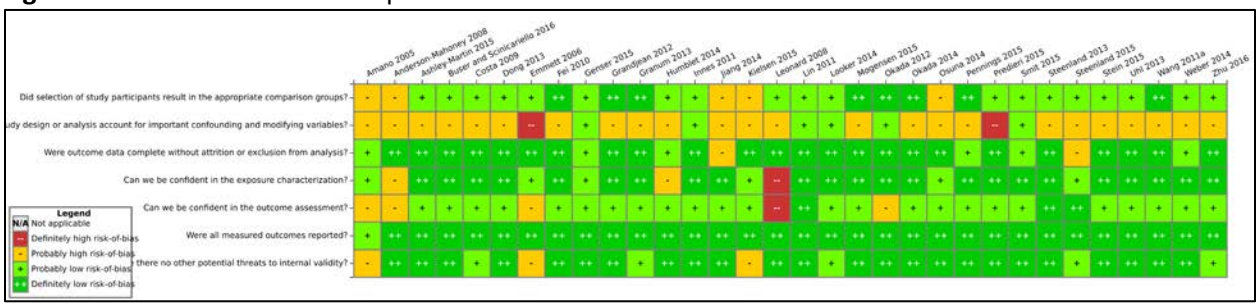
Yang J, Wang C, Nie X, Shi S, Xiao J, Ma X, Dong X, Zhang Y, Han J, Li T, Mao J, Liu X, Zhao J, Wu Q. 2015. Perfluorooctane sulfonate mediates microglial activation and secretion of TNF-alpha through Ca(2+)-dependent PKC-NF-small ka, CyrillicB signaling. *Int Immunopharmacol* 28(1): 52-60.

Zhu J, Qian W, Wang Y, Gao R, Wang J, Xiao H. 2015. Involvement of mitogen-activated protein kinase and NF-kappaB signaling pathways in perfluorooctane sulfonic acid-induced inflammatory reaction in BV2 microglial cells. *J Appl Toxicol* 35(12) 1539-49.

Appendix 3. Risk of Bias Heatmaps

Studies in Humans

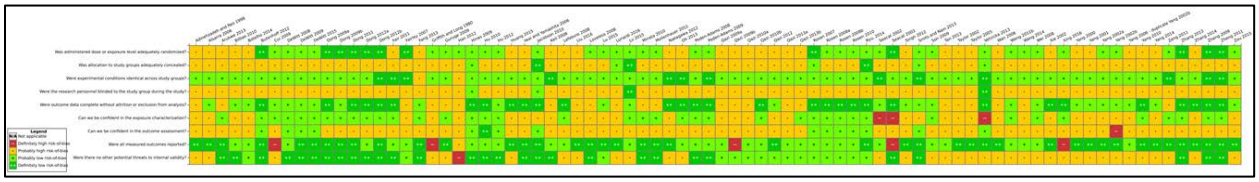
Figure A3-1. Risk of bias heatmap for all included studies in humans with PFOA or PFOS levels



(Individual study details and risk of bias ratings in HAWC *Figure A3-1 - Human RoB Heatmap*)

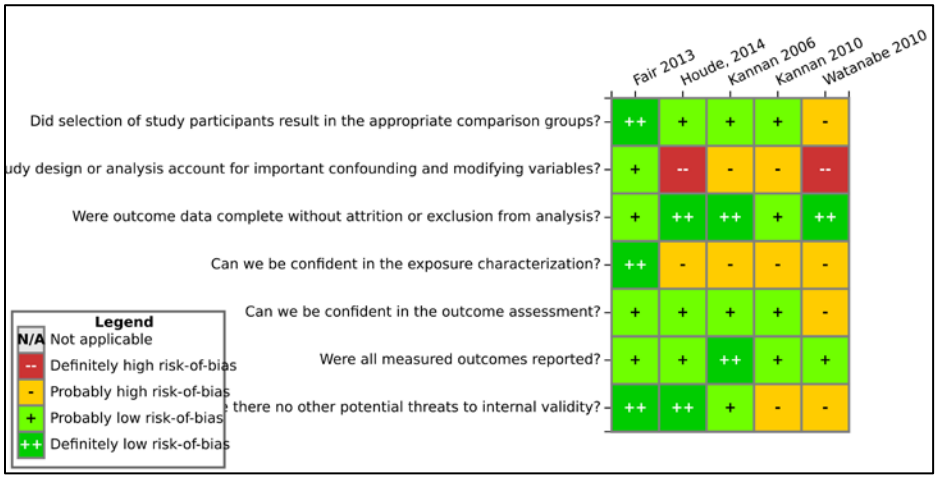
Studies in Non-human Animals

Figure A3-2. Risk of bias heatmap for all included studies in animals following PFOA or PFOS exposure



(Individual study details and risk of bias ratings in HAWC *Figure A3-2 - Animal Experimental RoB Heatmap*)

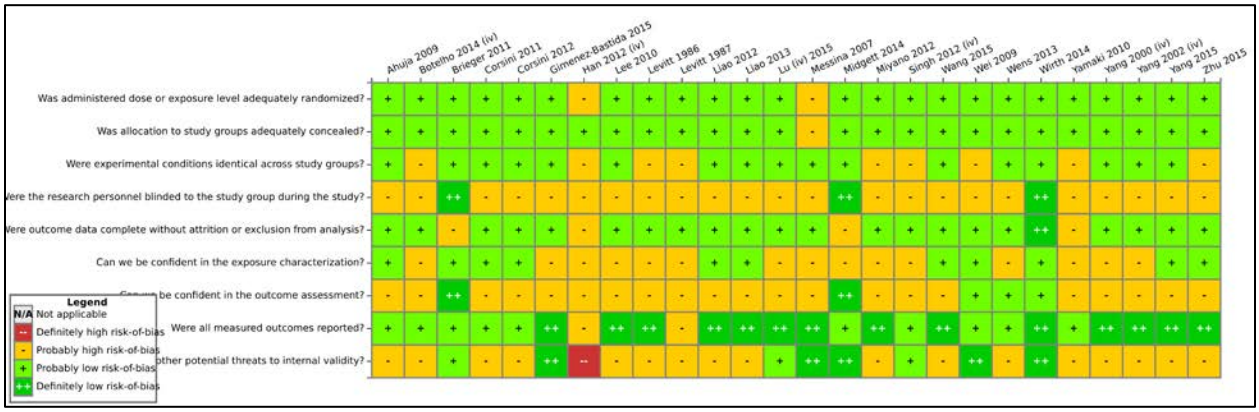
Figure A3-3. Risk of bias heatmap for all included wildlife studies with PFOA or PFOS levels



(Individual study details and risk of bias ratings in HAWC *Figure A3-3 - Animal Wildlife RoB Heatmap*)

In Vitro Experimental Studies

Figure A3-4. Risk of bias heatmap for all included in vitro experimental studies following PFOA or PFOS exposure



(Individual study details and risk of bias ratings in HAWC *Figure A3-4 - In vitro RoB Heatmap*)

Appendix 4. Additional Primary Immune Outcomes Endpoints

Delayed-type-hypersensitivity (DTH) Response

The DTH response is a measure of cell-mediated immunity important for protection against intracellular pathogens (Luster *et al.* 1992, Dietert 2010). The DTH response is dependent on antigen-specific sensitization of T cells and subsequent release of cytokines leading to inflammation and destruction of the corresponding tumor cells, fungal infected cells, or virus-infected cells. The DTH response in experimental animals is considered a direct equivalent to the tuberculin test in humans. Assays for DTH response are often included as part of immunotoxicity testing guidelines as a measure of cell-mediated immune function as part of comprehensive testing strategies (Luster *et al.* 1992, WHO 2012).

Human DTH Data for PFOA and PFOS

No human data were identified on potential association between PFOA and PFOS and the DTH response.

Animal DTH Data for PFOA and PFOS

Summary: There is very low confidence that exposure to PFOA or PFOS is associated with changes in DTH response in animals because there are very few studies and all the studies in mammals report no effect. There is only one mouse study on PFOA, one on PFOS and one rat study of PFOS (Dewitt *et al.* 2008, Lefebvre *et al.* 2008, Dong *et al.* 2011). In contrast to mammals, PFOA exposure of 2.1 mg/kg PFOA for 8 weeks was associated with suppression of the DTH response in Japanese quail (Smits and Nain 2013). This suppression contributes to very low confidence in the body of evidence suggesting more studies are necessary to conclude PFOA or PFOS have no effect on DTH across animal models or in mammals (Peden-Adams *et al.* 2009, Smits and Nain 2013). [Note: no confidence summary table was developed because the conclusion is based on few studies reporting no effect in mammals.]

Appendix 5. Observational Data

Observational data

Spleen and Thymus weight:
Changes in spleen or thymus weight may be indicative of immunosuppression as an observational measure that may be related to changes in lymphocyte numbers and potentially changes in B- and T-cell functional responses. PFOA or PFOS-related decrease in spleen or thymus weight may support reduced antibody response if it was reflected in changes in cellularity or changes in lymphocyte subpopulations and observed at the same or lower concentrations at which reduced antibody response was observed. Although there is consistent evidence for PFOA and PFOS-associated reduced relative weight of the spleen and thymus it is generally observed at higher doses than changes in the antibody response.

Spleen – relative weight examined to avoid effects of body weight and for comparability

- Consistent evidence of reduced of spleen weight for PFOA exposure in mice at higher doses (15 to 30 mg/kg/day), inconsistent below 10 mg. No effect reported in rats or monkeys (see Spleen Wt PFOA).
- Consistent evidence of reduced of spleen weight for PFOS exposure in mice at higher doses (20 to 40 mg/kg/day), most studies report reduced spleen weight at 10-20 mg/kg/day PFOS, inconsistent evidence below 5 mg. No effect reported in rats (see Spleen Wt PFOS).

Thymus - relative weight examined to avoid effects of body weight and for comparability

- Consistent evidence of reduced of thymus weight for PFOA exposure in mice at higher doses (15 to 50 mg/kg/day), inconsistent at 10 mg or below mg. No effect in rats or monkeys (see Thymus Wt PFOA).
- Consistent evidence of reduced of thymus weight for PFOS exposure in mice at higher doses (20 to 40 mg/kg/day), inconsistent below 20 mg. No effect in rats (see Thymus Wt PFOS).

Spleen and Thymus cellularity:
Changes in total cell numbers in the spleen or thymus may detect changes in lymphocyte numbers as an observational measure that may be related to immunosuppression of B- and T-cell functional responses. A PFOA or PFOS-related decrease in B-cell numbers would present a possible mechanism for reduced antibody response if it was observed at the same or lower concentrations at which reduced antibody response was observed. However, there is inconsistent evidence of changes in cellularity of the spleen or thymus and no change in cellularity was observed at doses of PFOA associated with lower antibody levels (DeWitt *et al.* 2016).

Spleen

- Inconsistent results for PFOA exposure and spleen cellularity based on available data in mice; there are no data from rats (see Spleen cellularity PFOA).
- Effect of PFOS exposure on spleen cellularity is unclear at lower doses. Consistent evidence of reduced spleen cellularity in mice at higher doses (20-40 mg/kg/day), inconsistent below 10mg. No data in rats (see Spleen cellularity PFOS).

Thymus

- Consistent evidence of reduced thymus cellularity for PFOA exposure in mice at higher doses (20-50 mg/kg/day), inconsistent below 20 mg. No data in rats (see Thymus cellularity PFOA).
- Consistent evidence of reduced thymus cellularity for PFOS exposure in mice at higher doses (20-40 mg/kg/day), inconsistent below 20 mg. No data in rats (see Thymus cellularity PFOS).

Cell Phenotyping:
Leukocyte phenotyping may detect changes key cell populations as an observational measure related to potential functional changes (e.g., macrophages, NK cells, or lymphocyte subpopulations). For example, PFOA or PFOS-related decrease in B-cell numbers would present a possible mechanism for reduced antibody response if it was observed at the same or lower exposure levels at which reduced antibody response was observed. However, there is inconsistent evidence of reduced B-cell (B220) number at higher exposure levels and no change in B-cell number was observed at doses of PFOA associated with lower antibody levels (DeWitt *et al.* 2016). Similarly, PFOS-associated changes in NK cell number were not consistent at exposure levels associated with changes in NK cell activity (Qazi *et al.* 2010a).

NK cells (CD16 or NK1.1)

- Few studies have evaluated the potential effect of PFOA and PFOS on NK cell numbers. Most studies reported no effect of PFOA (0.25 mg/kg to 7.5 mg/kg/day) or PFOS (0.25 mg/kg) in mice. Few studies and no effect of PFOA in rats (see NK cells (CD16 or NK1.1 or PanNK)).

B cells (B220 or CD16 or CD45RA)

- Effect of PFOA and PFOS exposure is unclear and mixed at lower doses. There is some evidence of reduced B-cell number at higher doses PFOA (20 mg/kg/day) or PFOS (20 mg/kg/day) in mice. Few studies and no effect of PFOS in rats (see B-cells (B220 or CD16)).

CD4

- Effect of PFOA exposure is unclear and mixed on CD4 T-cell number in spleen or thymus based on available studies in mice; there are no rat data. Effect of PFOS exposure is unclear and mixed at lower doses for both spleen and thymus. There is some evidence of reduced CD4 T-cell number at higher doses of PFOS (20 mg/kg/day) in mice. Few studies and no effect of PFOS in rats (see CD4 T-cells).

CD8

- Effect of PFOA exposure is unclear and mixed on CD8 T-cell number in spleen or thymus based on available studies in mice; there are no rat data. Similarly, the effect of PFOS exposure is also unclear and mixed on CD8 T-cell number in the spleen and thymus based on available studies in mice. Few studies and no effect of PFOS in rats (see CD8 T-cells).

DP (CD4+/CD8+)

- Effect of PFOA and PFOS exposures are unclear for spleen. There is some evidence that both PFOA and PFOS reduced CD4+/CD8+ T cells in the thymus at higher doses [e.g., ≥40 mg/kg/day PFOA and ≥20 mg/kg/day PFOS [DP(CD4+/CD8+)].

DN (CD4-/CD8-)

- Effect of PFOA and PFOS exposures is unclear for spleen or thymus at lower doses. There is some evidence that both PFOA and PFOS reduced CD4-/CD8- T cells in the spleen and thymus at higher doses [e.g., ≥40 mg/kg/day PFOA and 2 mg/kg/day PFOS; see DN(CD4-/CD8-)].

Lymphoproliferation:
B and T-cell lymphocyte proliferation is a component of several functional responses (e.g., B-cell proliferation is part of the development of an antibody response). However, non-specific proliferation (or lymphoproliferation) in response to mitogen stimulation by ConA (T-cells) or LPS (B-cells) is considered an observational endpoint because it is less predictive for immunotoxicity than actual functional immune measures. PFOA or PFOS-related decrease in B-cell proliferation would present a

possible mechanism for reduced antibody response if it was observed at the same or lower concentrations at which reduced antibody response was observed. However, reduced proliferation of B cells (LPS) is only observed at higher doses for PFOA or PFOS and not changed/no decrease at same or lower concentration than antibody effects observed.

ConA

- Effect of PFOA exposure is unclear but there are few studies and they are restricted to shorter exposure periods (i.e., maximum of 7 days). PFOS exposure appears to have no effect on ConA-induced lymphocyte proliferation at lower doses (1 mg/kg/day and lower). There is some evidence of suppressed ConA-induced lymphocyte proliferation at higher doses of PFOS (5-20 mg/kg/day) in mice. No data in rats (see Proliferation to ConA)

LPS

- Effect of PFOA and PFOS exposure is unclear and mixed at lower doses. There is some evidence of suppressed LPS-induced lymphocyte proliferation at higher doses PFOA (20 mg/kg/day) or PFOS (20 mg/kg/day) in mice. No data in rats (see Proliferation to LPS)

Cytokines:

Cytokine levels and cytokine release have been studies in multiple tissues and multiple culture conditions (e.g., with and without ConA stimulation). Given the heterogeneity in study design, tissues, and cell populations investigated it is difficult to evaluate whether or not there is a clear or consistent pattern for changes in these cell signaling molecules after exposure to PFOA or PFOS. However, in a pair of studies by the same research group, there is evidence that PFOS exposure was associated with a shift in cytokine balance away from TH_1 cytokines (reduced secretion of IL-2 and INFγ) and towards TH_2 cytokines (increased secretion of IL-4) in mice exposed to higher doses (0.833 to 20 mg/kg/day) (Dong *et al.* 2011, Zheng *et al.* 2011).

- IL1 – No data on PFOA. For PFOS, there are multiple studies (most by the same group) and the generally support increased IL-1 secretion. Studies cover mixed conditions, culture, spleen and peritoneal cavity, etc. (see Cytokine - IL1).
- IL2 – No data on PFOA. For PFOS, there are two studies and they support suppression at higher doses (0.833 to 20 mg/kg/day) (see Cytokine - IL2).
- IL4 – Two studies on PFOA with cells from the liver. For PFOS, there are multiple studies (most by the same group) and they generally support increased IL-4 at higher doses in splenocytes (0.833 to 20 mg/kg/day). Also several studies of liver with no effects (see Cytokine - IL4).
- IL5 – Data are from a single study of PFOS reporting no changes in IL-5 (see Cytokine - IL5).
- IL6 – Some evidence of increased IL-6 with PFOA exposure with different results by culture conditions and tissue type. For PFOS, there are inconsistent results across multiple culture conditions and tissues (see Cytokine - IL6).
- IL10 – Two studies on PFOA with inconsistent results that differ by sex. Two studies for PFOS, with inconsistent results (see Cytokine - IL10).
- IFN-γ– Two studies on PFOA from liver cells with inconsistent results but differed by culture conditions. For PFOS, there are multiple studies (by the same group for splenocyte studies) and they support increased decreased IFN-γ at higher doses (0.833 to 20 mg/kg/day). Also several studies of liver with inconsistent results (see Cytokine - IFN gamma).
- TNF-α – Multiple studies on PFOA with reduced secretion in splenocytes, and different results but across culture conditions and tissues spleen, serum, peritoneal cavity. For PFOS, there are multiple studies (most by the same group) and they generally support increased TNF-α at higher doses (0.833 to 20 mg/kg/day) (see Cytokine - TNF alpha).

Appendix 6. In Vitro Data

NK Cell Activity After In Vitro Exposure:

Two studies were identified that evaluated NK cell activity using cells of different origins and *in vitro* exposure to PFOA or PFOS: 1) a study using human peripheral blood and 2) a study using bottlenose dolphin peripheral blood as well as mouse spleen cells. Study details and results are included in the discussion of NK cell activity results and evidence synthesis above (see **Figure D24**). In brief, NK cell activity of peripheral blood cells was reduced after *in vitro* exposure to PFOS at the highest concentration 100000 ng/ml PFOS, and PFOA had no effect at the on NK cell activity at the concentrations tested (0, 1000, 10000, or 100000 ng/ml PFOA, (Brieger *et al.* 2011). *In vitro* exposure to PFOS (0 to 5000 ng/ml) had no effect on NK cell activity of the dolphin cells (Wirth *et al.* (2014). NK cell activity from the mouse spleen cells (n = 15 mice per dose group) was decreased by 30-45% at PFOS concentrations from 10-1000 ng/ml and increased 450% at 5000 ng/ml PFOS.

Cytokines After In Vitro Exposure:

Cytokine release with in vitro exposure to PFOA or PFOS has been examined in several studies using both human cells and non-human animal cells. As with *in vivo* exposure studies, there is considerable variation in cell sources and culture conditions (e.g., with and without ConA stimulation). Given the small number of studies and heterogeneity in cell sources and study design, it is difficult to evaluate whether or not there is a clear or consistent pattern for changes in these cell signaling molecules after exposure to PFOA or PFOS.

- IL4 – One study examined IL-4 release with PHA stimulation from human peripheral blood leukocytes cultured with exposure to PFOA and PFOS (0, 100, 1000, and 10000 ng/ml) (Corsini *et al.* 2011). The highest concentration of PFOA (10000 ng/ml) reduced IL-4; and all concentrations of PFOS (100-10000 ng/ml) reduced IL-4 (see In vitro cytokine - IL4).
- IL6 – Multiple studies investigated IL-6 following *in vitro* exposure to PFOA or PFOS. *In vitro* exposure of human peripheral blood to PFOA had no effect on IL-6 with or without PHA or LPS stimulation (Brieger *et al.* 2011, Corsini *et al.* 2011, Corsini *et al.* 2012). There is conflicting evidence for effects of *in vitro* exposure to PFOS on IL-6 released from leukocytes in human peripheral blood. Brieger *et al.* (2011) reported that *in vitro* PFOS exposure 1000-100000 ng/ml had no effect on basal, PHA-, or LPS-stimulated IL-6. In contrast, Corsini (2011, 2012) reported decreased IL-6 release from 100-10000 ng/ml PFOS from human peripheral blood. Microglia are resident macrophage-like cells that function in the brain and central nervous system. In contrast to decreased IL-6 in other immune cells, Zhu *et al.* (2015) reported *in vitro* exposure to PFOS resulted in increased IL-6 release from mouse microglial cells (see In vitro cytokine - IL6).
- IL8 – One study examined IL-8 release with LPS stimulation from human peripheral blood leukocytes and THP-1 cell line cultured with exposure to PFOA and PFOS (Corsini *et al.* 2011). Concentrations from 100 to 10000 ng/ml PFOA and PFOS had no effect on IL-8 release human peripheral blood leukocytes. However, using the THP-1 cell line, 100000 ng/ml PFOA and 1000 to 1000000 ng/ml PFOS reduced IL-8 (see In vitro cytokine - IL8).
- IL10 – One study examined IL-10 release with PHA stimulation from human peripheral blood leukocytes cultured with exposure to PFOA and PFOS (0, 100, 1000, and 10000 ng/ml) (Corsini *et al.* 2011). The highest concentration of PFOA (10000 ng/ml) reduced IL-10; and all concentrations of PFOS (100-10000 ng/ml) reduced IL-10 (see In vitro cytokine - IL10).
- IFN-γ– Two studies from the same group (Corsini *et al.* 2011, Corsini *et al.* 2012) examined IFN-γ release with PHA stimulation from human peripheral blood leukocytes cultured with exposure to

PFOA and PFOS (0, 100, 1000, and 10000 ng/ml). PFOA had no effect, and all concentrations of PFOS (100-10000 ng/ml) reduced IFN-γ (see In vitro cytokine - IFN gamma).

- TNF-α –Multiple studies investigated TNF-α following *in vitro* exposure to PFOA or PFOS. TNF-α was positively correlated with plasma levels of PFOA and PFOS, but *in vitro* exposure of human peripheral blood to PFOA had no effect on TNF-α with or without PHA or LPS stimulation (Brieger *et al.* 2011). In contrast, (Corsini *et al.* 2011) found that PFOA at 10000 ng/ml decreased TNF-α and PFOS at 100 ng/ml and higher decreased TNF-α from human peripheral blood. Reduced TNF-α secretion was also reported with PFOS exposure for mouse macrophages (Miyano *et al.* 2012). Microglia are resident macrophage-like cells that function in the brain and central nervous system. In contrast to decreased TNF-α in other immune cells, several studies reported *in vitro* exposure to PFOS resulted in increased TNF-α release from rodent microglial cells (Yang *et al.* 2015, Zhu *et al.* 2015) (see In vitro cytokine - TNF alpha).

National Toxicology Program
NTP Central Data Management, MD K2-05
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

http://ntp.niehs.nih.gov