Australian National University

The PFAS Health Study

Systematic Literature Review

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Abbreviations

17-HP	17 [alpha]-hydroxyprogesterone
ADHD	Attention deficit hyperactivity disorder
AFFF	Aqueous film forming foams
ALSPAC	Avon Longitudinal Study of Parents and Children
ANOVA	Analysis of variance
APFO	Ammonium perfluorooctanoic acid
APGAR	Appearance, Pulse, Grimace, Activity and Respiration
BMI	Body mass index
BraMat	A sub-cohort of the MoBa study
cm	Centimetres
CI	Confidence interval
df	Degrees of freedom
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DM	Diabetes mellitus
DNBC Study	Danish National Birth Cohort
EFSA	European Food Safety Authority
eGFR	Estimated glomerular filtration rate
EPA	Environmental Protection Authority
Et-PFOSA-AcOH	2-(N-ethylperfluorooctanesulfonate) acetic acid
EBGC Study	Ewha Birth and Growth Cohort
e2	Oestradiol
FAI	Free androgen index
FLEHS	Flemish human environmental health survey
FSH	Follicle stimulating hormone
FT	Free testosterone
g	Grams
GBCA Study	Genetics and Biomarkers study for Children and Adolescents
GDM	Gestational diabetes mellitus
GFR	Glomerular filtration rate
GM	Geometric mean

GP	General practitioner
HDL	High density lipoprotein
Hib	Haemophilus influenzae type B
Hokkaido Study	Hokkaido Study on Environment and Children's Health
HOMES Study	Health Outcomes and Measures of Environment Survey
HOMA-IR	Homeostatic model assessment - insulin resistance
HR	Hazard ratio
IgE	Immunoglobulin E
IGF1	Insulin-like growth factor-1
INUENDO	INUit-ENDOcrine Study – Biopersistent organochlorines in diet and human fertility
IQR	Interquartile range
IVF	In-vitro fertilisation
kg	Kilograms
LDL	Low-density lipoprotein
LGA	Large for gestational age
LH	Luteinizing hormone
LIFE Study	Longitudinal Investigation of Fertility and the Environment
LINC	Linking Endocrine Disrupting Hormones in Maternal Nutrition to Child Health Study
Me-PFOSA-AcOH	2-(N-methyl-perfluorooctanesulfonate) acetic acid
MIREC	Maternal-Infant Research on Environmental Chemicals
MISA	The Northern Norway Mother-and-Child contaminant Cohort Study
MoBa Study	Norwegian Mother and Child Study
n	Number of participants
NHANES Study	National Health and Nutrition Examination Survey
NSW	New South Wales
OBELIX	Obesogenic endocrine disrupting chemicals: linking prenatal exposure to the development of obesity later in life
OR	Odds ratio
p	Probability value
P	Proline
PFAS	Per- and poly- fluoroalkyl substances
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutane sulfonic acid

PFDA	Perfluorodecanoic acid
PFDoA	Perfluorododecanoic acid
PFDS	Perfluorodecane sulfonic acid
PFHpA	Perfluoroheptanoic acid
PFHpS	Perfluoroheptane sulfonic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PFOSA	Perfluorooctanesulfonamide
PFPeA	Perfluoropentanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFUdA	Perfluoroundecanoic acid
PI	Ponderal index
POSF	Perfluorooctanesulfonyl fluoride
PRL	Prolactin
Q	Q Statistic
Qld	Queensland
Q1	Quartile 1
Q4	Quartile 4
RR	Relative risk
SD	Standard Deviation
SE	Standard error
SGA	Small for gestational age
SHBG	Sex hormone binding globulin
SIR	Standardised incidence ratio
SMR	Standardised mortality ratio
T	Testosterone
T1	Tertile 1
T3	Tertile 3
T_3	Triiodothyronine

T_4	Thyroxine
TC	Testosterone cypionate
Tg	Thyroglobulin
TG	Triglyceride
TPOAb	Thyroid peroxidase antibody
TSH	Thyroid stimulating hormone
TTP	Time to pregnancy
US	United States
VLDL	Very low density lipoprotein

Plain Language Summary

This review examined all published research into the human health effects of exposure to perfluoroalkyl and polyfluoroalkyl substances, commonly known collectively as PFAS. PFAS chemicals are very resistant to heat and to degradation in the environment, and they persist for quite long periods in the human body. They were extensively used in fire-fighting foams, which were commonly used in fire drills at airports, and in household products, such as protective coatings on furniture and non-stick surfaces on cookware. PFOS (perfluorooctane sulfonic acid) and PFOA (perfluorooctanoic acid) were the two most commonly used PFAS chemicals.

We reviewed research published up until February 7th 2017. We found 221 separate scientific publications that reported new results of relevant research in humans. These publications covered effects on reproduction, on pregnant women and their newborn babies, on body metabolism, on major body systems, including brain and nerves, heart and blood vessels, airways and lungs and the immune system, on specific conditions such as overweight, diabetes and cancer, and on thyroid gland function. The people they studied included people who worked in plants manufacturing these chemicals, firefighters, people with higher than usual exposure because of contamination of water supplies and people in the general community, whose exposure was ascertained by measuring PFAS chemicals in their blood.

We found sufficient evidence that higher levels of PFOS or PFOA in a person's blood can lead to higher blood cholesterol levels. High blood cholesterol is associated with heart disease. PFOS and PFOA, however, appeared only to increase cholesterol levels by a small amount.

We found limited evidence that higher levels of PFAS in the blood resulted in slightly higher levels of uric acid in the blood. Uric acid is a normal body product and is removed by the kidneys. In a small number of studies, however, we also found limited evidence that high PFAS levels in the blood reduced kidney function or were associated with chronic kidney disease. Since PFAS chemicals are excreted by the kidneys it is possible PFAS does not cause poor kidney function, rather that poor kidney function caused by something else causes increase in PFAS levels in blood. This possibility of "reverse causation" might also explain the association of higher uric acid levels with higher PFAS levels in blood.

We found limited evidence in a small number of relevant studies that PFAS exposure caused kidney and testicular cancers and that higher levels of PFAS in the blood resulted in lower levels of antibodies than usual following vaccination against some vaccine preventable infections.

We found inadequate evidence that PFAS caused other health effects.

While there have been many studies into the health effects of PFAS it is uncertain whether or not PFAS are harmful to human health. In the few areas in which there is evidence for a possibly causal association of PFAS with an effect on human health the association is either uncertain or apparently weak.

Executive Summary

Background

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a diverse family of fluorinated organic chemicals that have been produced since the 1950s. The most commonly known PFAS are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). The high chemical, thermal and biological stability of these substances have led to their use in a wide variety of consumer products and industrial applications, including fire-fighting foams.

The extensive use, distribution, manufacture and disposal of PFAS in the 1950s through to the 2000s has resulted in widespread exposure of humans, animals and the environment. The general population is exposed to background levels of PFAS through food, drinking water and house dust. As a result, PFAS can be detected in blood serum samples of the general human population. Certain populations have higher levels of PFAS exposure, particularly those who have produced or worked with these chemicals. Some communities living in environments that have experienced exceptional contamination may also be exposed to high levels of PFAS. Once absorbed into the body, PFAS bio-accumulate and may take years to be excreted from the body.

Epidemiological studies have investigated a range of possible health outcomes resulting from exposure to PFAS. However, the public health significance of the chemical exposure remains unclear. In this report, we systematically review the health literature to describe currently known human health effects of PFAS chemicals and examine the consistency of evidence regarding the relationship between exposure to PFAS and different health outcomes.

Key findings

We conducted a comprehensive search of the health and grey literature published up until February 7th 2017 for reports of the results of research into the association of human exposure to PFAS chemicals with health outcomes using standard search terms and common reference databases. We found 221 papers that met the systematic review criteria examining 148 different individual health outcomes in 12 main categories of health effects:

- Neonatal, infant and maternal outcomes;
- Reproductive outcomes;
- Metabolic outcomes;
- Thyroid outcomes;
- Neurodevelopmental and neurophysiological outcomes;
- Cancers;
- Diabetes;
- Cardiovascular outcomes;
- Overweight and outcomes;
- Immunological outcomes;
- Skeletal outcomes; and
- Respiratory outcomes

We used a systematic framework to review each paper, extract data and rate the risk of potential bias. We considered whether it was possible to pool study results in a meta-analysis where we identified five or more studies on a particular health outcome.

Of the 148 health outcomes investigated, we found *sufficient evidence* of an association between two PFAS chemicals and elevated blood cholesterol. The consequent increase in blood cholesterol from PFAS exposure is likely to be low. We found *limited evidence* of an association between two PFAS chemicals and seven health effects, namely high blood uric acid concentration, impaired glomerular filtration rate, chronic kidney disease, kidney cancer, testicular cancer and impacts on vaccine derived immunity for diphtheria and rubella. The overall body of evidence (number of relevant studies) for the metabolic outcomes was much greater, than for the renal outcomes, cancers or effects on vaccination.

PFOA and PFOS were associated with higher blood uric acid levels (hyperuricemia). Six of seven studies reported that PFOA exposure was positively associated with uric acid levels. Similarly, four of six studies reported that elevated blood PFOS levels were associated with hyperuricemia. Results for both were significant in adults and in children and adolescents. We were unable to pool study results in a meta-analysis.

PFOA was associated with kidney cancer in two out of six relevant studies and with testicular cancer in two out of five relevant studies. These findings were statistically significant or marginally so in several studies of both cancers and showed evidence of a dose-response relationship for both cancers.

For immunological effects of PFAS exposure, there was evidence of inverse associations between PFAS chemicals and antibody levels of diphtheria and rubella after vaccination of children and adults, although this was from a very small number of studies.

We found *inadequate evidence* for a health effect for the majority of individual health outcomes, including reduced infant birth weight. We were able to conduct meta-analyses on a restricted number of studies of birth weight, birth length and infertility, which did not change our conclusions about the inconsistency of findings from published papers. In many instances, there was a large body of evidence in support of lack of any association.

The majority of studies we included in this review were evaluated to have a moderate to high risk of bias that could have influenced published findings. It is important to consider some of the limitations of literature reviews, including this one, which include: potential to miss recently published papers, difficulties in accessing international government reports, differences in the nature of exposed populations, and variability in the nature of reviews conducted when a number of reviewers were used.

The health effects of PFAS have been extensively studied. However, there is still a need for high-quality research to increase certainty of the findings for outcomes for which there is suspicion but limited or inconsistent findings. In our review of 221 papers, we found sufficient evidence of an association of PFOA and PFOS with hypercholesterolemia, limited evidence of an association of PFOA and PFOS exposure with hyperuricemia, impaired glomerular filtration rate, chronic kidney disease, antibody response to diphtheria and rubella vaccination, and kidney and testicular cancer. Our review highlights additional areas for epidemiological research to further our understanding of the health effects of these chemicals.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a diverse family of fluorinated organic chemicals that have been produced commercially since the 1950s. (1) PFAS have a carbon backbone with one or more fluorine substitutions and functional end groups which provide specific properties. (2) The carbon-fluorine bond is extremely strong, (3) giving PFAS high chemical, thermal and biological stability. The fluorinated carbon tail is hydrophobic as well as oleophobic, while the functional end group is hydrophilic, resulting in substances with highly effective surface tension-lowering properties. Perfluoroalkyl acids have all hydrogen atoms substituted with fluorine, while polyfluoroalkylacids have one or more but not all hydrogen atoms substituted with fluorine.

Due to their stability and hydrophobic and lipophilic properties, PFAS have been used in a wide variety of consumer products, including surface treatments for textiles, non-stick coatings for cookware, grease-repellent food packaging and paints; and in industrial applications, such as in the metal plating industry, in hydraulic fluids and as key ingredients in aqueous film forming foams (AFFF). The AFFF have been used extensively as flame-retardants for firefighting, particularly in aviation settings. The extensive use of these chemicals and their persistence has led to concerns about environmental and human health impacts.

The general population is exposed to background levels of PFAS through food consumption (mainly dairy products, fish and meat), drinking water and house dust. (4-6) The extensive use, distribution, manufacture and disposal of PFAS chemicals, and their effects on wildlife and humans (7-12), has resulted in substantial scientific investigation, community concern and publicity in the media. Since the early 2000s, major manufacturers have phased out production of key long-chain PFAS compounds, although it is likely that substantial production of PFAS still occurs in low- and middle-income countries. (2, 13, 14)

Concerns over the potential for PFAS to adversely affect human health arise from the ease that they are absorbed into and distributed through the body. PFAS can be detected in blood serum samples from the general human population. (9, 15, 16) The most widely studied PFAS chemicals are perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid also known as perfluorooctanesulfonate (PFOS). People in communities with high levels of PFOS and/or PFOA in their drinking water have been found to have serum PFOA and PFOS concentrations above those reported for the general population. (17-19) PFAS may be passed to infants through breastmilk. (20) Additionally, prenatal exposure to PFAS can occur through the placenta. (21)

PFOA and PFOS bind to serum proteins, especially albumin, with high affinity. (22-24) PFAS bioaccumulate once absorbed into the blood stream via digestive and gas-exchange pathways, due to their biochemical stability. The distribution of PFAS in tissue is not only species and sex dependent, but also dependent on the characteristics of the ‘head’ chemical, the length of the perfluoroalkyl chain (the ‘tail’) and the exposure dose. (25-27) The PFAS ‘head’, or foundation chemical, has an electric charge, either positive or negative. The charge determines what chemicals will attach to it. The chemicals that attach to the ‘head’ regulate where in the body the PFAS will bioaccumulate. Generally, PFAS tend to accumulate in tissues with a large blood supply, including the liver, kidneys and lungs. (28)

One of the concerns with PFAS chemicals is the length of time they remain in the human body. The half-life of a substance in the body is the length of time required for the body to eliminate half of the substance by normal physiological processes. The biological half-life in human serum varies with the type of PFAS, with estimates of 3.8 years for PFOA, 5.4 years for PFOS and 8.5 years for perfluorohexane sulfonate (PFHxS). (29) However, ongoing background exposure to PFAS makes it difficult to estimate the half-lives of these chemicals. Studies in the United States, have shown substantial declines in PFAS concentrations since the early 2000s, when industries began phasing-out production of these PFAS chemicals. (30)

The potential health effects of PFAS exposure have been studied in animals and humans. Information from animal studies, which generally use high oral doses of PFAS, have indicated potential effects on pre- and postnatal development and the liver and immune system. Studies into reproductive effects have indicated that the effects depend on the specific PFAS chemical studied. Importantly, there are considerable differences in the toxicokinetics of PFAS in different animal species and humans, and the half-life of PFAS in some animals is much shorter than in humans. (31)

The toxicity of PFAS in humans is poorly understood, although PFAS with longer carbon chains are thought to be more toxic than those with shorter carbon chains. (31, 32) The majority of human health investigations have been on PFOA, PFOS and to a lesser extent, PFHxS. There have been a limited number of studies examining the burden of PFAS in the human body, with studies showing accumulation in the liver, spleen, kidney and lungs. There have been a range of proposed mechanisms for adverse health effects of PFAS, many of which relate to endocrine disruption potentially affecting male and female reproduction and thyroid function. (33, 34) Human health research into PFAS has focused on: reduced foetal growth and development, decreased fertility and reproductive hormone levels, increased cholesterol levels, negative immunological effects and cancer.

Key studies on human health

PFAS chemicals have been used for industrial and domestic purposes since the 1950s. However, minimal attention was given to their potential effects on human health until the early 2000s. (2) A growing awareness of the chemicals' potential toxicity and accumulative nature has resulted in a dramatic increase in epidemiological research into them. Despite the advancement of scientific methods of testing for PFAS concentrations in blood and increased public interest in understanding health effects, findings remain largely inconsistent. (20)

PFOA and PFOS have been the most commonly studied PFAS, although many studies have investigated exposure to a wider range of PFAS, including PFHxS and perfluorononanoic acid (PFNA). Research into the health effects of PFAS has been conducted mainly through cross-sectional and cohort studies, with a large number of earlier studies using data from existing longitudinal studies. These include the C8 Health Project, the National Health and Nutrition Examination Survey (NHANES), and the Health Outcomes and Measures of Environment (HOME) Study, all conducted in the United States. European studies include the Danish National Birth Cohort (DNBC) and the Inuit-Endocrine (INUENDO) Cohort Study. In addition, there have been many studies on PFAS health effects from birth cohort studies in Japan and Taiwan. Some studies have reported on human exposure to PFAS at different ages, including the blood concentration in neonates.

The C8 Health Project was established in 2005 as the result of a settlement from a class action lawsuit against the DuPont Company. (35) The DuPont Works Plant in Parkersburg, USA, contaminated the mid-Ohio river with PFOA from the 1950s. The epidemiological study was conducted from 2005-2013 on approximately 69,000 people living in Ohio and West Virginia who received contaminated drinking water. The study provided a large pool of data from a population that was exposed to PFOA over an extended period of time. Interviews, questionnaires and blood samples were collected from residents allowing the investigation of a range of health outcomes. The C8 Health Project Science Panel suggested the following health effects may be associated with exposure to PFOA and related chemicals: hypercholesterolemia; pregnancy-induced hypertension; thyroid disease; testicular and kidney cancer; and ulcerative colitis. (35)

The NHANES study is another key study dataset for examining the association between PFAS exposure and health outcomes. Operating since the 1960s, the NHANES annually assesses the health and nutritional status of approximately 5,000 adults and children from a nationally representative sample in the United States of America. Blood samples collected as part of NHANES have been tested for PFAS since 1999, with the exception of 2001 to 2003. (36) The repeated cross-sectional nature of NHANES has allowed for assessment of time trends in PFAS levels in the population. In addition, NHANES allows assessment of the relationships of much lower levels of PFAS with different health outcomes than has been possible in studies of highly exposed populations.

Many birth cohorts have been used to study the health effects of PFAS. The HOME Study is one example. Established in the early to mid-2000s, it is a prospective pregnancy and birth cohort study in metropolitan Cincinnati, Ohio, designed to determine whether environmental exposure to chemicals, such as PFAS, in early life would affect children's health. (37) The DNBC began in 1996 with interviews conducted with women twice during pregnancy and again when their children were 18 months and six years of age. (38) Blood samples were collected from maternal blood twice during pregnancy, and umbilical cord blood shortly after birth. By 2002, 100,000 participants had been registered with the study and follow-up interviews were conducted at seven and 11 years after the initial data collection. (39) The INUENDO Cohort Study was a birth cohort formed from four European populations—Sweden, Poland Ukraine and Greenland—that began in 2002 with over 2000 pregnant women participating. Questionnaires on exposure and blood samples were collected from the participating children and their parents. (40) Other important birth cohorts have been conducted in countries Taiwan, Japan and Korea.

Previous systematic reviews

Several systematic reviews of the effects of PFAS on specific health outcomes have been conducted, mostly focusing on foetal growth and health outcomes in children. (21, 41-47) These have included:

- Foetal growth (3 studies)
- Neurodevelopmental and neurobehavioural outcomes (1 study)
- Health outcomes in children (1 study)
- Immunological health conditions (1 study)
- Cancer risk (1 study)
- Thyroid function in pregnant women and children (1 study)

Of these eight reviews, three focussed specifically on the relationship between PFOA and foetal growth, with one also examining PFOS. (21, 41, 42) In two related reviews, Johnson et al. (21) and Lam et al. (42) examined 18 human studies as part of a novel systematic review methodology incorporating evidence from human epidemiological studies and animal studies—the Navigation Guide. They concluded there was sufficient evidence that PFOS exposure reduces foetal growth, but indicated that the magnitude of reduction may not be clinically significant. Bach et al. (41), reviewed 14 studies and concluded that there was insufficient data to confirm a link between PFOA and PFOS exposure and impaired foetal growth. They found that there was inconsistent evidence of a link with PFOS, with some studies showing an association and others finding none. The results for PFOA were more consistent with all studies reviewed showing reduced foetal growth, although not all associations were statistically significant.

Roth and Wilks (46) reviewed eight studies focussing on the relationship of PFAS with neurodevelopmental and neurobehavioural outcomes in children and infants and concluded that the evidence did not support a link between the chemicals and these outcomes. Rappazzo et al. (47) identified 64 studies looking at the relationship between prenatal and/or childhood exposure to PFAS and health outcomes in children. They examined six categories of health outcomes, including: immunological, cardiometabolic, neurodevelopmental, thyroid, renal and reproductive effects. The authors concluded there was evidence to suggest an association between increased PFAS levels in children and dyslipidaemia (high lipid levels in the blood), asthma diagnosis, decreased adaptive immune response, reduced renal function, and older age at menarche.

Chang et al., (45) reviewed 24 papers looking at the relationship between exposure to PFOA and PFOS and several immune-related health outcomes including: biomarker levels or gene expression patterns, atopic or allergic disorders, infectious diseases, vaccine responses, and chronic inflammatory or autoimmune conditions. Due to the number of conditions studied and the few papers in some categories, the authors were unable to reach a conclusion about a causal relationship between PFOA and PFOS and any immune-related health outcome.

Chang et al. (44) assessed 18 papers looking at the association between PFOA and PFOS and cancer incidence and mortality. The results were inconclusive with some studies finding positive associations for prostate, kidney, testicular and thyroid cancers, and others inverse

associations. Many of the positive associations were found in community-based settings, but not in occupationally-exposed workers, which could suggest the findings may have been due to chance, bias or uncontrolled confounding. The authors concluded that the evidence does not support a causal association between exposure to PFOA and PFOS and cancer in humans.

Ballesteros et al. (43) reviewed 10 studies examining the relationship between PFAS and thyroid function in women (*n*=7), children (*n*=2) and in both groups (*n*=1). The study found some evidence of a positive association between PFHxS and PFOS exposure and concentrations of Thyroid Stimulating Hormone (TSH) in maternal blood. They also found that increased PFNA levels in boys aged 11 years or older were associated with increased TSH in blood.

The present study

In this study, we systematically review the existing literature reporting possible human health effects of exposure to PFAS chemicals. This review compiles and synthesises published and unpublished studies into the human health effects of PFAS. The study was designed to assist in identifying potential health-effect targets for future epidemiological studies and to elucidate gaps in knowledge. The Australian Government Department of Health funded the Australian National University to conduct this independent study as part of the PFAS Health Study.

Methods

The systematic review followed a research protocol that defined the research questions, identified search terms and databases for the scientific and grey literature searches, and specified how recovered literature was reviewed. The protocol was developed in consultation with a research librarian to ensure a comprehensive literature search.

Research question

In this systematic review, we had one main research question:

- Is exposure to the PFAS group of chemicals associated with the development of human health outcomes?

Search strategy

The search strategy closely followed the PRISMA (2009) flow design (Appendix 1). Two independent reviewers searched databases containing scientific literature during 23 January 2017 through to 7 February 2017.

Search terms

The search strategy explored the electronic databases and grey literature using the following search terms:

1. Search Line 1 – Chemical Search Terms

Pfas OR pfoa OR pfos OR pfhxs OR polyfluoroalkyl* OR perfluoroalkyl* OR perfluorooctan* OR perfluorohexan*

2. Search Line 2 – Participant Search Term

Human*

3. Search Line 3 – Exposure and Outcome Search Terms

Expos* OR health* OR outcome* OR disease*

4. Search Line 4 – 5 – Exclusion Search Terms

NOT foetal alcohol OR fetal alcohol

NOT malaria

Databases used

The following electronic databases were searched:

- CAB Abstracts through Ovid platform
- Cochrane Library
- PubMed including Medline
- SciFinder Scholar
- Scopus
- Web of Science
- Wiley Online Library

Grey literature sources

Two independent reviewers searched the grey literature (listed below) during February and March 2017. The search was completed on 15 March 2017. A third reviewer assisted the search on the Swedish Environment Protection Agency pages as the results were published in Swedish.

The following lists the grey literature searched:

- Australian Government Department of Defence
- Australian Government Department of Health
- New South Wales (NSW) Department of Health
- NSW Department of Primary Industries
- NSW Environment Protection Authority
- Queensland (Qld) Department of Agriculture and Fisheries
- Qld Department of Health
- United States (US) Environmental Protection Agency
- US Centers for Disease Control and Prevention
- US Department of Health and Human Services
- C8 Science Panel
- National Research Council of Canada
- World Health Organization Library Database
- Swedish Environmental Protection Agency
- European Food Safety Authority
- Google indexed web sites

The grey literature search provided additional research results for inclusion in the systematic review. Excluding the C8 Science Panel website, examination of the 14 remaining websites selected for this review yielded 2005 document returns. Most of these were fact sheets or health advisories that were excluded after initial examination. The NSW Environment

Protection Authority site returned results for more than 500 documents or pages; however, the search facility only allowed the first 100 to be viewed. The Google search returned over 400,000 results; however, only the first 22 pages, with 10 results per page were able to be viewed. Twenty-four papers were selected for further assessment to determine their suitability for inclusion in this study. The majority of those 24 papers comprised environmental and/or exposure reports on specific sites of interest for PFAS chemicals; reports on early findings for the 3M and DuPont workers which were published subsequently, or reports containing literature reviews on the health effects of PFAS exposure. Two reports by Morel Symons et al., (48, 49) were not found in the peer reviewed literature, but were determined to be relevant to this study.

All reports on the C8 Science Panel website were considered to be relevant and were assessed for eligibility. This website separately listed 'Study publications' and 'Probable links reports'. The list of study publications was hand-searched to ensure that all relevant publications had been captured for this review; no new papers were found. The list of probable links reports was also hand-searched to uncover any previously non-identified studies. The listed studies were cross referenced with the initial review database to identify 14 additional studies for inclusion.

Inclusion & exclusion criteria

Participants and exposure

The systematic review focused specifically on human health outcomes. Studies reporting on animal-related outcomes or environmental effects were excluded if there was no statement of findings relevant to human PFAS chemical exposure.

Methodology and outcomes

Studies were excluded from the review if there was no evidence that the study was primarily epidemiological research on human health or examined quantitatively the association between PFAS exposure and some health or health-related effect. Studies that estimated PFAS exposure for a population based on occupation or residential location only were included in the systematic review, but excluded from the quantitative meta-analyses due to the difficulty of aligning their results with those of studies that used directly measured PFAS levels.

Full-text reports

Only full-text reports were used in this review. All references identified through the database searches were exported into the citation database EndNote X7.4™.

Data collection and screening

For quality control purposes, two reviewers independently completed every stage of the collection and screening process. The reviewers then compared their results to ensure that all relevant papers had been included in the systematic review. A flow diagram of the search strategy and results at each stage can be seen in Appendix 1.

Hand-searching of included literature

The final step in locating all relevant literature was to hand-search the reference lists of each paper reviewed for this study and of any relevant systematic review paper identified during the initial search for this study to ensure that all possibly relevant literature was identified for inclusion or exclusion. Systematic reviews themselves were excluded from our review process in the belief that we would cover individually all relevant primary reports that any previous systematic review had covered.

The hand-searching identified an additional 13 papers that were potentially eligible for inclusion in the review. These papers were reviewed for eligibility in the same way as all other papers were reviewed; four were excluded by this process and the remaining nine were incorporated into this study.

The hand-searching process also identified many grey literature reports from the EPA, EFSA, 3M and DuPont. The reports that could be located were also assessed for eligibility, but most were excluded from this review as they were reviews and not epidemiological studies in their own right. Four reports were reviewed for eligibility, three were excluded as ineligible and the remaining paper was included in this study.

Quality assessment

Study design and risk of bias assessment

Full-text studies eligible for review were evaluated based on their study design. A set of 'risk of bias' tools developed by Professor Bruce Armstrong and Professor Dianne O'Connell for *Clinical Practice Guidelines: PSA testing and early management of test-detected prostate cancer* (50), was further developed for use with eligible studies on PFAS. These risk of bias assessment tools were developed in 2013 and are the first published set of risk of bias tools designed specifically for the evaluation of classical observational epidemiological studies. Other commonly-used tools for assessing the potential for bias to influence study results in systematic reviews were developed for use with studies of clinical interventions and were considered unsuitable for this study.

We reviewed and revised the 2013 tools for use in this study and enhanced the nested case-control study tool and developed a case-cohort study tool. We developed table templates for each study type (content summarised in narrative form in Appendix 2) which corresponded closely to that study type's risk of bias assessment tool. This enabled recording of the risk of bias assessments simultaneously with extraction of other study information from each scientific paper under review. The tools are included in Appendix 3 through to Appendix 6.

Analysis of quantitative results

The statistical findings of each study were evaluated and compared based on continuous or categorical measurements of PFAS concentrations in blood serum. By categorical we mean numerical measurements that had been categorised for analysis. The study team developed criteria for the evaluation and data to include in summary tables. The minimally (raw values) and maximally adjusted results for each study were recorded in separate tables.

For each study using a categorical measurements of PFAS, the calculated point estimate (including the odds ratio (OR), relative risk (RR), hazard ratio (HR), difference in means or

regression coefficient) was recorded for each PFAS quantile, including the upper and lower confidence intervals. Under the circumstances, where studies did not report PFAS exposure using quantiles a continuous measure of PFAS exposure was used. In the absence of full reporting of the calculated point estimate and confidence intervals in a study, the calculated probability value (p) was recorded.

Assessment of consistency of evidence

The International Agency for Research on Cancer's (IARC's) criteria for evaluating the strength of evidence for carcinogenicity provided by studies of cancer in human beings (see sections B2 and B6a in (51)) were adapted by Professor Bruce Armstrong for use in this systematic review.

The evidence relevant to each separate health effect of PFAS exposure studied in humans has been classified into one of the following categories:

Sufficient evidence of a health effect: A causal relationship has been established between exposure to PFAS and the health effect in humans. A positive (direct) or negative (inverse) relationship has been observed between the exposure and the health effect in studies in which chance, bias and confounding could be ruled out with reasonable confidence.

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

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

Evidence suggesting lack of a health effect**:** There are several adequate studies covering the full range of levels of exposure that humans are known to encounter that are mutually consistent in not showing a positive (direct) or negative (inverse) association between exposure to the agent and any studied health effect in humans at any observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals that include the null value (e.g. a relative risk of 1.0). Levels of bias and confounding that might obscure an effect should be ruled out with reasonable confidence, and there should be results of studies that have sufficient length of follow-up from initial exposure and sufficient statistical power for a material effect to be observable.

Meta-analysis

We undertook meta-analyses where there were five or more studies with exposure and outcomes assessments that were reasonably comparable. Where relevant, separate meta-analyses were undertaken for continuous, categorical and time to event outcomes, and for exposures evaluated as continuous or categorical measures with comparable units of measurement. Where exposure was categorised into quantiles, we conducted meta-analyses comparing the highest quantile to the lowest quantile regardless of the number of quantiles used in each study. We used measures of effect from adjusted analyses whenever available.

Exposure concentrations for birth outcomes could be obtained from either maternal or umbilical cord blood. As the concentrations from different blood sources may not be comparable, where relevant three of meta-analyses were undertaken pooling studies using maternal blood, studies using cord blood, and all studies.

We used the Q statistic to test whether heterogeneity among studies was greater than expected by chance at a significance level of 0.1; and the I^2 statistic to measure the percentage of variation in the effect measure due to between-study heterogeneity. No formal tests for publication bias were undertaken due to small numbers of studies. When there was either statistical heterogeneity or $I^2 > 50\%$, indicating substantial heterogeneity between studies, a pooled estimated is provided for completeness, however, such results should be interpreted cautiously. Meta-regression was not undertaken to investigate causes of heterogeneity due to the small number of studies for all outcomes.

All meta-analyses were undertaken using fixed effects, with sensitivity analyses using random effects. Meta-analyses were undertaken using Stata statistical version 13.1.

Key findings

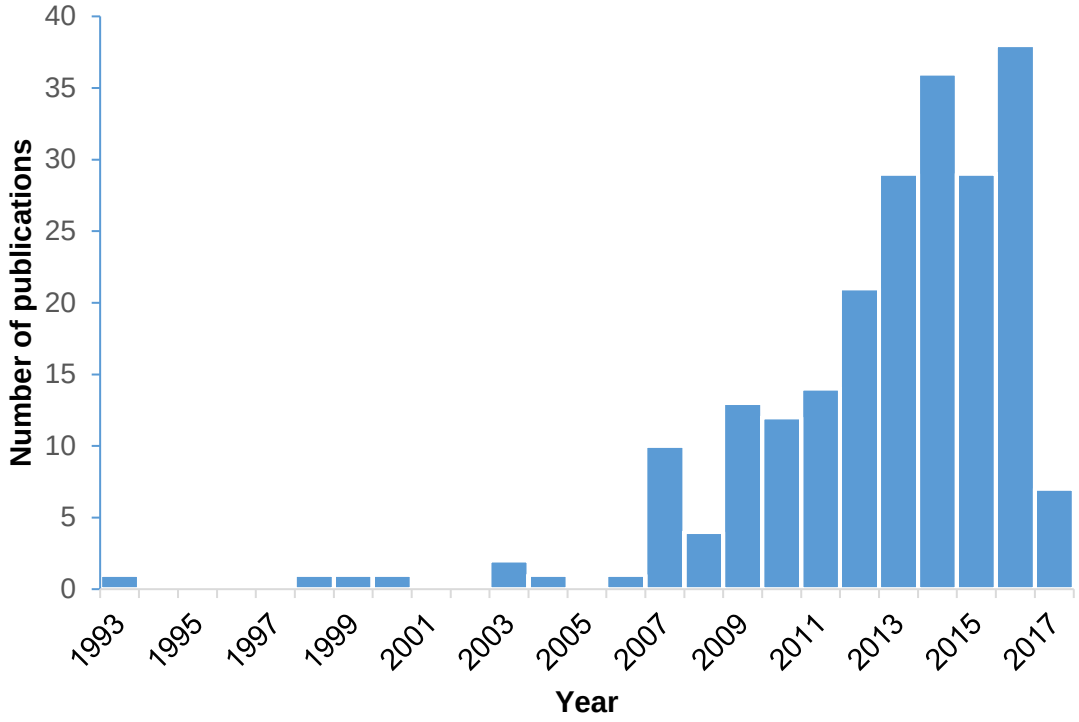
Identification of studies

We identified a total of 7,205 papers from database searches. There were 3,227 duplicates that were then excluded, leaving 3,978 papers eligible for review. An additional 2,714 results from the grey literature brought the total identified papers to 6,692. Of all the papers, 6,474 could be excluded after both reviewers completed an abstract level analysis based on the eligibility criteria of the systematic review. The research team found 207 papers eligible for full text evaluation. Once all papers had been assessed for inclusion in this review, a hand-search of the reference lists of the included papers was conducted. This included related systematic reviews, and identified an additional 14 papers previously not discovered in the original database search. Nine of these papers met the criteria for this review, giving 221 papers in total.

Overview of studies

The search strategy yielded a diverse range of epidemiological studies related to the human health effects of exposure to PFAS. There was a dramatic increase in published papers after 2007 (Figure 1). The search results highlighted the health conditions and diseases possibly linked to living or working in an environment contaminated by PFAS. While the search identified the large quantity of studies that have been conducted on PFAS exposure, it also emphasised the scope of the literature and the number of questions on human health that remain unanswered.

Figure 1. Number of epidemiological papers included in the systematic review, by publication year, PFAS Health Study, 2017.



The effects of several exposure routes were explored through the literature, including direct contact with PFAS through occupational exposure and prolonged contact with PFAS through the consumption of contaminated water and food. Prenatal exposure routes were further investigated in a large proportion of the results, as determined through maternal serum concentrations of PFAS during the first and second trimester, or umbilical cord serum concentrations of PFAS at birth. In many studies, exposure was quantified based on blood serum concentrations of PFAS; however, several studies statistically modelled estimated historical exposure from specific chemicals based on occupation or residential location.

Key health outcomes associated with PFAS

The literature in general supported a relationship between exposure to PFAS and elevated blood concentrations of PFAS in humans, resulting in a wide range of health outcomes being studied. Papers reviewed for this study can be categorised into 12 main health outcome areas;

1. Neonatal, infant and maternal outcomes;
2. Reproductive outcomes;
3. Metabolic outcomes;
4. Thyroid outcomes;
5. Neurodevelopmental and neurophysiological outcomes;
6. Cancers;
7. Diabetes;
8. Cardiovascular outcomes;
9. Overweight and obesity;
10. Immunological outcomes;
11. Skeletal outcomes; and
12. Respiratory outcomes.

The health outcome categories are listed in descending order of numbers of papers identified, where the greatest number of eligible papers reported on foetal and neonatal effects of PFAS exposure and the least number of papers reported on respiratory effects. In some instances, the findings of the papers overlapped categories. For example, reproductive health outcomes can affect prenatal health outcomes. When this occurred, the papers were listed in the section where they had the greatest effect on health outcome, and relevant results included in other sections.

Within the 12 categories, 146 sub-categories of health outcomes were identified in the literature. While there may have been many papers in a particular health outcome category, this did not necessarily reflect strength of evidence; rather, it could simply have reflected ease with which the health outcome could be studied.

Neonatal, infant and maternal outcomes

We identified 38 papers investigating the association of prenatal PFAS exposure with health outcomes in neonates, infants and their mothers. The scope of prenatal effects of PFAS investigated was extensive, with findings published in relation to 28 health outcomes and 12 exposures. The studies predominantly focussed on the relationship between prenatal exposure to PFOA, PFOS, PFHxS and PFNA with adverse birth outcomes including low birth weight, preterm birth and pregnancy loss. All papers were found to have moderate or high risk of bias. The papers are summarised in Appendix 7. Studies that provided quantitative evidence for birth weight, birth length, head circumference and preterm birth were considered for meta-analysis.

Measurements at birth

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Birth weight		
	Umbilical cord; PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFUdA, PFTrDA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA	Inadequate evidence
Small for gestational age		
	Umbilical cord; PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA	Inadequate evidence
Large for gestational age		
	Maternal; PFOA, PFOS	Inadequate evidence
Placental weight		
	Maternal; PFOA, PFOS	Inadequate evidence
Birth length		
	Umbilical cord; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpS, PFDoA, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Ponderal index		
	Umbilical cord; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS, PFNA, PFOSA, PFDA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence

Head circumference at birth		
	Umbilical cord; PFOA, PFOS, PFHxS, PFNA, PFUdA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS, PFHpS, PFNA, PFDA, PFUdA, PFDoA, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Abdominal circumference at birth		
	Maternal; PFOA, PFOS	Inadequate evidence
Chest circumference at birth		
	Maternal; PFOA, PFOS	Inadequate evidence
APGAR score		
	Maternal; PFOA	Inadequate evidence

Birth weight

Twenty-nine papers investigated the association between prenatal exposure to PFAS and birth weight measurements. (52-80) Predominately, the papers reported no significant association between concentrations of PFAS and birth weight, though there is a small body of conflicting evidence to suggest that PFOA and PFOS measurements in maternal serum are negatively associated with birth weight, meaning an increase in maternal blood concentration of PFAS is associated with a lower birth weight. However, overall the findings relating to umbilical cord and maternal blood concentrations of PFAS are inconsistent and provide inadequate evidence for a causal relationship between PFAS exposure levels and decreased or increased birth weight.

Umbilical cord blood studies

Eleven papers reported the relationship between umbilical cord concentrations of PFAS and birth weight measurements, all of which reported results for PFOS and PFOA. (53, 54, 56, 58, 60, 63, 65-67, 74, 80) De Cock et al. (58) and Kwon et al. (65) were the only studies to report a statistically significant association between PFAS levels in umbilical cord serum and changes in birth weight. De Cock et al. (58) identified a significant positive association between the highest tertile of PFOS concentrations in the umbilical cord and the birth weight of males (regression β (Tertile 3 (T3)-Tertile 1 (T1)) birth weight (grams (g)) (95% CI); 724.4 (193.83, 1254.97)) in a cohort study of 91 mother-infant pairs enrolled in the obesogenic endocrine disrupting chemicals linking prenatal exposure to the development of obesity later in life (OBELIX) study in the Netherlands. Kwon et al. (65) reported the opposite from a cohort of 268 mother-infants pairs in the Ewha Birth and Growth Cohort (EBGC) in South Korea, finding a significant negative association between birth weight and umbilical cord measurements of PFOA (regression β (continuous) birth weight (g) (95% CI); -77.93 (-153.56, -2.30)), PFOS (regression β (continuous) birth weight (g) (95% CI); -49.41 (-95.57, -3.25)), PFNA (regression β (continuous) birth weight (g) (95% CI); -77.02 (-135.30, -18.73)), PFDA (regression β (continuous) birth weight (g) (95% CI); -101.24 (-184.80, -17.67)) and PFUdA (regression β (continuous) birth weight (g) (95% CI); -83.63 (-153.94, -13.33)).

Across seven papers that investigated additional PFAS, no statistically significant association was reported between umbilical cord levels of PFHxS, PFUdA, PFDoA and PFTrDA and birth weight, with the exception of one study. (54, 56, 65-67, 74, 80) In a cross-sectional study of 70 pregnant women from Gyeongbuk County in South Korea, Lee et al. (66) found a negative

association with PFHxS and birth weight (OR low birth weight (<median- ≥median) (95% CI); 0.26 (0.08–0.85)).

Maternal blood studies

The association between maternal serum concentrations of PFAS during pregnancy and birth weight measurements was investigated in 21 papers. (52, 55, 57, 59, 61-64, 66, 68-73, 75-80) A significant negative association between maternal concentrations of PFOA and birth weight was reported by: Andersen et al. (52) (regression β (continuous) birth weight (g) (95%CI); -12.8 (-24.5, -1.2)), from a randomly selected sample of 1,400 mother-infant pairs in the Aarhus Birth Cohort; Lenters et al. (68) (regression β (continuous) birth weight (g) (95% CI); -63.77 (-122.83, -4.71)), in a birth cohort study of 1,710 couples from Greenland, Poland and Ukraine; Maisonet et al. (69) (regression β (T3-T1) birth weight (g) (95% CI); -133.45 (-237.37, -29.54)), from 447 mother-infant pairs enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC); and Wu et al. (79) (estimated change in mean birth weight (g) per 10-fold increase in PFOA (95% CI); -267.30 (-573.27, -37.18)), from a study of 160 pregnant women in China. Fourteen other papers, however, reported no association between PFOA exposure levels and birth weight. (55, 57, 59, 62, 63, 66, 70, 71, 73, 75-78, 80)

Inconsistent results were further reported for the relationship between maternal serum levels of PFOS and birth weight: Kishi et al. (64) (regression β (Quartile 4 (Q1)-Quartile 1 (Q1)) birth weight (g) (95% CI); -186.6 (-363.4, -9.8)) from a cohort study of 306 mother-infant pairs from the Hokkaido Study on Environment and Children's Health (Hokkaido Study); Maisonet et al. (69) (regression β (T3-T1) birth weight (g) (95% CI); -140.01 (-238.14, -41.89)); and Washino et al. (77) (partial regression β (continuous) birth weight (g) (95% CI); -148.8 (-297.0, -0.5)), from a cohort study of 514 mother-child pairs from the Hokkaido Study. However, the findings presented by Kishi et al. (64) were only statistically significant in female neonates. Further, Stein et al. (75) reported that increased maternal PFOS levels was associated with delivering a neonate with a low birth weight (OR (>90th percentile- <50th percentile) birth weight <5.5 pounds (95% CI); 1.8 (1.2, 2.8)). Twelve papers reported no association between PFOS levels in maternal serum and birth weight. (52, 54, 55, 57, 59, 61-63, 66, 68, 71, 78)

In relation to additional PFAS exposures, Maisonet et al. (69) reported a significant negative association between maternal PFHxS levels and birth weight (regression β (T3-T1) birth weight (g) (95% CI); -107.93 (-206.18, -9.69)). However, Bach et al. (55), Hamm et al. (62), Lenters et al. (68) and Monroy et al. (80) reported there was no association between PFHxS exposure and birth weight. The Taiwan Maternal and Infant Cohort Study of 223 mother-infant pairs by Wang et al. (76) was the only study to find a negative association between birth weight and maternal serum concentrations of PFNA (regression β (continuous) birth weight (kg) (95% CI); -0.08 (-0.16, 0.0)), PFDA (regression β (continuous) birth weight (kg) (95% CI); -0.14 (-0.26, -0.02)), PFUdA (regression β (continuous) birth weight (kg) (95% CI); -0.06 (-0.11, -0.01)) and PFDoA (regression β (continuous) birth weight (kg) (95% CI); -0.12 (-0.21, -0.02)). However, these associations were significant only for female neonates. (76) Four other papers on the maternal levels of PFNA, PFDA, PFUdA or PFDoA reported no significant associations with birth weight. (55, 68, 71, 80)

Birth weight meta-analysis

Of the 28 papers reporting results on the relationship between birth weight and PFAS: 21 papers presented 23 sets of results for PFOA; (52-60, 62, 63, 65-74) 19 papers presented 21

sets of results for PFOS; (52, 53, 55-60, 62, 64-69, 74, 75, 77, 78) eight papers presented nine sets of results for PFHxS; (55, 62, 65-69, 74)and seven papers presented eight sets of results for PFNA. (55, 56, 65, 66, 68, 74, 76) Each of Kishi et al. (64) and Wang et al. (76) presented results separately for male and female neonates, and Lee et al. (66) presented results separately for maternal and umbilical cord blood exposure assessment; thus there are 24 sets of results overall across the relevant PFAS exposures.

Studies used a variety of means to present data for PFAS. Three studies were not appropriate for meta-analysis: Arbuckle et al. (54) as concentration was an outcome, and birth weight an explanatory variable in analyses; and Lee et al. (66) and Stein et al. (75) as birth weight was dichotomised. No studies that modelled prenatal PFAS exposure based on residential location or occupation were considered for this meta-analysis.

PFOA meta-analysis

There were 21 papers, which presented 19 sets of results, which were potentially eligible for meta-analysis of PFOA exposure. (52-60, 62, 65-69, 74-79) Five studies presented results for categorised PFOA concentrations and birth weight, four of which used maternal blood, (55, 57, 62, 69) and one of which used umbilical cord blood. (58) We conducted a meta-analysis for all five studies which categorised PFOA, combining results from PFOA exposure assessment for both umbilical cord and maternal blood. Six studies presented results for (natural) log transformed exposure; however, four (53, 56, 65, 67) assessed exposure from umbilical cord blood measurements and two from maternal blood measurements. (68, 79) We conducted a meta-analysis for all five studies with categorised PFOA exposure and for all six studies with log transformed PFOA exposure, combining results from PFOA exposure assessment for both maternal and umbilical cord blood. For both meta-analyses, there were too few studies to present results separately for umbilical cord versus maternal blood.

For categorised PFOA there was high heterogeneity in study effects (I^2=47.00%; Q statistic (Q) =7.55; degrees of freedom (df) =4; p=0.109). The pooled regression coefficient was -9.44 (95% CI=-47.05, 28.18) (Figure 2). These results provide little overall evidence for any trend in birth weight with increasing exposure to PFOA. The apparently lower birth weight with higher PFOA exposure reported by Maisonet et al. (69) is an outlier and likely to be the main reason for the high heterogeneity in study effects. The result from the random effects model was a pooled regression coefficient of -14.50 (95% CI=73.76, 44.77; p=0.63) which is consistent with the fixed effects model in showing little evidence of an association in either direction.

For continuous log transformed PFOA concentration, there was substantial heterogeneity in study effects (I^2=69.80%; Q=16.58; df=5; p=0.005). The pooled fixed effects regression coefficient was -0.03 (95% CI=-0.25, 0.18; p=0.77) (Figure 3), which provides no overall evidence for any trend in birth weight with increasing exposure to PFOA. The strong null result of Lee et al. (67) is the dominant result in this analysis; the results of the other studies in the meta-analysis are consistent with a possible inverse association between PFOA in maternal or cord blood and birth weight.

The pooled effect for the random effects model was statistically significant at -44.25 (95% CI=-85.31, -3.18; p=0.035) and overall consistent with a possible inverse association between PFOA in maternal or cord blood and birth weight.

Due to the substantial heterogeneity and the combination of results from maternal and umbilical cord blood, these results for meta-analyses of the association between PFOA and birth weight should be interpreted with caution.

Figure 2. Fixed effects meta-analysis of the effects of PFOA on birth weight for studies reporting categorised outcomes.

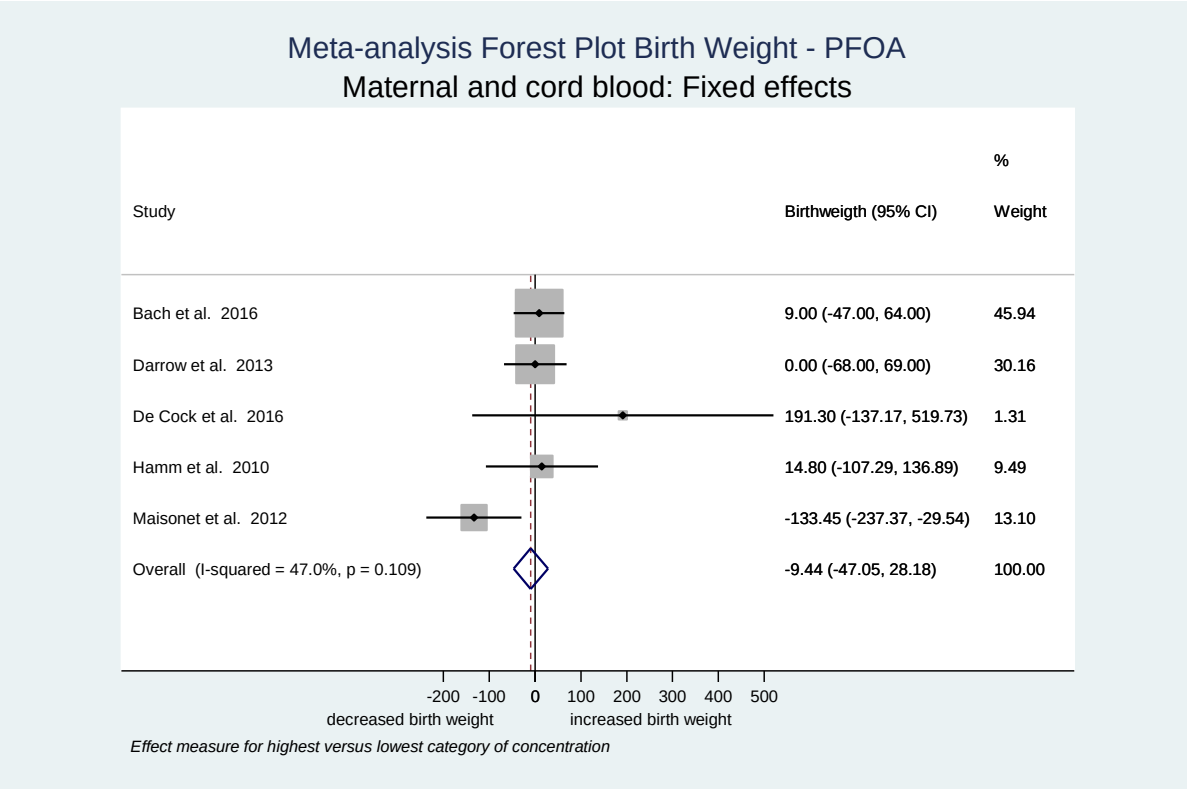
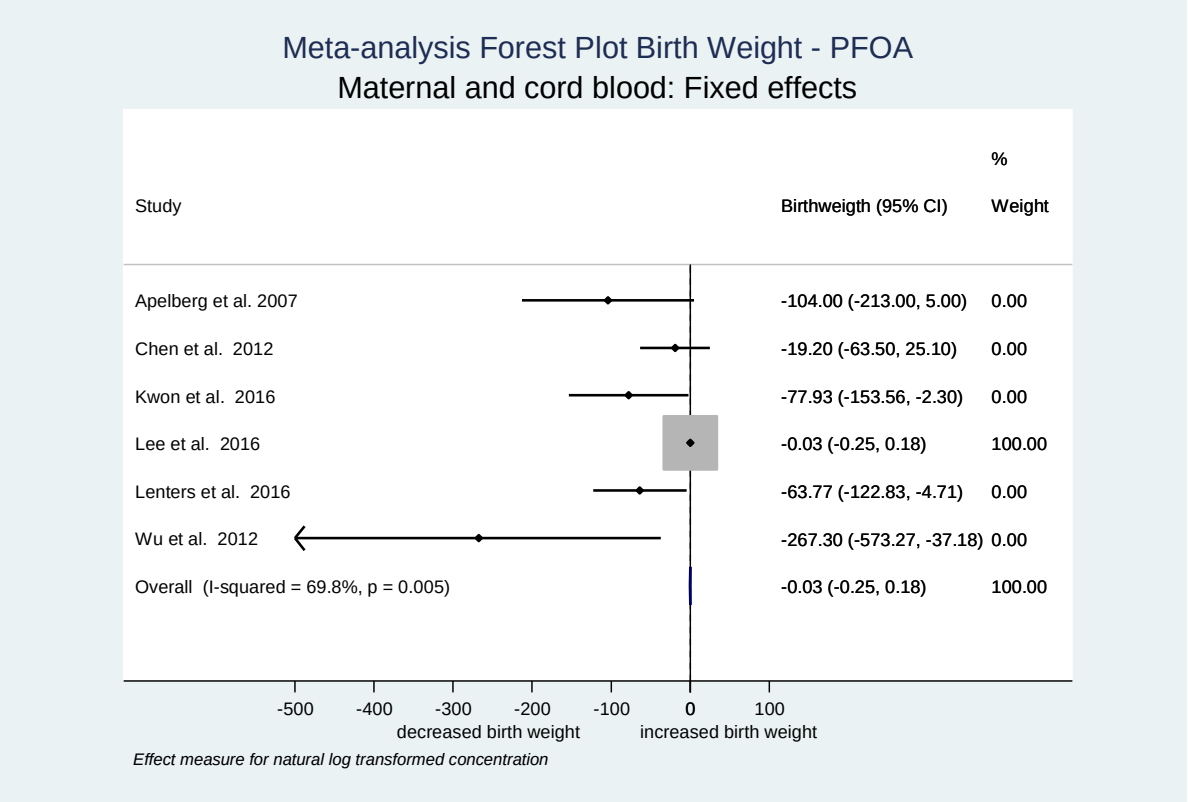


Figure 3. Fixed effects meta-analysis of PFOA on birth weight for studies reporting continuous outcomes.

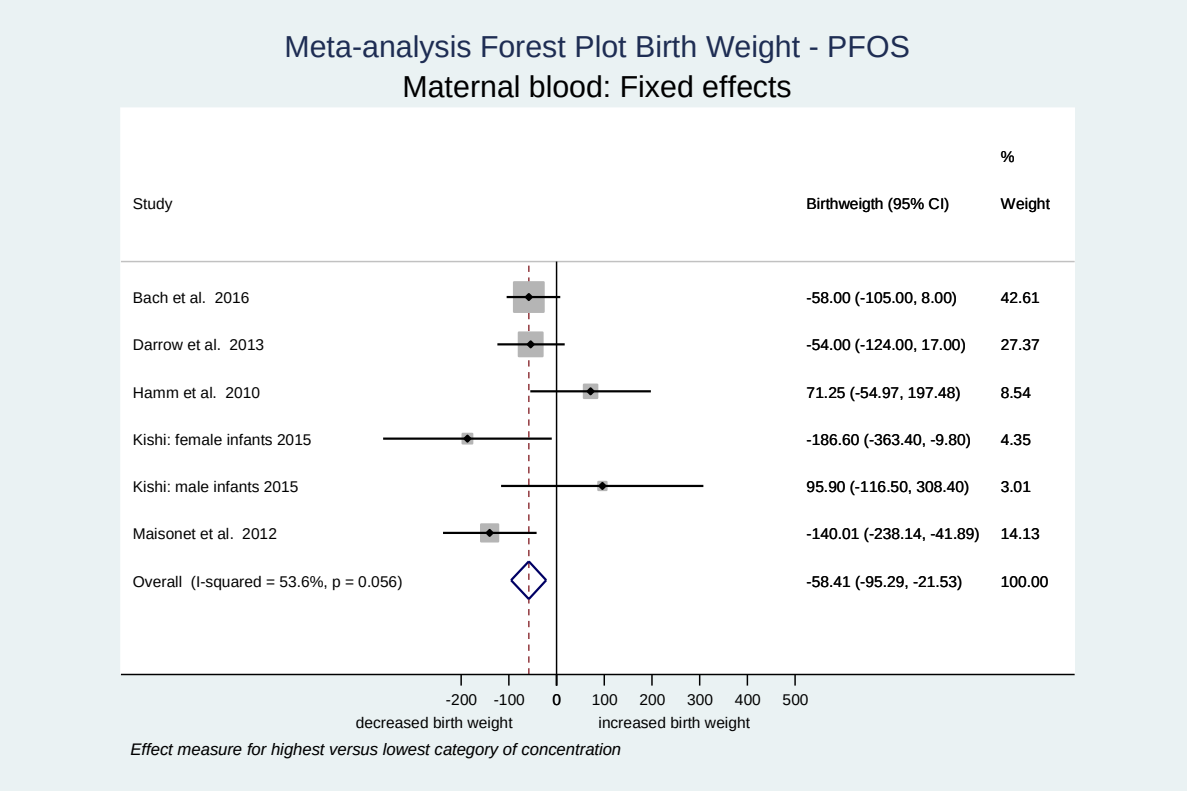


PFOS meta-analysis

There were 19 papers which were potentially eligible for meta-analysis of PFOS exposure. (52, 53, 55-60, 62, 64-69, 74, 75, 77, 78) There were seven papers presenting eight sets of results for categorised PFOS; one was excluded as it reported z-scores of birth weight. All of the remaining papers, except De Cock et al. (58), included data for PFOS measured in maternal blood serum. Therefore a meta-analysis was undertaken on the six sets of results from five papers assessing PFOS in maternal blood. One of these six studies presented results separately for male and female neonates, both of which are included in the analysis. The forest plot for the fixed effects meta-analyses is shown in Figure 4. There was substantial heterogeneity across the seven comparable studies assessing the relationship between categorised PFOS and birth weight (I^2=53.6%; Q=10.77; df=5; p=0.056). The overall measure of effect was statistically significant (pooled regression coefficient for the highest versus lowest category -58.41 (95% CI=-95.29, -21.53; p=0.002)), providing strong evidence for an inverse association between maternal blood concentrations of PFOS category and birth weight. A random effects analysis resulted in a similar point estimate but a wider confidence interval and higher p (pooled estimate of regression coefficient -55.03 (95% CI=-117.03, 6.97; p=0.082)) and provides weak evidence for an inverse association between categories of PFOS in maternal blood and birth weight.

The funnel plot did not demonstrate substantial publication bias for PFOS, however, these graphs are generally difficult to interpret when the number of studies is small.

Figure 4. Fixed effects meta-analysis of the effects of PFOS on birth weight for studies reporting categorised outcomes.



Evaluation

The reported associations between prenatal exposure to PFAS and birth weight across the 29 papers were largely inconsistent. Overall findings differed between umbilical cord and maternal serum concentrations. Generally, the findings for the relationship between umbilical cord levels of PFAS and birth weight were not statistically significant. In contrast, the association between maternal levels of PFOA, PFOS and PFHxS during pregnancy and birth weight were reported to be inverse and statistically significant in a number of studies. (52, 64, 66, 68, 69, 75, 77, 79) In addition, an inverse association between PFAS concentration and birth weight is supported, although only weakly, by the meta-analyses.

Most studies reported no statistically significant association between maternal PFAS concentrations and birth weight, and the meta-analyses included results from only 11 of the 28 studies. Further, of the eight studies that reported a statistically significant inverse association between maternal PFAS concentrations and birth weight, seven papers were evaluated to have a high risk of bias. Therefore, there is inadequate evidence to suggest a causal relationship between prenatal exposure to PFAS and increased or decreased birth weight.

Small for gestational age

Small for gestational age (SGA) is a classification method for birth weight in relation to gestational age. A neonate is defined as SGA if their birth weight is below the 10th percentile for their gestational age. Six papers (55, 59, 62, 73, 76, 78) examined the relationship between prenatal exposure to PFAS and SGA. Overall, the papers reported no statistically significant

association between serum concentrations of PFAS and SGA. However, Chen et al. (56) demonstrated an increased risk of SGA with higher levels of PFOS in the umbilical cord at birth (OR (per log increase in PFOS) SGA (95% CI); 2.27 (1.25, 4.15)), in a birth cohort panel study of 429 mother-infant pairs enrolled in the Taiwan Maternal and Infant Cohort Study, and Wang et al. (76) stated a positive finding for maternal concentrations of PFDA (OR (per log increase in PFDA) SGA (95% CI); 3.14 (1.07, 9.19)) and PFUdA (OR (per log increase in PFUdA) SGA (95% CI); 1.83 (1.01, 3.32)), in 223 mother-infant pairs from the same cohort as Chen et al., (56) although during a different time frame. The results reported by Wang et al. (76) were specific to female neonates however, with no statistically significant relationship between PFAS and SGA in males. No other studies investigated the association between these three specific exposures and SGA.

Chen et al. (56) further reported the association between umbilical cord concentrations of additional PFAS at birth and SGA. The authors demonstrated no significant association between PFOA, PFNA and PFUdA measurements in umbilical cord serum and the health outcome, in contrast to their findings for prenatal exposure to PFOS. Fei et al., (59) Hamm et al., (62) Savitz et al., (73) Wang et al., (76) and Whitworth et al. (78) reported no significant relationship between maternal concentrations of PFOA, PFOS, PFHxS, PFNA and PFDoA and SGA.

The results from the six studies largely conclude that prenatal exposure to PFAS is not significantly associated with SGA. Although Chen et al. (56) and Wang et al. (76) provide evidence for a statistically significant association between specific PFAS exposures and the odds of SGA in neonates, we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias. Further studies and quantitative analyses are required to identify whether the results are replicable in other exposed populations.

Large for gestational age

In contrast to the term SGA, large for gestational age (LGA) refers to a birth weight measurement above the 90th percentile for a neonate's gestational age. Whitworth et al. (78) examined the association between prenatal exposure to PFAS and LGA. The authors did not identify any significant associations between PFOA and PFOS measurements in maternal serum and LGA.

Placental weight

Fei et al. (81) investigated the relationship between prenatal exposure to PFAS and placental weight at birth. The study reported no significant association between maternal concentrations of PFOA and PFOS during pregnancy and the placental weight of mothers.

Birth length

The association between prenatal exposure to PFAS and birth length was reported in 11 papers. (53, 55, 56, 66, 69, 71, 74, 76, 77, 79, 81) Predominately, the relationship between umbilical cord and maternal concentrations of PFAS and birth length was not reported to be statistically significant. Results that demonstrated a significant relationship between PFAS exposure and birth length are overall conflicting, and do not present a trend in the same direction for umbilical cord and maternal exposure measurements.

Umbilical cord blood studies

Four studies investigated the association between umbilical cord concentrations of PFAS and birth length. (53, 56, 66, 74) Chen et al. (56) reported a positive association between log PFNA levels in the umbilical cord at birth and birth length (regression β (continuous) birth length (centimetres (cm)) (95% CI); 0.16 (0.05, 0.27)), from the birth cohort panel study of 429 mother-infant pairs enrolled in the Taiwan Maternal and Infant Cohort Study, which was the only significant finding across the four studies. The relationship between PFOA and PFOS concentrations in the umbilical cord and birth length was reported to be non-significant in all studies, and Lee et al. (66) and Shi et al. (74) further stated there was no association for PFHxS. Chen et al. (56) found no significant relationship between umbilical cord levels of PFUdA and birth length, and Shi et al. (74) stated no association for PFNA, PFDA and PFUdA.

Maternal blood studies

The effect of maternal serum concentrations of PFAS on birth length was investigated in eight papers. (55, 66, 69, 71, 76, 77, 79, 81) In a longitudinal study of parents and children in the United Kingdom, Maisonet et al. (69) reported a significant negative association between PFOS (regression β (T3-T1) birth length (cm) (95% CI); -0.63 (-1.11, -0.15)) and PFHxS (regression β (T3-T1) birth length (cm) (95% CI); -0.82 cm (-1.29, -0.34)) concentrations in maternal serum and birth length, but no association with PFOA. Wu et al. (79) presented a negative association between maternal PFOA concentration and birth length (estimated mean change in birth length (cm) per one log-unit increase in PFOA (95% CI); -1.91 (-3.31, -0.52)), from a birth cohort of approximately 160 pregnant women in China. Across the papers by Bach et al. (55), Fei et al. (81), Lee et al. (66), Robledo et al. (71), Wang et al. (76) and Washino et al. (77), maternal exposure levels of PFOA, PFOS, PFNA, PFDA, PFUdA, PFHpS, PFDoA, PFOSA, 2-(N-methyl-perfluorooctanesulfonate) acetic acid (Me-PFOSA-AcOH) and 2-(N-ethylperfluorooctanesulfonate) acetic acid (Et-PFOSA-AcOH) were not associated with birth length. Robledo et al. (71) further found no association between the paternal PFAS concentrations and birth length.

Birth length meta-analysis

Nine papers which determined the relationship between PFOA and birth length were considered for meta-analysis. (53, 55, 56, 66, 69, 76, 77, 79, 81) Eleven sets of results were presented, as Lee et al. (66) provided results for PFOA measured in both maternal and umbilical cord blood serum, and Wang et al. presented results separately, by infant sex. Seven papers assessed the relationship between PFOS and birth length (53, 55, 56, 66, 69, 77, 81); presenting eight sets of results, as Lee et al. (66) provided results for both maternal and umbilical cord blood PFOS.

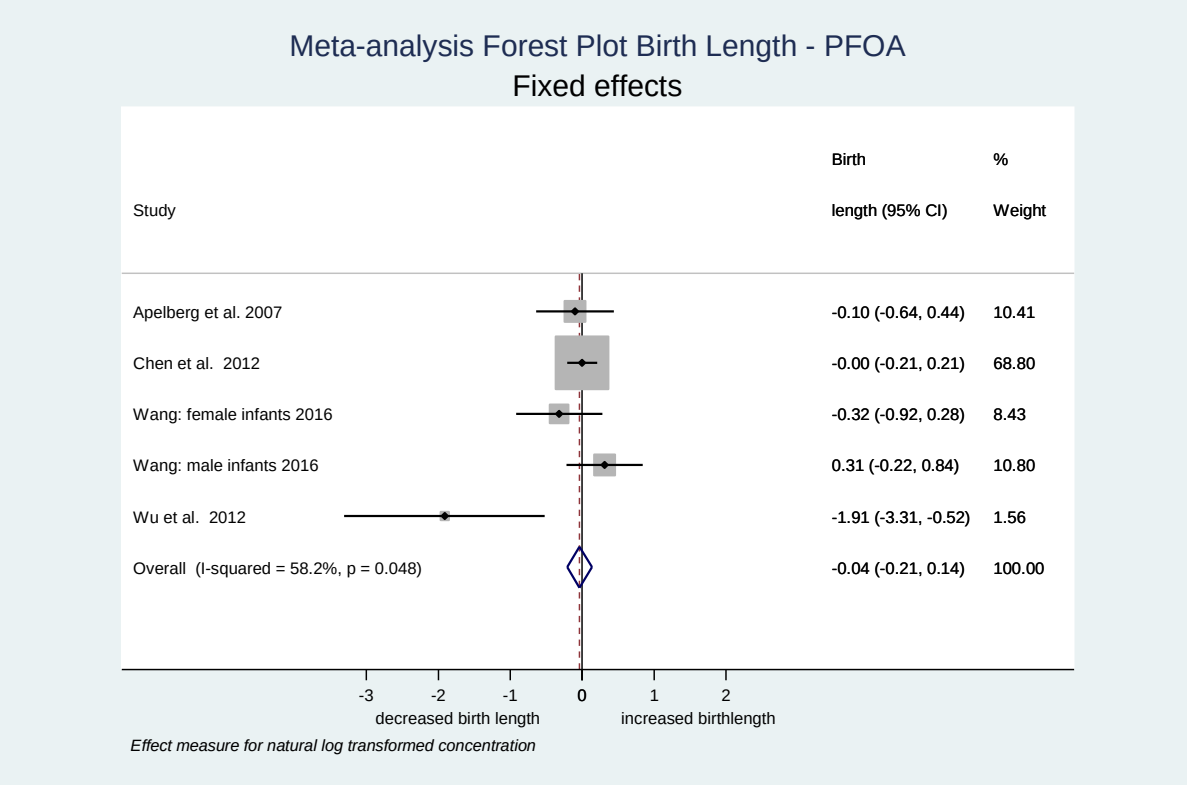
Lee et al. (66) dichotomised birth length and this paper was therefore, ineligible for inclusion in the meta-analysis. Of the eight remaining papers (with nine sets of results) on PFOA exposure, four considered PFOA as a categorical variable and seven (with eight results) considered PFOA as a continuous variable. Four studies presented five sets of results for log transformed PFOA, thus providing an adequate number of studies for meta-analysis; with two papers using PFOA measurements of umbilical cord blood and two (with three results) using maternal blood measurements. As the number of papers reporting results separately by type of blood was inadequate to conduct a meta-analysis we present only the results which combine umbilical cord and maternal exposure measurements.

There was substantial heterogeneity in the study effects for PFOA (I^2=58.2%; Q=9.58; df=4; p=0.048). The overall measure of effect was not statistically significant (pooled regression coefficient -0.036 (95% CI=-0.210, 0.138; p=0.690) (Figure 5). Results for random effects models were consistent with those of fixed effects, with similar pooled point estimate but wider confidence intervals (pooled regression coefficient -0.125 (95% CI: -0.487–0.236), p=0.50).

These results should be interpreted with caution, due to the between-study heterogeneity and because results were combined for cord and maternal blood.

Excluding the paper by Lee et al. (66) which dichotomised birth length, six potentially eligible papers investigated the association between PFOS and birth length. Four considered PFOS exposure as a categorical variable and five considered PFOS exposure as a continuous variable. The continuous values of PFOS were (natural) log transformed in two papers, log to base 10 transformed for one paper and untransformed for two papers. Thus, there were an inadequate number of papers with comparable outcome and PFOS exposure measures for inclusion in a meta-analysis.

Figure 5. Fixed effects meta-analysis of the effects of PFOA on birth length for studies reporting categorised outcomes.



Evaluation

Overall, the findings reported by the eleven papers support a non-significant association between prenatal exposure to PFAS and birth length. Where significant results were reported for prenatal exposure to PFOA and PFOS, the direction of effect was conflicting and there is a larger body of evidence to suggest that there is no association between exposure and this health outcome. As there was only a single paper reporting this statistically significant

association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Ponderal index

The ponderal index (PI) is a paediatric measure of leanness using height or length and weight, and is similar to body mass index (BMI). Seven papers investigated the association between prenatal exposure to PFAS and PI at birth. (53, 56, 66, 69, 71, 74, 79) Conclusively, the papers reported no association between maternal and paternal concentrations of PFAS and neonatal PI. In contrast, the reported relationship between umbilical cord levels of PFAS at birth and PI were inconsistent across the papers.

Umbilical cord blood studies

Apelberg et al. (53), Chen et al. (56), Lee et al. (66) and Shi et al. (74) reported the association between umbilical cord concentrations of PFOA and PFOS and PI at birth. In a cross-sectional study of 293 infants in Baltimore, Apelberg et al. (53) found a significant negative association between PI and umbilical cord concentrations of PFOA (regression β (Q4-Q1) PI (g/cm^3) (95% CI); -0.039 (-0.077, -0.001)) and PFOS (regression β (Q4-Q1) PI (g/cm^3) (95% CI); -0.062 (-0.104, -0.021)). In contrast, Chen et al. (56), Lee et al. (66), and Shi et al. (74) showed no significant association between PFOA and PFOS levels and PI. Chen et al. (56) reported one negative finding from their study set within the Taiwan Birth Panel Study, the association between PFNA and PI (regression β (continuous) PI (g/cm^3) (95% CI); -0.02 (-0.03, -0.004)), which conflicted with the conclusions by Shi et al. (74), who reported no association for PFNA. Reported associations between additional PFAS and PI were all non-significant. Chen et al. (56), Lee et al. (66) and Shi et al. (74) did not demonstrate any association between PFHxS, PFUdA and PFDA in the umbilical cord and PI at birth.

Maternal blood studies

The association between maternal concentrations of other PFAS and PI was reported by Lee et al. (66), Maisonet et al. (69), Robledo et al. (71) and Wu et al. (79). Together, the papers found no significant associations between maternal serum concentrations of PFOA, PFHxS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH and Et-PFOSA-AcOH during pregnancy and PI at birth. Lee et al. (66) reported a negative finding for PFOS (OR (≥ median versus < median) (95% CI); 0.22 (0.05, 0.90)) in a cross-sectional study of 70 pregnant women from Gyeongbuk County in South Korea. Robledo et al. (71) also found no association between paternal serum concentrations of PFAS and PI.

The results from the seven papers suggest that there was overall no significant association between prenatal PFAS concentrations and PI at birth, however, the evidence is conflicting for umbilical cord measurements of PFOS. Two out of four papers reported a significant negative association between umbilical cord PFOS concentrations and PI, though both papers reported no association between birth weight or birth length separately. Therefore, it is unknown whether the significant negative association between the exposure and PI is driven by the weight or length of neonates at birth. As the available data did not allow for a meta-analysis, further investigation into umbilical cord concentrations of PFOS and PI measurements may be important.

Head circumference

Eight papers investigated the relationship between prenatal exposure to PFAS and measurements of neonatal head circumference at birth. (53, 55, 56, 66, 71, 76, 77, 81) Predominately, the studies reported no significant association between PFAS and head circumference at birth, however the overall findings differed for maternal and umbilical cord measurements.

Umbilical cord blood studies

Apelberg et al. (53), Chen et al. (56) and Lee et al. (66) each investigated the relationship between PFOA and PFOS concentrations in umbilical cord serum at birth. Apelberg et al. (53) reported a significant negative association between head circumference and umbilical cord levels of PFOA (estimated change (cm) (Q4-Q1) (95% CI); -0.23 (-0.42, -0.04)) and PFOS (estimated change (cm) (Q4-Q1) (95% CI); -0.27 (-0.48, -0.06)) in a cross-sectional study of infants from Baltimore. In the Taiwan Birth Panel Study, Chen et al. (56) found a negative association between head circumference and prenatal exposure to PFOS (estimated change (cm) (per log increase in PFOS) (95% CI); -0.25 (-0.46, -0.05)), though no association for PFOA, PFNA and PFUdA. Lee et al. (66) concluded no association between umbilical cord and maternal levels of PFOA, PFOS and PFHxS and neonatal head circumference.

Maternal blood studies

In addition to Lee et al. (66), five papers also reported no significant association between maternal concentrations of PFOA and PFOS and head circumference measurements at birth. (55, 71, 76, 77, 81) The studies by Bach et al. (55), Robledo et al. (71) and Wang et al. (76) further stated there was no relationship between the outcome and several other PFAS, including PFHxS, PFHpS, PFNA, PFDA, PFUdA, PFOSA, Me-PFOSA-AcOH and Et-PFOSA-AcOH. Robledo et al. (71) also determined the same results for the relationship between paternal PFAS exposure levels and head circumference measurements. The only inverse association between maternal exposure and the health outcome was reported by Wang et al. (76) for PFDoA concentrations (estimated change (cm) (Q4-Q1) (95% CI); -0.38 (-0.74, -0.02)) in female infants only, based on a sample from the Taiwan Birth Panel Study.

The findings from the eight papers suggest that increased concentrations of PFOS in the umbilical cord at birth may result in reduced head circumference measurements in neonates, with two out of three papers reporting a significant, inverse association between the exposure and health outcome. In contrast, maternal concentrations of PFAS do not appear to be significantly associated with head circumference measurements at birth, with the exception of the negative findings reported by Wang et al. (76) for PFDoA. Whilst there is conflict between the results for umbilical cord and maternal concentrations of PFOA and PFOS, it is important to consider that the negative associations reported by Apelberg et al. (53) and Wang et al. (76) were evaluated to have a high risk of bias. The results by Chen et al. (56) were associated with a moderate risk of bias, and therefore are considered to be more reliable than those reported by Apelberg et al. (53) for umbilical cord exposure levels.

Head circumference meta-analysis

We considered seven papers that reported the association between prenatal exposure to PFOA or PFOS and head circumference measurements at birth for meta-analysis. Apelberg et al. (53), Bach et al. (55), Chen et al. (56), Fei et al. (81), Lee et al. (66) and Washino et al.

(77) investigated both PFOA and PFOS exposures, while Wang et al. (76) considered PFOA only. Lee et al. (66) further investigated exposure measured in both umbilical cord and maternal blood and Wang et al. (76) presented infant sex-specific results only. PFOA and PFOS concentrations were obtained from umbilical cord blood for three papers and from maternal blood for five papers. However, there were an inadequate number of papers with comparable PFAS measures for inclusion in a meta-analysis.

Abdominal circumference

Fei et al. (81) reported on the association between prenatal exposure to PFAS and neonatal abdominal circumference measurements. The authors found no significant association between the concentration of PFOA and PFOS in maternal serum and abdominal circumference at birth.

Chest circumference

Washino et al. (77) investigated the relationship between prenatal exposure to PFAS and neonatal chest circumference measurements. The study reported no significant association between the concentration of PFOA and PFOS in maternal serum and chest circumference at birth.

APGAR score

APGAR is a mnemonic frame of reference to assess a neonate's vital signs one minute and five minutes post birth. The five signs assessed are appearance (skin colour), pulse, grimace (reflex irritability), activity (muscle tone) and respiration. Each sign receives a score of 0−2 and the five scores are added to give a score out of ten. Scores of seven or above are normal while scores of six and lower indicate that medical attention is required.

Wu et al. (79) examined the association between prenatal exposure to PFOA and neonatal APGAR scores five minutes after birth. The study of approximately 160 pregnant women in China reported a significant negative association between PFOA concentrations in maternal serum and APGAR scores (estimated change in score (per log increase in PFOA) (95% CI); -1.37 (-2.42, -0.32)). As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Delivery outcomes

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Preterm birth		
	Umbilical cord; PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS	Inadequate evidence
Gestational age		
	Umbilical cord; PFOA, PFOS	Inadequate evidence
	Maternal; PFOA, PFHxS	Inadequate evidence
Miscarriage		
	PFOA, PFOS, PFHxS, PFNA, PFDA	Inadequate evidence
Stillbirth		
	PFOA	Inadequate evidence
Pregnancy loss (unspecified)		
	PFOA, PFOS, PFNA, PFOSA, PFDA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Mode of delivery (vaginal delivery compared to caesarean)		
	Umbilical; PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Delivery complications		
	PFOA	Inadequate evidence
Gender outcomes of pregnancy (male compared to female)		
	Maternal; PFOA, PFOS, PFNA, PFDA, PFOSA, Et-PFOS-AcOH, Me-PFOS-AcOH	Inadequate evidence

Preterm birth

Ten papers investigated the association between prenatal PFAS exposure and preterm birth. (54, 55, 57, 59, 62, 70, 72, 73, 75, 78) For all studies, preterm birth was defined as a mother who gave birth to their child before 37 weeks gestation, Savitz et al. (73) also investigated whether prenatal exposure to PFAS resulted in birth before 32 weeks gestation. Elevated PFAS levels in maternal serum were not consistently associated with preterm birth; however, the relationship between umbilical cord measurements and preterm birth provide statistically significant evidence for a significant positive association between the exposure and health outcome.

Umbilical cord blood studies

Arbuckle et al. (54), in a small cohort study of approximately 100 women giving birth in Ottawa, Canada found that elevated PFOS in umbilical cord blood was associated with pre-term birth (estimated change in gestational age (per log increase PFOS) (SE); -0.966 (0.35)). Similarly, Chen et al. (56) reported that elevated PFOS in umbilical cord blood was associated with pre-

term birth (OR (per log increase PFOS) (95% CI); 2.45 (1.47–4.08)). Both studies reported non-significant associations for PFOA, PFHxS, PFNA and PFUdA.

Maternal blood studies

In contrast, Whitworth et al. (78) report a significant negative relationship between maternal concentrations of PFOA (OR (Q4-Q1) (95% CI); 0.1 (0.03–0.6)) and PFOS (OR (Q4-Q1) (95% CI); 0.1 (0.03–0.6)) during pregnancy and preterm birth from the Norwegian Mother and Child Cohort Study of 901 women. Hamm et al. (62), from a small cohort study of 252 pregnant women in Alberta, Canada, reported a significant negative association between PFHxS and preterm birth (risk ratio (T3-T1) (95% CI); 0.31 (0.11–0.90)). Results related to maternal PFAS exposure levels were conflicting; however, seven papers (57, 59, 62, 70, 72, 73, 75) reported no statistically significant association between maternal PFOA and preterm birth and four papers stated there was no statistically significant relationship with PFOS. (57, 59, 62, 75)

The 10 papers which investigated the relationship between prenatal exposure to PFAS and preterm birth present inadequate evidence for an association. While the statistically significant results presented by Arbuckle et al. (54) and Chen et al. (56) suggest a positive association between PFOS exposure levels and preterm birth, these results are opposed by the association reported by Whitworth et al. (78). Further, the statistically significant negative association between maternal concentrations of PFHxS and preterm birth was only a single paper reporting this statistically significant association. Thus, we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Preterm birth meta-analysis

We considered seven papers which reported on the association between blood concentrations of PFOA and PFOS and preterm birth for meta-analysis. (54, 56, 57, 59, 62, 75, 78) Chen et al. (56), Darrow et al. (82), Fei et al. (59), Hamm et al. (62), Stein et al. (75) and Whitworth et al. (78) measured PFOA and PFOS exposure levels; and, Arbuckle et al. (54) presented findings related only to PFOS. Due to differences in the categorisation of PFAS measurement there were an inadequate number of results for inclusion in a meta-analysis.

Gestational age

Gestational age relates to the number of weeks a mother is pregnant before giving birth to their child, and is a continuous measurement across preterm, full-term and post-term births. Five studies examined the relationship between prenatal PFAS exposure and gestational age. (53, 62, 69, 70, 79) Results were conflicting for the association between maternal and umbilical cord measurements of PFAS and gestational age. Specifically, the findings for umbilical cord concentrations of PFOS and maternal levels of PFHxS are inconclusive for gestational age. However, results related to prenatal exposure to PFOA and gestational age are, predominately non-significant.

Umbilical cord blood studies

Apelberg et al. (53) investigated the association between umbilical cord measurements of PFAS and gestational age and reported no significant association between PFOA and PFOS concentration in umbilical cord blood and gestational age.

Maternal blood studies

Hamm et al. (62), Maisonet et al. (69), Nolan et al. (70) and Wu et al. (79) investigated the association between maternal PFAS concentrations during pregnancy and gestational age. Hamm et al. (62) reported a significant positive association for maternal PFHxS levels and gestational age in a small Canadian cohort study of 252 pregnant women (natural logarithm slope (95% CI); 0.22 (0.03, 0.42)). Wu et al. (79) found a significant negative association for PFOA (change in gestational age (days) (per log increase PFOA) (95% CI); -15.99 (-27.72, -4.25)) in a small cohort study of 167 pregnant women in China. As Hamm et al. (62), Maisonet et al. (69) and Nolan et al. (70) each concluded no association between maternal concentrations of PFOA and gestational age, the results for Wu et al. (79) provide inadequate evidence of an association. Results are conflicting for the relationship between maternal PFHxS and gestational; age, as Maisonet et al. (69) did not identify an association between elevated maternal blood concentration and increased length of gestation.

The five papers that reported the relationship between prenatal exposure to PFAS and gestational age largely concluded that umbilical cord and maternal concentrations of PFOA were not consistently associated with gestational age. In contrast, the findings for maternal levels of PFHxS are conflicting, as one paper identified a positive significant association and one paper reporting no association with gestational age. We considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Miscarriage

Four papers reported the association between prenatal exposure to PFAS and the occurrence of miscarriage among pregnant women. (72, 75, 82, 83) Three papers concluded that there were no significant associations between maternal concentrations of PFAS during pregnancy and miscarriage. Jensen et al. (83) analysed data for 392 women from Odense, Denmark enrolled in a cohort study and identified that the matched OR of a miscarriage for women with the highest quartile of PFNA compared to the lowest quartile was 37.9 (95% CI; 9.9, 145.2) and 3.71 (95% CI; 1.60, 8.60) for PFDA. Exposure levels of PFHxS were not found to be significantly associated with miscarriage occurrences, though also showed a positive trend. The study by Jensen et al. (83) was evaluated to have a high risk of bias due to participation rates for the cohort and potential for missing exposure data on cases where only 67% of mothers reporting stillbirth had serum stored for analysis. As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Stillbirth

Two papers reported on the association between prenatal exposure to PFAS and stillbirth in pregnant women. (72, 73) Both studies found no significant association between PFOA concentrations in maternal blood and stillbirth occurrences. Each analysis reported on the association between modelled estimates of PFOA exposure, rather than blood serum measurements, and therefore, both studies were evaluated to have a high risk of bias.

Pregnancy loss (unspecified)

Buck Louis et al. (84) investigated the association between prenatal exposure to PFAS and all definitions of pregnancy loss in women, specifically a change from a positive to a negative pregnancy test, clinical confirmation of pregnancy loss or the onset of menstrual bleeding. The study did not further define or categorise pregnancy loss throughout the analyses. The findings of the research indicate that maternal concentrations of PFOA, PFOS, PFNA, PFOSA, PFDA, Me-PFOSA-AcOH and Et-PFOSA-AcOH were not significantly associated with pregnancy loss in women. As the study investigated instances of self-reported pregnancy loss, the results were determined to have a high risk of bias.

Mode of delivery

In the cohort study of just over 100 Canadian women, Arbuckle et al.(54) reported the association between prenatal PFAS exposure and mode of delivery during pregnancy, comparing instances of vaginal deliveries to caesarean sections. The study reported a significant positive association between log umbilical cord concentrations of PFOA, PFOS and PFNA and vaginal deliveries (logistic regression coefficient (reference-caesarean) (SE); -0.511 (0.15), -0.463 (0.17) and -0.375 (0.15) respectively). However, PFHxS exposure levels were not associated with vaginal deliveries. As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Delivery complications

Nolan et al. (85) investigated prenatal exposure to PFAS and labour and delivery complications in women. The cross-sectional study of 1,548 highly exposed women in Washington County, Ohio, reported a significant positive association between estimated maternal exposure to PFOA and occurrences of dysfunctional labour, including cervical, foetal, uterine and iatrogenic complications (OR (95% CI); 5.37 (1.31, 22.0). Nolan et al. (85) further examined the association between modelled PFOA exposure and 16 additional labour and delivery complications, including precipitous labour, prolonged labour, excessive bleeding and seizure during labour, anaesthetic complications, foetal distress, breech birth, cephalopelvic disproportion, umbilical cord prolapse, placenta previa, abruptio placenta, membrane rupture, meconium and febrile. Overall, the study indicated that parental exposure to PFOA was not associated with adverse delivery complications for pregnant women. Nolan et al. (85) reported findings associated with estimated maternal exposure to PFAS, rather than blood serum measurements.

Gender outcomes

In a cohort study of 223 women enrolled in the Longitudinal Investigation of Fertility and the Environment (LIFE) study, Bae et al. (86) investigated the relationship between prenatal exposure to PFAS and the odds of a pregnant woman giving birth to a male. The study reported no significant association between maternal concentrations of PFOA, PFOS, PFNA, PFDA, PFOSA, Et-PFOSSA and Me-PFOSSA. The investigators found a significant negative association between paternal PFNA and Me-PFOSSA exposure levels and the odds of giving birth to a male (OR (T3-T1) (95% CI); 0.43 (0.21, 0.88) and 0.34 (0.13, 0.89) respectively).

Bae et al. (86) concluded that these significant associations may have been due to chance and the study was evaluated as having a high risk of bias. As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Maternal outcomes

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Preeclampsia	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpS	Inadequate evidence
Eclampsia	PFOA	Inadequate evidence
Pregnancy induced hypertension	PFOA, PFOS	Inadequate evidence
Gravidity	Umbilical cord; PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Parity	Umbilical cord and maternal; PFOA, PFOS, PFHxS	Inadequate evidence

Preeclampsia

Preeclampsia is a complication of pregnancy, which usually occurs after 20 weeks of pregnancy. It is characterized by high blood pressure and signs of damage to other organs, such as the kidneys and the liver.

Three papers reported the association between maternal exposure to PFAS and a diagnosis of preeclampsia in pregnant women. (72, 75, 87) Savitz et al. (72) reported a positive relationship between maternal exposure levels of PFOA and preeclampsia in 11,737 pregnancies in women from the C8 Health Project, however, results were not statistically significant. Starling et al. (87) and Stein et al. (75) found no association between exposure to PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpS and preeclampsia. The studies evaluated suggest that elevated levels of PFAS in pregnant women are not associated with preeclampsia.

Eclampsia

Eclampsia is a progression of preeclampsia during pregnancy. The condition is diagnosed in women with preeclampsia that begin to have seizures, which often result in a mother delivering their child before full-term. Nolan et al. (85) studied the effect of maternal exposure to PFAS and eclampsia in pregnant women and found no statistically significant association.

Pregnancy induced hypertension

Pregnancy induced hypertension is defined as systolic blood pressure (SBP) >140 mmHg and diastolic blood pressure (DBP) >90 mmHg. It effects 6-10% of pregnancies, and is often an indication of preeclampsia. Darrow et al. (57) and Nolan et al. (85) investigated the association between maternal exposure to PFAS and a diagnosis of pregnancy induced hypertension. The studies reported no significant association between maternal concentrations of PFOA and pregnancy induced hypertension in women, and Darrow et al. (82) further reported no significant results for maternal PFOS exposure.

Gravidity and parity

Gravidity refers to the number of times a woman has become pregnant, with nulligravida meaning that the woman has never been pregnant, and multigravida meaning multiple pregnancies, regardless of the outcome. Parity refers to the number of times a woman's

pregnancy has lasted to a viable gestational age. The term ‘nulliparous’ refers to a woman who has not given birth.

In a cohort study of approximately 100 deliveries by Canadian women, Arbuckle et al. (54) reported on the association between prenatal exposure to PFAS and gravidity. The study reported a significant negative association between umbilical cord measurements of PFOS and PFHxS and gravidity (logistic regression coefficient (SE); -0.182 (0.05), and -0.215 (0.07) respectively). In this study many tests results for PFHxS were below the analytical methods limit of detection. Arbuckle et al. (54) found no significant association between umbilical cord measurements of PFOA and PFNA and gravidity. Lee et al. (66) investigated the association between prenatal exposure to PFAS and parity, and found no significant relationship between maternal and umbilical cord concentrations of PFOA, PFOS and PFHxS and the health outcome. Arbuckle et al. (54) and Lee et al. (66) investigated different pregnancy outcomes making it difficult to interpret overall findings. As Arbuckle et al. (54) was the only study to report a statistically significant negative association between PFOS and PFHxS exposures and gravity, we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Neonatal and infant diagnoses

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Congenital abnormalities	Maternal; PFOA, PFOS	Inadequate evidence
Cerebral palsy	Maternal; PFOA, PFOS, PFHxS, PFNA. PFDA, PFHpS	Inadequate evidence

Congenital anomalies

Four studies investigated the association between prenatal exposure to PFAS and congenital anomalies in neonates. (72, 75, 85, 88) All studies investigated maternal exposure levels of PFAS on the health outcome, with Nolan et al. (85), Savitz et al. (72) and Stein et al. (88) using estimated PFAS exposure and not concentrations of PFAS in maternal serum. Overall, investigators did not identify significant associations between exposure to PFAS and congenital anomalies, with the exception of one study identifying a significant positive association between PFOA exposure and congenital brain defects. (88)

Savitz et al. (72) and Stein et al. (75) investigated the association between maternal exposure to PFAS and birth defects of any definition in neonates. Both studies reported no significant relationship between PFOA exposure levels and birth defects, and Stein et al. (76) further concluded no association for PFOS. In a cohort of 10,105 mother-infant pairs from the C8 Health Project, Stein et al. (88) investigated the effect of maternal exposure to PFOA on 8 birth defects. The study reported a significant positive association between modelled PFOA exposure and brain defects in neonates (crude OR (95% CI); 2.6 (1.2–5.4)), but did not identify significant findings for craniofacial, heart, gastrointestinal, genitourinary, kidney, limb, and eye defects. Nolan et al. (70) determined the relationship between estimated maternal exposure to PFOA and 12 congenital anomalies and reported no significant association between the exposure and health outcomes. The 12 congenital anomalies included heart malformation, circulatory malformation, anencephalus, spinabifida, tracheoesophageal fistula, omphalocele, cleft lip, polydactyly, Down syndrome and club foot. Congenital anomalies of any definition and other congenital anomalies not listed were also studied by Nolan et al. (70) The 4 studies report no significant associations between parental exposure to PFAS and an array of congenital anomalies. The findings for maternal exposure to PFOA and congenital abnormalities present inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Cerebral Palsy

Liew et al. (89) investigated the association between prenatal exposure to PFAS and occurrence of cerebral palsy in infants among 156 offspring diagnosed with cerebral palsy from the DNBC Study (1996-2002). The authors found a significant positive relationship between maternal concentrations of PFOA, PFOS and PFHpS and cerebral palsy diagnosis in male infants only (risk ratio (95% CI); 2.1 (1.2–3.6), 1.7 (1.0–2.8) and 1.5 (1.0–2.2) respectively). They did not identify a significant association between PFHxS, PFNA and PFDA and cerebral palsy. Liew et al. (89) found no relationship between maternal concentrations of PFAS and cerebral palsy in females. As the study by Liew et al. (89) was only a single study reporting this statistically significant association we considered this to be inadequate evidence

of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Growth during infancy

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Weight	Maternal; PFOA, PFOS, PFHxS	Inadequate evidence
Height	Maternal; PFOA, PFOS	Inadequate evidence
BMI	Maternal; PFOA, PFOS	Inadequate evidence

Weight

Two papers reported on the relationship between prenatal exposure to PFAS and weight measurements for children during early infancy. (52, 69) In a randomly selected sample of 1,400 mother-infant pairs from the Aarhus Birth Cohort, Andersen et al. (52) reported a significant negative association between maternal concentrations of PFOS and weight measurements in infants at 12 months old (estimated change in weight (g) (95% CI); -5.8 (-10.4, -1.2)). Investigators reported effect modification by sex, with the association observed in male but not in female infants. The study further found no statistically significant association between maternal PFOA and PFOS and weight at five months, and no statistically significant association between PFOA levels and weight at 12 months. In the ALSPAC cohort study, Maisonet et al. (69) reported a statistically significant positive association between maternal PFOS concentrations and weight measurements at 20 months in girls (estimated change in weight (g) (T3-T1) (95% CI); 579.82 (301.40, 858.25)). The study also reported no statistically significant findings for the relationship between maternal PFOA and PFHxS and weight at 20 months.

It is difficult to compare the findings of Andersen et al. (52) and Maisonet et al. (69) given the contrasting measurements used to define changes in weight during infancy. Thus, we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Height

Andersen et al. (52) determined the association between prenatal exposure to PFAS and height measurements in infants during their first year of life. The study reported no significant relationship between concentrations of PFOA and PFOS in maternal serum and the height of infants at 5 and 12 months old.

Body Mass Index

Andersen et al. (52) reported the association between prenatal exposure to PFAS and BMI measurements for infants in their first year of life. The study of 1,400 randomly sampled mother-infant pairs enrolled in the Aarhus Birth Cohort found no statistically significant relationship between maternal levels of PFOA and PFOS and BMI calculations in infants at 5 months, though reported a significant negative association between PFOS and BMI at 12 months (z Score (95% CI); -0.007 (-0.011, -0.002)). These significant changes in BMI are likely attributable to changes in weight measurements found in the study. As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Reproductive outcomes

We evaluated 34 papers examining the association of PFAS exposure with reproductive outcomes in children and adults. The studies primarily examined the association between prenatal and adulthood exposure levels of PFOA, PFOS, PFHxS and PFNA and adverse reproductive outcomes. A wide-range of reproductive effects were investigated in the studies, including concentrations of reproductive hormones, time to pregnancy in women (fecundity) and semen quality in men. Most reviewed papers were determined to have a high risk of bias. The papers are summarised in Appendix 8. Time to pregnancy and infertility outcomes were eligible for meta-analysis in relation to PFOA and PFOS exposure.

Reproductive hormone levels

Testosterone (T)

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
T levels in male adults	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA PFHpS	Inadequate evidence
T levels in female adults	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
T levels in boys	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHxA, PFDoA, PFUdA, PFTEDA, PFBS	Inadequate evidence
T levels in girls	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHxA, PFDoA, PFUdA, PFTEDA, PFBS	Inadequate evidence
Free Testosterone (FT) levels in male adults	PFOA, PFOS	Inadequate evidence

Testosterone (T) is a male sex hormone, which is important for male reproductive development. Total T refers to T measured in blood, while Free Testosterone (FT) refers to T that is not bound to sex hormone binding globulin. Eleven studies investigated the effect of elevated PFAS exposure levels on concentrations of T in males and females. (34, 90-99) Of these studies, three related to T levels in male adults, three related to T levels in children and four related to the effects of prenatal exposure to PFAS (two related to T levels in children and adults, separately). One study related to the effects of PFAS exposure on T levels in adolescents and young adults aged 12 to 30-years old. Overall, these studies suggest opposing effects of PFAS exposure on T concentrations in males and females; elevated PFAS exposure was correlated with reduced levels of T in males and higher levels of T in females.

Male adult studies

In a cohort of 247 healthy Danish males, Joensen et al. (99) found a significant negative association between T for a 1 ng/mL increase in PFOS (regression coefficient (95% CI); -0.010 (-0.020, 0.000)). The study also reported a negative association between free T and

PFOS (regression coefficient 1 ng/mL increase β (95% CI); -0.016 (-0.026, -0.006)). Joensen et al. (99) reported no significant findings related to PFOA, PFHxS, PFNA, PFDA and PFHpS exposures. In an earlier study in a cohort of 105 Danish males, Joensen et al. (34) reported no significant relationship between PFOA and PFOS exposure and measurements of T. Olsen et al. (93) reported no association between PFOA exposure and T and FT in a cohort of male 3M employees. Raymer et al. (94) found no association between PFOA and PFOS exposures and T in a study of 256 men who visited the Duke University's Medical Centre's IVF Clinic between the years 2002–2005. However, Raymer et al. (94) reported a positive correlation between serum concentrations of PFOA and free T levels in men (Spearmen correlation *r* (p); 0.155 (0.015)). The study found no significant results related to PFOS exposure. Tsai et al. (96) found no significant relationship between PFOA, PFOS, PFNA and PFUdA and T in males aged 18 to 30-years old.

Male child studies

In a study of children aged 6 to 9-years old enrolled in the C8 Health Project, Lopez-Espinosa et al. (91) found a significant negative association between T levels and PFOA (adjusted difference (%) per IQR increment of PFOA (95% CI); -4.9 (-8.7, -0.8)) and PFOS (adjusted difference (%) per IQR increment of PFOS (95% CI); -5.8 (-9.4, -2.0)) in males. In Taiwanese males aged 13–15-years old enrolled in the Genetics and Biomarkers study for Childhood Asthma (GBCA) study, Zhou et al. (98) reported a significant negative association between PFOS (multivariate linear regression β (95% CI); -0.0029 (-0.0055– -0.0003)), PFNA (multivariate linear regression β (95% CI); -0.4233 (-0.6998– -0.1467)), PFDA (multivariate linear regression β (95% CI); -0.2565 (-0.4135– -0.0994)) and PFHxA (multivariate linear regression β (95% CI); -0.3095 (-0.5942– -0.0248)) and T concentrations in serum. However, the study found no association between PFOA, PFHxS, PFBS, PFDoA and PFTEDA exposures and T concentrations. Tsai et al. (96) reported no relationship between PFOA and PFOS exposures and T levels in Taiwanese males aged 12–17 years old, though found no significant effects related to PFNA and PFUdA exposures.

Pregnant female studies

Toft et al. (95) investigated the effects of prenatal exposure to PFOS on hormone levels in mothers pregnant with males registered on the Danish National Patient Registry. The study, which included 270 cases of cryptorchidism, 75 causes of hypospadias and 300 healthy controls, found a significant increase in T in amniotic fluid related to elevated PFOS exposure levels (% increase T1-T3 (95% CI); 18 (7, 29)). Through a prospective investigation of the Sapporo Cohort of the Hokkaido Study, Itoh et al. (90) investigated the effect of prenatal PFAS exposure on umbilical cord measurements of reproductive hormones in neonates. The study reported no significant relationship between PFOS and PFOA exposures and T level at birth.

Male and female adolescent studies

In a study of Danish males aged 19 to 21-years old enrolled in the Aarhus Birth Cohort, Vested et al. (97) did not find any associations between prenatal exposure to PFOA and PFOS and T levels in adolescent males. In 72 adolescent females from the ALSPAC cohort study, Maisonet et al. (92) found that prenatal exposure to PFOA (adjusted linear regression β T1-T3 (95% CI); 0.24 (0.05, 0.43)), PFOS (adjusted linear regression β (T3-T1) (95% CI); 0.18 (0.01 – 0.35)) and PFHxS (adjusted linear regression β (T3-T1) (95% CI); 0.18 (0.00, 0.35)) increased

T concentrations. Maisonet et al. (92) did not find evidence of associations between PFNA and T in this cohort.

Female child and adolescent studies

In girls aged 6–9-years old, Lopez-Espinosa et al. (91) found a negative relationship between PFOS exposure and T (adjusted difference (%) per IQR increment of PFAS (95% CI); -6.6 (-10.1, -2.8)). Tsai et al. (96) reported a significant negative association between PFOS and T levels (mean ln (T) level (SE); Q1=3.97 (0.23) and Q4=3.61 (0.36)) for 12–17-year old females. Tsai et al. (96) found no effect of PFOA, PFNA and PFUdA on T levels in girls aged 12–17-years old, and no association between PFOA, PFOS, PFNA and PFUdA and T in women aged 18–30 years old. In females aged 13–15 years old, Zhou et al. (98) found increased exposure levels of PFDoA were negatively associated with serum measurements of T (multivariate linear regression ln(T) β (95% CI); -0.0119 (-0.0227, -0.0010)). The study reported no association for PFOS, PFOA, PFBS, PFDA, PFHxA, PFHxS, PFNA and PFTEDA and T in females aged 13–15-years old.

In summary, the 11 evaluated studies provide conflicting evidence for the association between PFAS exposure levels and serum concentrations of T. While the results are inconsistent for PFOA, PFOS, PFHxS and PFNA exposures, the 4 studies which reported significant findings suggest that elevated PFAS levels result in decreased T levels in males, but the direction of change in T levels in adolescent females is unclear. However, there is no evidence to the effect of PFAS on T levels in adult females. Further, the association between PFAS and T concentrations differs between measurements in children, adolescents and adults, and therefore, results were not combined for males and females, or across the lifespan. Due to this, the significant effects of PFDA and PFHxA levels in boys, and PFDoA levels in girls were defined as limited evidence. Therefore, the literature does not support an association between elevated PFAS levels and changes in T.

Oestradiol (e2)

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
e2 levels in male adults	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFHxA, PFTEDA, PFBS	Inadequate evidence
e2 levels in female adults	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFOSA	Inadequate evidence
e2 levels in boys	PFOA, PFOS	Inadequate evidence
e2 levels in girls	PFOA, PFOS	Inadequate evidence

Oestradiol (e2) is an oestrogen and is the principal female sex hormone. The effect of PFAS exposure on e2 levels in males and females was investigated in 10 studies. (34, 90, 91, 93, 94, 97-101) Overall, the literature does not suggest an association between elevated PFAS levels and increased e2 in males and females, however, results are inconsistent for PFOA, PFOS and PFHxS exposures.

Male studies

In 225 Taiwanese adolescents aged 13–15-year olds, Zhou et al. (98) reported that serum concentration of e2 significantly increased with elevated levels of PFOA (multivariate linear regression β (95% CI); 0.0921 (0.0186, 0.1656)) and PFHxS (multivariate linear regression β (95% CI); 0.0462 (0.0020, 0.0905)) in males. The study stated no association related to PFOS, PFBS, PFDA, PFDoA, PFHxA, PFNA and PFTEDA exposures, and further, no effect in females. Two studies by Joensen et al. (34, 99) stated no association between PFOA and PFOS and e2 levels in males, and Joensen et al. (99) further stated no significant findings related to PFHxS, PFNA, PFDA and PFHpS exposures. Raymer et al. (94) reported no association between PFOA and PFOS exposures and e2 levels in males and Olsen et al. (93) found no significant results related to PFOS exposure. Therefore, there is inconsistent evidence presented for the association between PFOA and PFHxS and e2 levels in male adults across these 5 studies.

Female studies

Barrett et al. (100) reported a negative association between PFOS and PFOSA exposures and e2 levels in reproductive-age women enrolled in the Energy Balance and Breast Cancer Aspects study; however, results were significant only for nulliparous women. The study reported non-significant results for e2 and PFOA, PFNA, PFDA, PFUdA and PFHxS exposures. Knox et al. (101) also found a significant negative association between PFOS and e2 concentrations in women enrolled in the C8 Health Project (perimenopausal age group – β (*p* value); -3.65 (*p* <0.0001); menopausal age group – β (*p* value); -0.83 (*p*=0.007)). Thus, several PFAS are not associated with changes in e2 levels in women, however, there is limited evidence to suggest that PFOS and PFOSA is negatively associated with e2 level, as reported by Barrett et al. (100) and Knox et al. (101) While the study conducted by Barrett et al. (100) was associated with a low risk of bias assessment, the study by Knox et al. (101) was evaluated to have a high risk of bias.

Child and adolescent studies

Lopez-Espinosa et al. (91) concluded that elevated PFOS concentrations in serum were related to decreased levels of e2 in males aged 6 to 9-years old (adjusted difference (%) per IQR increment of PFAS (95% CI); -4.0 (-7.7, -0.1)). Itoh et al. (90) in the Sapporo cohort of the Hokkaido study found that increased maternal PFOS concentration significantly increased e2 levels in male (adjusted regression coefficient β (95% CI); 0.372 (0.057, 0.687)), but not female infants. Vested et al. (97) reported no significant findings related to prenatal PFOA and PFOS exposure levels and e2 in male adolescents. There is inconsistent evidence of an effect of PFAS on e2 in boys and no evidence related to the effect of PFOS and PFOA on e2 levels in girls.

Luteinizing hormone (LH)

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
LH levels in male adults	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpS	Inadequate evidence
LH levels in female adults	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
LH levels in boys	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
LH levels in girls	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence

Luteinizing hormone (LH) is a hormone that stimulates secretion of sex steroids from the testicles or ovaries. Seven studies investigated the association between increased PFAS exposure levels and LH serum concentrations in men and women. (34, 90, 93, 94, 96, 97, 99) In 249 men of reproductive age, Raymer et al. (94) reported a positive correlation between serum concentrations of PFOA and LH levels (Spearmen correlation *r* (*p*); 0.162 (0.011)) and PFOS (Spearmen correlation *r* (*p*); 0.121 (0.057)). Joensen et al. (99) did not find any association between PFOA, PFOS, PFHxS, PFNA, PFDA and PFHpS exposures and LH in male adults. Joensen et al. (34) had also previously found no effect of PFOA and PFOS exposures on LH levels in males. Olsen et al. (93) found no association between occupational exposure to PFOA and LH concentrations in males and Tsai et al. (96) reported no association between PFOA, PFOS, PFNA and PFUdA and LH in men and women aged 18–30-years old. In summary, the literature does not suggest a significant association between PFAS exposures and changes in LH in adults, although Raymer et al. (94) did report positive associations with PFOA and similar results for PFOS of borderline statistical significance.

Vested et al. (97) concluded that an increased prenatal exposure to PFOA was related to an increased level of LH (adjusted β IU/L (SE); 0.04 (0.02)) in adolescent males, but there was no association for PFOS. Tsai et al. (96) found no significant effects related to PFOA, PFOS, PFNA and PFUdA in males and females aged 12–17-years old and Itoh et al. (90) did not find any association between prenatal exposure to PFOA and PFOS and LH levels in infants. There is inconsistent evidence for a positive association between PFOA and LH levels in adolescent males, and no evidence suggesting an effect in adolescent females or infants.

Follicle-stimulating hormone (FSH)

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
FSH levels in male adults	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpS	Inadequate evidence
FSH levels in female adults	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
FSH levels in boys	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
FSH levels in girls	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence

Follicle-stimulating hormone (FSH) is a hormone that manages the menstrual cycle and stimulates maturation of the ovaries in females and is involved in the development of testes and stimulates the production of sperm in males. The effect of PFAS exposure on serum FSH concentrations was investigated in seven studies. (34, 90, 93, 94, 96, 97, 99) The literature does not support an association between PFAS and FSH levels in adults. Joensen et al. (34, 99) found no association between PFOA, PFOS, PFHxS, PFNA, PFDA and PFHpS exposures and FSH levels in males. Tsai et al. (96) found no significant relationship between PFOA, PFOS, PFHxS and PFNA and FSH levels in males and females aged 18–30 years old. Raymer et al. (94) also reported no association between PFOA and PFOS exposures and FSH in males and Olsen et al. (93) stated no significant results related to PFOA and FSH.

In contrast, results are conflicting for the effect of PFAS exposure on FSH in adolescents. Tsai et al. (96) found that increasing PFOS was associated with a negative trend in FSH concentrations in Taiwanese males aged 12–17-years old (p for trend <0.05) and that serum FSH level in the 12–17-year old females was also decreased (p for trend <0.01) with increasing categories of PFUdA concentration. In contrast, Vested et al. (97) concluded that an increased prenatal exposure to PFOA was related to increased levels of FSH in male adolescents (adjusted β IU/L (SE); 0.06 (0.02)); however, found no association for PFOS. Itoh et al. (90) found no association between prenatal exposure to PFOA and PFOS and FSH levels in infants. In summary, Tsai et al. (96) and Vested et al. (97) report opposing results for the effect of PFOA and PFOS exposures and FSH levels in male adolescents, and therefore, these exposure-effect associations remain unclear.

Sex hormone-binding globulin (SHBG)

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
SHBG levels in male adults	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpS	Inadequate evidence
SHBG levels in female adults	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
SHBG levels in boys	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
SHBG levels in girls	PFOA, PFOS, PFHxS, PFNA, PFUdA	Inadequate evidence

Sex hormone binding globulin (SHBG) is a glycoprotein with high affinity for other hormones, such as testosterone and oestradiol. Seven studies investigated the association between PFAS exposure levels and SHBG in males and females. (34, 90, 92, 93, 96, 97, 99) Conclusively, the results did not support a significant effect related to increased PFAS exposure and SHBG levels in males of all ages. Joensen et al. (99) investigated the association between PFAS exposure and SHBG levels in male semen, and reported no significant findings related to PFOA, PFOS, PFHxS, PFNA, PFDA and PFHpS. Joensen et al. (34) found no association between PFOA and PFOS and SHBG concentrations in males. Tsai et al. (96) stated no significant relationship between PFOA, PFOS, PFHxS and PFNA and SHBG levels in males and Olsen et al. (93) found no effects related to PFOA exposure levels. Vested et al. (97) found no association between prenatal exposure to PFOA and PFOS and SHBG levels in male adolescents.

Tsai et al. (96) reported a significant decrease in SHBG hormones related to elevated levels of PFOA (mean PFAS level (SE); Q1–3.50 (0.24) and Q4–2.96 (0.34)) in females aged 12–17-years old, but did not find a significant effect in women aged 18–30-years old. In contrast, Maisonet et al. (92) did not find evidence of associations between prenatal exposure levels of PFOA, PFOS, PFHxS and PFNA and SHBG levels in adolescent girls. Itoh et al. (90) stated no association between prenatal exposure to PFOA and PFOS and SHBG levels in infants. Thus, the literature does not support an association between PFAS and SHBG levels in children or adults, although results are inconsistent for PFOA and SHBG levels in female adolescents, as reported by Tsai et al. (96).

Other reproductive hormones

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
FAI levels	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHpS	Inadequate evidence
T: LH levels	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHpS	Inadequate evidence
FAI: LH levels	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHpS	Inadequate evidence
FT: LH levels	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHpS	Inadequate evidence
Inhibin B levels	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHpS	Inadequate evidence
P4 levels	PFOA, PFOS	Inadequate evidence
PRL levels	PFOA, PFOS	Inadequate evidence
INSL-3 levels	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Cortisol levels	PFOA	Inadequate evidence
DHEAS levels	PFOA	Inadequate evidence
17-HP levels	PFOA	Inadequate evidence
IGF-1 levels	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
P levels	PFOA, PFOS, PFHxS PFNA, PFDA, PFUdA, PFOSA	Inadequate evidence

Nine studies reported on the effect of PFAS on additional reproductive hormones in men and women. (34, 90, 91, 93-95, 97, 99, 100) Joensen et al. (99) concluded a significant negative association between PFOS exposure levels in male adults and concentrations of Free Androgen Index (FAI) and ratios of T: LH, FAI: LH and FT: LH in their semen. The study reported no significant findings related to PFOA, PFHxS, PFNA, PFDA and PFHpS exposures, and no association between all 6 PFAS exposures and inhibin B levels in semen. Similarly, Joensen et al. (34) found no effects of elevated PFOA and PFOS levels and inhibin B concentrations in male semen. Vested et al. (97) found no association between prenatal exposure to PFOA and PFOS and inhibin B and FAI hormone levels in male adolescents.

Among male infants, Itoh et al. (90) found a significant negative association between PFOS and Progesterone (P4) (adjusted regression coefficient β (95% CI); -0.344 (-0.678, -0.010)) and Inhibin B (adjusted regression coefficient β (95% CI); -0.439 (-0.620, -0.257)). Itoh et al. (90) also found negative associations between PFOS and P4 (adjusted regression coefficient β (95% CI); -0.552 (-0.894, -0.210)) and prolactin (PRL) (adjusted regression coefficient β (95% CI); -0.491 (-0.764, -0.218)) concentrations in female infants. Itoh et al. (90) did not find significant results related to PFOA exposure and inhibin B and Insulin-like Factor 3 (INSL-3). Raymer et al. (94) reported no association between PFOA and PFOS exposures and PRL in males. Olsen et al. (93) stated no association between PFOA and several hormone levels including cortisol, dehydroepiandrosterone sulfate (DHEAS), 17-alpha-hydroxyprogesterone (17-HP) and PRL in male adults.

Lopez-Espinosa et al. (91) investigated hormones in 2,292 6–9 year old children in the C8 Health Project. They found that elevated PFOS and PFNA concentrations in serum were related to decreased levels of Insulin-like Growth Factor 1 (IGF-1) in male children (adjusted difference (%) per IQR increment of PFOS (95% CI); -5.9 (-8.3, -3.3)) and (adjusted difference (%) per IQR increment of PFNA (95% CI); -3.5 (-6.0, -1.0)). Similarly, the same effect was seen in females between PFOS and PFNA and IGF-1 levels (adjusted difference (%) per IQR increment of PFOS (95% CI); -5.6 (-8.2, -2.9)) and (adjusted difference (%) per IQR increment of PFNA (95% CI); -3.8 (-6.4, -1.2)). Toft et al. (95) found a significant reduction in INSL-3 concentration in amniotic fluid related to elevated PFOS exposure levels (% decrease T1 vs T3 (CI); 40 (69–11)), though there were no significant results related to PFOA, PFHxS and PFNA. Further, Barrett et al. (100) reported a negative association between PFOS and PFOSA exposures and proline (P) in nulliparous women. The study reported non-significant results for P levels and PFOA, PFNA, PFDA, PFUdA and PFHxS exposures.

In summary, the nine studies suggest that across all PFAS exposures, there is inconsistent evidence to support an association between elevated PFOS levels and decreased levels of reproductive hormones in men and women of all ages. While some evidence is conflicting, negative associations were reported for FAI, T: LH, FAI: LH, FT: LH, P4, PRL, IGF-1, INSL-3 and P. In contrast, there is not a large body of evidence to suggest other PFAS have a significant effect on reproductive hormone levels in men and women, or adults and children.

Time to pregnancy (TTP), fecundity and infertility

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
TTP	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpS, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Infertility	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpS	Inadequate evidence

TTP and fecundity

Nine papers reported on the association between elevated PFAS exposure levels and Time to Pregnancy (TTP) in women. (102-110) In summary, the literature did not support an association between serum PFAS concentrations and a longer time to pregnancy, however, 2 studies reported significant results related to PFOA, PFOS and PFHxS exposures.

Fei et al. (104) reported a significant negative association between TTP in women enrolled in the DNBC and PFOA (fecundity OR Q1-Q4 (95% CI); 0.60 (0.47, 0.76)) and PFOS (fecundity OR Q1-Q4 (95% CI); 0.74 (0.58, 0.93)) exposures. The fecundity OR estimates the odds of conception in a cycle among exposed compared to the unexposed. Through a cohort of 2,001 Canadian women enrolled in the Maternal-Infant research on Environmental Chemical (MIREC) study, Velez et al. (107) reported a significant reduction in fecundity related to PFOA (adjusted fecundity OR per 1 SD increase (95% CI); 0.89 (0.83, 0.94)) and PFHxS (adjusted fecundity OR per 1 SD increase (95% CI); 0.91 (0.86, 0.97)) exposures; however, found no association for PFOS.

Bach et al. (102) concluded no significant association between PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA and PFHpS exposures and fecundity. Buck Louis et al. (103) found that fecundity was not affected by PFOSA exposure. Jørgensen et al. (105) stated no association between PFOA, PFOS, PFHxS and PFNA and TTP and Lum et al. (106) further reported no relationship between PFOA, PFOS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH and Et-PFOSA-AcOH. Vestergaard et al. (108) stated no significant effects related to eight PFAS exposures and TTP more than six months, including PFOA, PFOS, PFHxS, PFNA, PFDA, Me-PFOSA-AcOH, Et-PFOSA-AcOH and PFOSA. Two studies by Whitworth et al. (109, 110) observed no associations between PFAS and TTP among women. Thus, there is more evidence to suggest there is no association between PFAS exposure levels and time to pregnancy across the nine evaluated studies, despite the inconsistent findings reported by Fei et al. (104) and Velez et al. (107).

Infertility

The effect of PFAS exposure on infertility in women was investigated in 4 studies. (21, 102, 104, 107) In contrast to the findings for TTP, 3 of the 4 evaluated studies found elevated PFAS exposure levels are associated with infertility in women. Fei et al. (104) reported a significant positive relationship between exposure to PFOA (adjusted OR Q1-Q4 (95% CI); 2.54 (1.47, 4.39)) and PFOS (adjusted OR Q1-Q4 (95% CI); 1.77 (1.06, 2.95)) and infertility. Velez et al.

(107) found a positive association for PFOA (adjusted OR per 1 SD increased (95% CI); 1.31 (1.11, 1.53)) and PFHxS (adjusted OR per 1 SD increased (95% CI); 1.27 (1.09–1.48)) exposures, though stated no relationship between PFOS levels and infertility. Jørgensen et al. (105) reported a significant increase in infertility in relation to elevated PFNA levels for 938 women from Greenland, Poland and Ukraine enrolled in the INUENDO cohort (pooled-analysis continuous log-scale OR (95% CI); 1.53 (1.08, 2.15)). The study concluded no significant effects related to PFOA, PFOS and PFHxS exposure levels. Bach et al. (102) stated no association between infertility and seven PFAS including PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA and PFHpS. Despite most evaluated studies reporting a significant positive association between PFAS levels and infertility, overall, the evidence is inconsistent, specifically for PFOA, PFOS, PFHxS and PFNA exposures.

TTP and infertility meta-analyses

Six articles reported data on time to pregnancy. (102, 104, 105, 107, 108, 110) The Jørgensen et al. (105) study was conducted in three countries, which we considered as separate studies, thus there are eight relevant studies in total. All studies reported the association between both PFOA and PFOS and outcomes; PFHxS was not considered by Fei et al. (104) and PFNA was not considered by Fei et al. (104) and Velez et al. (107), thus there were eight potential studies for PFOA and PFOS, seven for PFHxS and six for PFNA.

Exposures were assessed from maternal blood samples taken during pregnancy for all studies, except for Vestergaard et al. (108) where exposure was assessed prior to women becoming pregnant. The follow-up period for Vestergaard et al. (108) was only six months, compared to 12–13 months for all other studies and was not considered comparable for inclusion in the meta-analyses.

Five studies used categorised exposure for PFOA and PFOS and met the eligibility criteria for the minimum number of studies required to undertake a meta-analysis. There were an inadequate number of studies which categorised PFHxS or PFNA exposure, or with comparative continuous scale measures of any of the PFAS measures for inclusion in a meta-analysis. Two meta-analyses were conducted for time to pregnancy outcomes (fecundity and infertility) for PFOA and PFOS exposures categorised in the highest quantiles relative to the lowest quantiles.

There was substantial heterogeneity in study effects regarding fecundity for both PFOA (I^2=77.30%; Q=17.65; df=4; p=0.001) (Figure 6) and PFOS (I^2=50.6%; Q=8.10; df=4; p=0.088) (Figure 7). The overall measures of effect were non-significant at the 5% level for PFOA (pooled fixed effects OR (95% CI); 0.92 (0.82, 1.03); p=0.16) and PFOS (pooled fixed effects OR (95% CI); 0.92 (0.82, 1.03); p=0.16). Results for random effects models were consistent with those of fixed effects, with similar pooled point estimates but wider confidence intervals for PFOA (pooled OR (95% CI); 0.91 (0.70, 1.18); p=0.46) and PFOS (pooled OR (95% CI); 0.89 (0.75–1.06); p=0.20). Due to the substantial heterogeneity associated with this outcome, these results should be interpreted with caution.

Figure 6. Fixed effects meta-analysis of fecundity OR for PFOA.

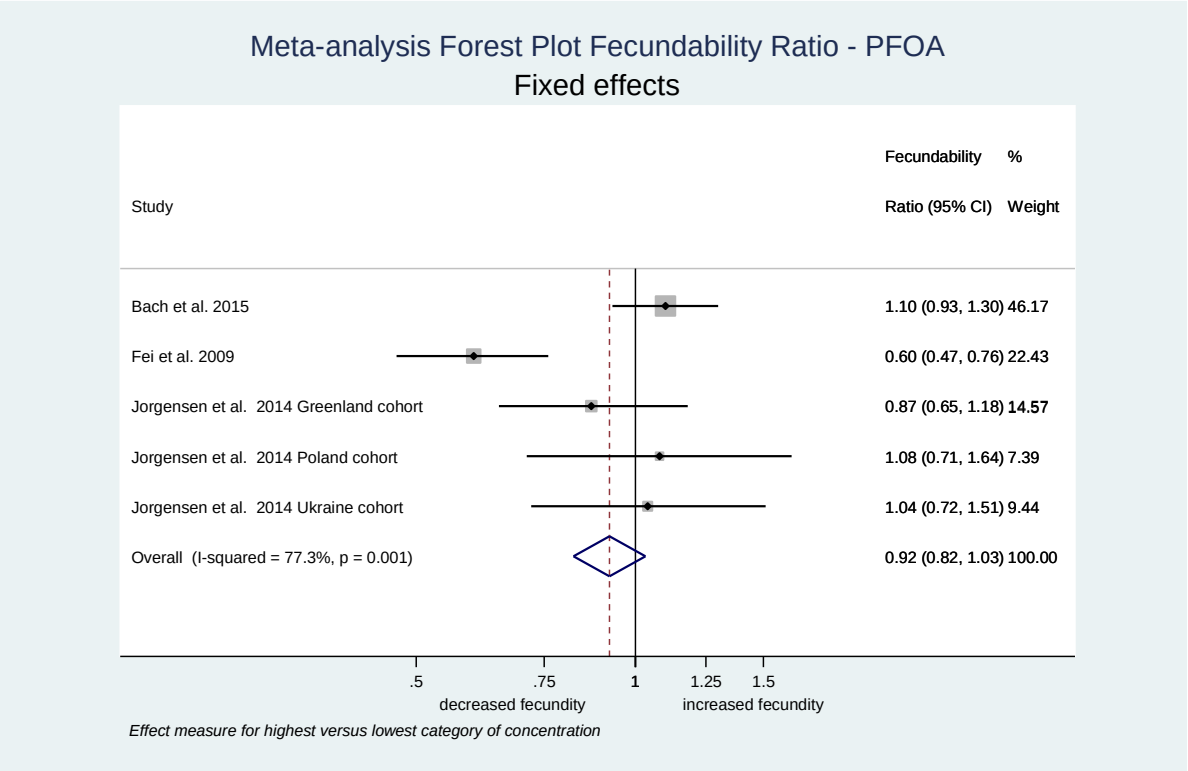
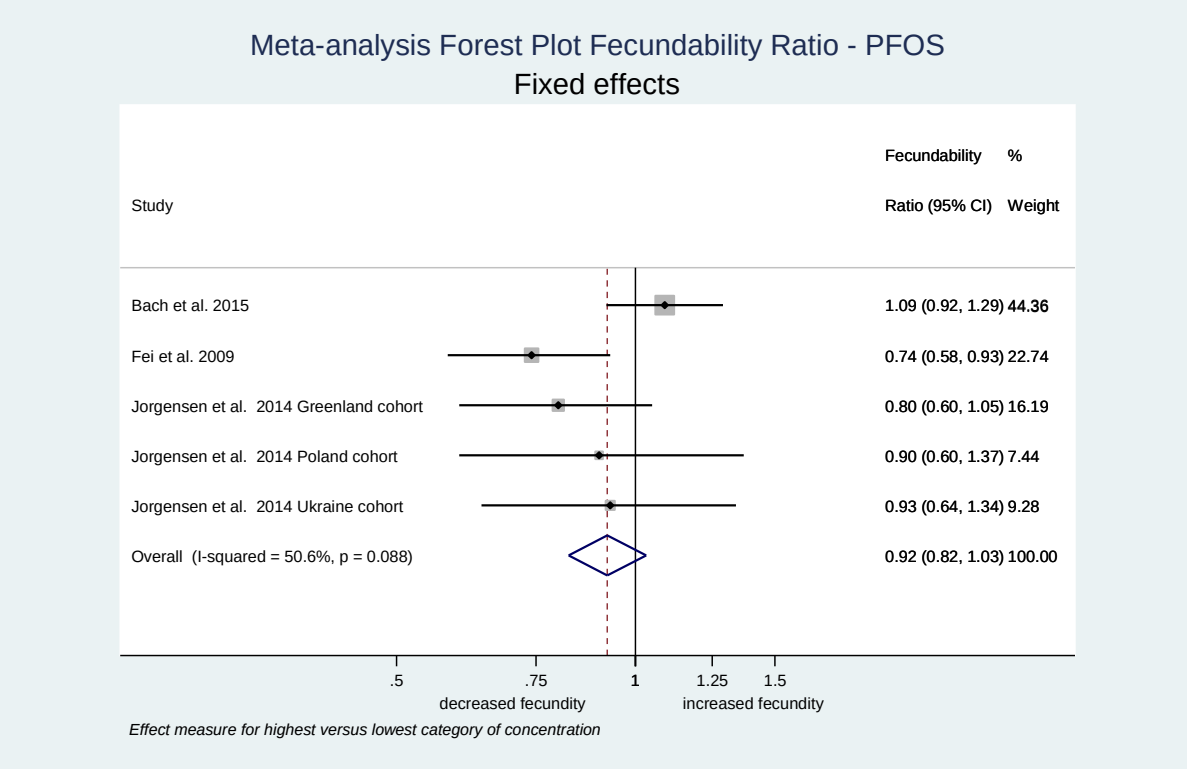


Figure 7. Fixed effects meta-analysis of fecundity OR for PFOS.



There was substantial heterogeneity in study effects for infertility for PFOA (I^2=71.10%; Q=13.86; df=4; p=0.008) (Figure 8) and PFOS (I^2 = 62.50%; Q=10.68; df=4; p=0.030) (Figure 9). The overall measures of effect were non-significant (pooled OR (95% CI); 1.22 (0.93–1.60), p=0.15 and 1.18 (0.91–1.53), p=0.22 for PFOA and PFOS, respectively). Results for random effects models were consistent with those of fixed effects, with similar pooled point estimate but wider confidence intervals (pooled OR (95% CI); 1.3 (0.79–2.30), p=0.28 and 1.32 (0.83–2.10), p=0.24 for PFOA and PFOS, respectively). Due to the substantial heterogeneity associated with this outcome, these results should be interpreted with caution.

Figure 8. Fixed effects meta-analysis of infertility for PFOA.

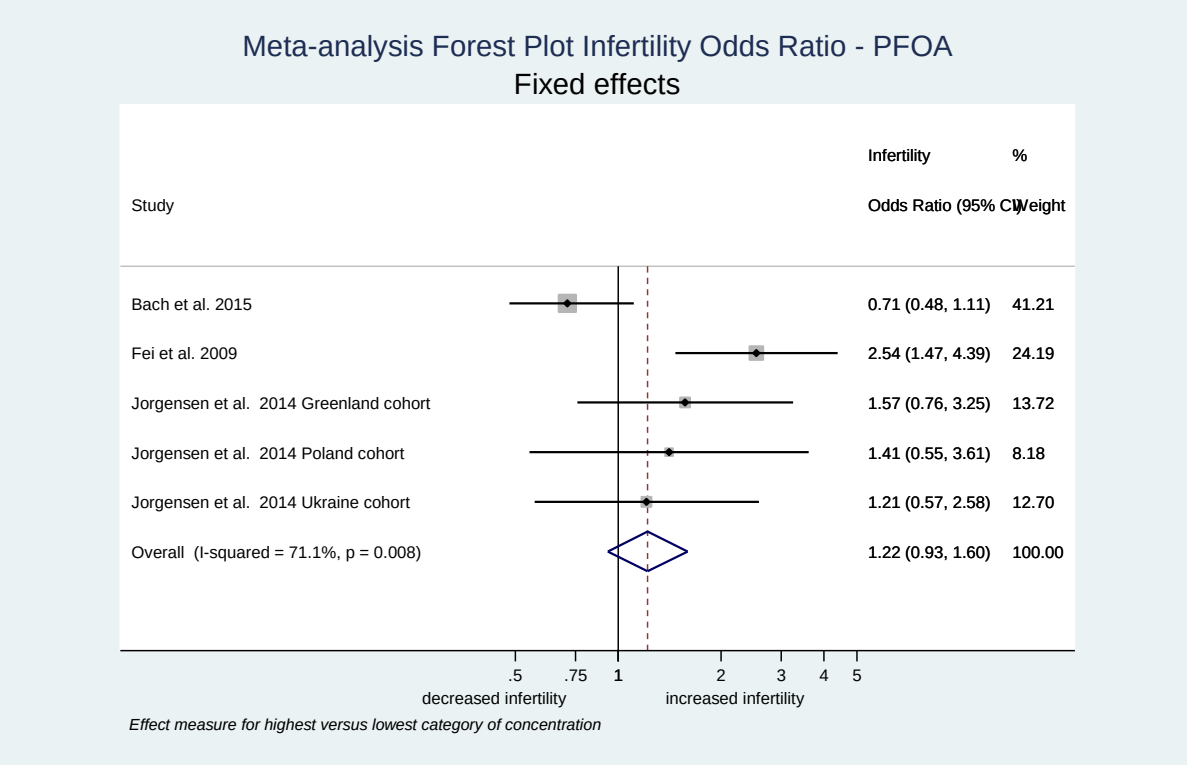
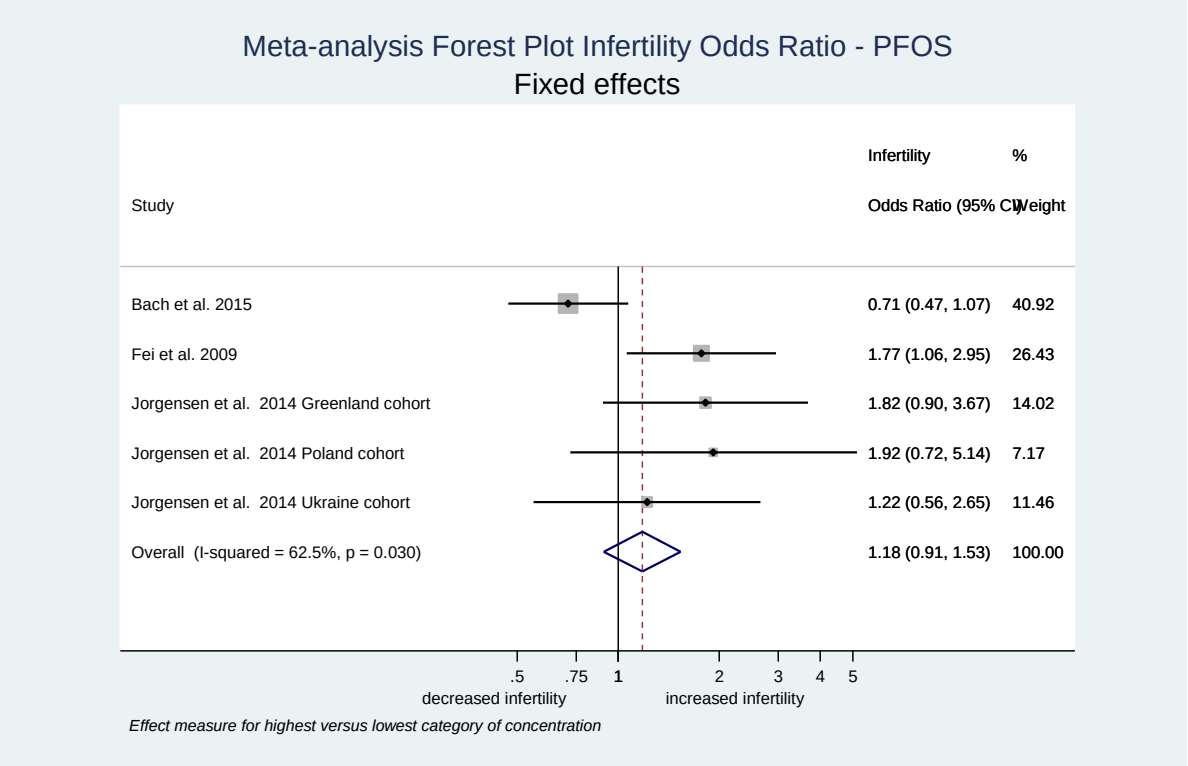


Figure 9. Fixed effects meta-analysis of Infertility for PFOS.



Evaluation

The reported associations between prenatal exposure to PFAS and TTP, fecundity and infertility outcomes across the nine studies were largely inconsistent for PFOA, PFOS, and PFHxS. Generally, the findings for the relationship between these outcomes and PFDA, PFUdA, PFHpS showed no evidence of an association. For TTP and fecundity there was also no evidence of an association with PFNA, Me-PFOSA-AcOH, Et-PFOSA-AcOH. The meta-analyses included results from only 3 of the 9 studies for TTP and fecundity and 3 of the 4 studies for infertility. Of the three studies reporting a statistically significant negative association between maternal PFAS concentrations and TTP, fecundity and fertility, all except the study conducted by Velez et al. (107) were evaluated to have a high risk of bias. Currently, there is inconclusive evidence to identify if exposure to PFAS chemicals negatively effects TTP, fecundity and infertility.

Sperm characteristics

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Sperm counts		
Concentration	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHpS, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Total number	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHpS, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Proportion of normal sperm	PFOA, PFOS, PFHxS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Proportion of immature sperm	PFOA, PFOS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Sperm morphology		
Abnormal head characteristics	PFOA, PFOS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Abnormal tail characteristics	PFOA, PFOS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Abnormal neck or midpiece characteristics	PFOA, PFOS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Sperm motility	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHpS, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Sperm DNA stability	PFOA, PFOS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence

Six studies determined the effect of PFAS exposure on sperm quality in males of reproductive age Buck Louis et al., (111) Joensen et al., (34) Joensen et al., (99) Raymer et al., (94) Toft et al., (112) and Vested et al. (97)). Across the studies, sperm quality was measured based on a number of indicators; sperm counts and concentration, sperm morphology, sperm motility and sperm DNA characteristics. Overall, the studies present conflicting evidence for the association between elevated PFAS levels and adverse sperm qualities; however, a larger number of characteristics were measured in relation to nine PFAS exposures. Therefore, there are a number of exposure-effect associations defined to have limited evidence.

Buck Louis et al. (111) reported significant association between exposure to PFOA, PFOS, PFNA, PFDA, PFOSA and Me-PFOSA-AcOH and 17 indicators of sperm quality in men enrolled in the LIFE study. Higher levels of PFOA exposure were associated with increase sperm motility (µm/sec) (curvilinear velocity—estimated difference per 1-unit increase in PFOA (95% CI); 4.98 (0.14, 9.83)). Exposure to PFDA and PFOSA showed a significant negative association with sperm head length (µm) and perimeter (µm), respectively (estimated difference per 1-unit increase in PFAS (95% CI); -0.16 (-0.30, -0.01)) and -1.25 (-2.28, -0.23),

respectively); however the study also concluded a significant positive association between PFOA and the acrosome area of the sperm head (%) (estimated difference per 1-unit increase in PFOA (95% CI); 1.30 (0.18, 2.89)). Further, PFOSA exposure was negatively related to sperm head area (μm^2) (estimated difference in semen quality per 1-unit increase in PFOSA (95% CI); -2.3 (-4.05, -0.54)).A negative association was also found in relation to sperm chromatin (DNA) stability with PFOSA (high DNA stainability (%)—estimated difference per 1-unit increase in PFOSA (95% CI); -15.15 (-26.56, -3.75)) and Me-PFOSA-AcOH (high DNA stainability (%)—estimated difference per 1-unit increase in Me-PFOSA-AcOH (95% CI); -2.55 (-4.67, -0.44)) exposures.

Buck Louis et al. (111) also found PFOA (estimated difference per 1-unit increase in PFOA (95% CI); -2.77 (-5.54, -0.00)) and PFOS (estimated difference per 1-unit increase in PFOS (95% CI); -2.27 (-4.21, -0.34)) were negatively associated with coiled tail (%). PFNA was positively related to the percent of normal sperm (estimated difference per 1-unit increase in PFNA (95% CI); 3.9 (0.56, 7.23)) and negatively associated with coiled tail (%) (estimated difference per 1-unit increase in PFNA (95% CI); -4.03 (-7.77, -0.29)). PFDA was negatively associated with coiled tail (%) (estimated difference per 1-unit increase in PFDA (95% CI); -7.60 (-14.01, -1.19)). PFOSA was positively related to bicephalic sperm (%) (estimated difference per 1-unit increase in PFOSA (95% CI); 4.13 (0.15, 8.11)) and the number of immature sperm and (estimated difference per 1-unit increase in PFOSA (95% CI); 90.88 (51.27, 130.5)). Me-PFOSA-AcOH was positively associated with neck or midpiece abnormalities in sperm (estimated difference in semen quality per 1-unit increase in Me-PFOSA-AcOH (95% CI); 5.01 (0.72, 9.3)) and the number of immature sperm (estimated difference in semen quality per 1-unit increase in Me-PFOSA-AcOH (95% CI); 18.72 (11.61, 25.83)). Buck Louis et al. (111) found no association between Et-PFOSA-AcOH and sperm quality.

In a study of 588 men from Greenland, Poland and Ukraine enrolled in the INUENDO cohort, Toft et al. (112) reported significant associations between PFAS exposures and sperm quality. Increased PFOS exposure was associated with a reduced proportion of normal cells in sperm (% lower T1 vs T3 (95% CI); 35 (4, 66)). They also found elevated PFOA exposure was related to an increased proportion of motile sperm (% lower T1 vs T3 (95% CI); 19 (1, 39)) and that increased PFHxS exposure levels were associated with a reduced concentration of normal sperm in semen (proportion of normal sperm (%) (T3-T1) (95% CI); -35 (-70, -1). Toft et al. (112) reported no significant association between elevated PFNA exposure and sperm quality indicators in males, and none of the four PFAS were related to sperm concentration, total sperm count or volume of semen. In contrast, Vested et al. (97) concluded that an increased prenatal exposure to PFOA was related to a reduced sperm concentration and total sperm count (adjusted β (SE); -0.11 million/mL (0.04) and -0.20 million (0.06), respectively). The study found no association between elevated PFOA levels and semen volume and the percentage of normal or progressive spermatozoa in semen, and further no effects of PFOS exposure on sperm quality.

Joensen et al. (34) found exposure to combined PFOA and PFOS was significantly associated with a decreased number of normal sperm in semen (6.2 million normal spermatozoa in men with high PFOA and PFOS, compared to 15.5 million in men with low levels of PFAS; p=0.03), based on a study of 105 Danish males from the general population. The study found no association between lower sperm concentrations or counts in the men. However, in an update of this study in 2008–2009, Joensen et al. (99) reported no association between PFOA, PFOS,

PFHxS, PFNA, PFDA and PFHpS and sperm quality in Danish men. Raymer et al. (94) further concluded semen quality was unaffected by elevated levels of PFOA, PFOS, PFHxS, PFNA, PFDA and PFHpS.

Across the six studies, the association between PFAS exposure levels and adverse sperm characteristics is unclear. Buck Louis et al. (111) presented evidence for a significant association between elevated levels of PFOA, PFDA, PFOSA and Me-PFOSA-AcOH and abnormal morphology of the head and neck regions of sperm; however, also stated that PFOA, PFDA and PFOSA were associated with a reduced number of sperm with coiled tails, meaning that PFAS exposures are not related to abnormal morphology of the tail section of sperm. Buck Louis et al. (111) further identified an association between increased PFOSA and Me-PFOSA-AcOH exposure levels and decreased DNA stability in sperm, as well as an increased proportion of immature sperm in semen samples. While Buck Louis et al. (111) showed that increased PFAS exposures may be associated with a reduced sperm quality, these findings have not been replicated to date and provide limited evidence. In the same way, the significant association reported by Toft et al. (112) for elevated PFHxS levels and a reduced proportion of normal sperm in a semen sample provides limited evidence. However, the studies conducted by Buck Louis et al. (111) and Toft et al. (112) were associated with a low and moderate risk of bias assessment, respectively.

Menstruation and menopause

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Menstrual cycle length	PFOA, PFOS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH and Et-PFOSA-AcOH	Inadequate evidence
Endometriosis	PFOA, PFOS, PFHxS, PFNA, PFDA	Inadequate evidence
Onset of menopause	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Hysterectomy rate	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence

Menstrual cycle length

Lum et al. (106) and Lyngsø et al. (113) investigated the effect of PFAS exposure on the menstrual cycle in women of reproductive age. Lum et al. (106) reported that higher levels of PFOA were associated with a shorter menstrual cycle (adjusted OR T1 vs T3 (95% CI); 0.98 (0.96, 1.00)) in cohort study of women from Michigan and Texas, USA, recruited between 2005 and 2009, after cessation of contraception use. The study further found higher levels of PFDA were related to a longer menstrual cycle in women; however, results were significant only when comparing moderate and low exposure levels (adjusted OR T1 vs T3 (95% CI); 1.03 (1.00, 1.05)). Lum et al. (106) did not find an association between PFOS, PFNA, PFOSA, Me-PFOSA-AcOH and Et-PFOSA-AcOH and changes in menstrual cycle length. In contrast, Lyngsø et al. (113) found a significant association between elevated serum concentrations of PFOA and longer menstrual cycles (adjusted OR of a long cycle Q1 vs Q4 (95% CI); 1.8 (1.0, 3.3)) in pooled estimates of 1,623 pregnant women from Greenland, Poland and Ukraine, enrolled in the INUENDO cohort between June 2002 and May 2004. As stated by Lum et al.(106), Lyngsø et al. (113) reported no relationship between PFOS and menstrual cycle length in women before pregnancy. Therefore, these results present conflicting evidence for the association between PFOA and menstrual cycle length in women.

Endometriosis

Endometriosis is a common disease in women of reproductive age where endometrial growth occurs outside of the uterus. Buck Louis et al. (114) and Campbell et al. (115) studied the relationship between increase PFAS exposure and endometriosis in women. Buck Louis et al. (114) concluded a positive association between exposure to PFOA (multivariate OR (95% CI); 1.62 (0.99, 2.66)) and PFOS (multivariate OR (95% CI); 1.99 (0.91, 4.33)) and endometriosis in a cohort of women aged 18 to 44-years old scheduled to have a laparoscopy or laparotomy between 2007 and 2009 in selected regions of the USA. Exposure to PFOA and PFOS were further found to increase the chance of a women having moderate to severe endometriosis in the cohort of women, however results were not statistically significant. Buck Louis et al. (114) did not find an association between PFHxS, PFNA and PFDA exposures and endometriosis. Campbell et al. (115) identified a positive association with PFOS (OR (Q4-Q1) (95% CI); 3.48 (1.00, 12.00)) and endometriosis in women aged 20 to 50-years old enrolled in the NHANES study, along with a positive, though non-significant, association for PFOA and PFNA. In agreement with Buck Louis et al. (114), Campbell et al. (115) found no association between PFHxS and endometriosis.

These studies suggest an association between elevated PFOA and PFOS exposures and increased rates of endometriosis in women, however, evidence is inadequate. While the study conducted by Buck Louis et al. (114) was only associated with a moderate risk of bias, the study by Campbell et al. (115) was evaluated to have a high risk of bias.

Onset of menopause

The association between PFAS exposure and the age of menopause onset was reported in three studies by Dhingra et al. (116), Knox et al. (101) and Taylor et al. (117) Knox et al. (101) found a significant positive association between PFOA and PFOS exposures and an increased odds of having experienced menopause in women aged 18 to 65-years old (perimenopausal age group: (OR PFOA (95% CI); 1.4 (1.1, 1.8)) and (OR PFOS (95% CI); 1.4 (1.1, 1.8)); and menopausal age group:(OR PFOA (95% CI); 1.7 (1.3, 2.3)) and (OR PFOA (95% CI); 2.1 (1.6, 2.8))). Taylor et al. (117) reported a dose-response association between menopause and PFOA (adjusted HR T1 vs T3 (95% CI); 1.36 (1.05, 1.75)), PFNA (adjusted HR T1 vs T3 (95% CI); 1.47 (1.08, 1.87)) and PFHxS (adjusted HR T1 vs T3 (95% CI); 1.70 (1.36, 2.12)) in women aged 20 to 65-years old enrolled in the NHANES study. The study reported no significant relationship between PFOS exposure levels and trends in menopause onset. Conversely, Dhingra et al. (116) reported that earlier age at menopause was not associated with PFOA exposure.

From these three studies, there is inadequate evidence of an association between elevated serum levels of PFOA, PFOS, PFNA and PFHxS and an earlier onset of menopause in women. In relation to these findings, Taylor et al. (117) suggest that reverse causation may affect the relationship between PFAS exposure and menopause with increases in PFAS concentration being associated with the rate of natural menopause in women. Further, all three studies were evaluated to have a high risk of bias.

Hysterectomy

Taylor et al. (117) investigated the effects of PFAS exposure on rates of hysterectomy in women. The study reported a significant relationship between increased rates of hysterectomy in women and PFOA (HR (T3-T1) (95% CI); 2.81 (2.12, 3.71)), PFOS (HR (T3-T1) (95% CI); 2.56 (1.90, 3.43)), PFHxS (HR (T3-T1) (95% CI); 3.50 (2.72, 4.50)) and PFNA (HR (T3-T1) (95% CI); 1.78 (1.33, 2.37)) exposures; however, as there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias. Further investigation is required before a clear conclusion can be made for the effect of PFAS on hysterectomy rates.

Onset of puberty

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Age at menarche in girls	PFOA, PFOS, PFHxS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence
Pubertal maturation in boys	PFOA, PFOS, PFHxS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH	Inadequate evidence

Three studies examined the effect of PFAS exposure on the onset of puberty in male and female adolescents. (118-120) Lopez-Espinosa et al. (120) investigated age at menarche and pubertal maturation in children aged 8 to18-years old enrolled in the C8 Health Project and reported a negative association between PFAS exposure and each health outcome. The authors reported a later age of pubertal maturation in boys with higher levels of PFOS exposure (delay of 190 days between the highest and lowest quartile of PFOS exposure) and a later age of menarche in girls in relation to increased serum concentrations of PFOA (130 days of delay) and PFOS (138 days of delay). Similarly, in a prospective cohort study of mother-infant pairs from Denmark, Kristensen et al. (119) concluded that daughters exposed to higher levels of PFOA in utero had a 5.3 (95% CI; 1.3, 9.3 and p=0.01) month delay of menarche compared with the reference group of lower PFOA. In contrast, Christensen et al. (118) found no association between the onset of puberty and prenatal exposure to eight PFAS; PFOA, PFOS, PFHxS, PFNA, PFDA, PFOSA, Me-PFOSA-AcOH and Et-PFOSA-AcOH. Therefore, results are conflicting for the association between PFAS exposure and age at menarche and pubertal maturation in adolescents, specifically for PFOA and PFOS exposures, which were significantly associated with the health outcomes in 2 of the 3 evaluated studies.

Congenital cryptorchidism and hypospadias

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Congenital cryptorchidism	PFOA, PFOS	Inadequate evidence
Congenital hypospadias	PFOS	Inadequate evidence

Jensen et al. (121) and Toft et al. (95) investigated the effect of prenatal exposure to PFAS on the absence of testes in male infants at birth. Jensen et al. (121) reported no statistically significant association between umbilical cord measurements of PFOA and PFOS at birth and congenital cryptorchidism diagnosis (absence of one or both testes) in male infants. Similarly, Toft et al. (95) found no association between prenatal PFOS exposure and congenital cryptorchidism or hypospadias (a disorder of the urethra where the urinary opening is not at the usual location on the head of the penis).

Metabolic effects

The effect of PFAS exposure on metabolic outcomes in children and adults was investigated in 45 evaluated papers. The majority of studies centred on occupational exposure to PFOA and PFOS, with the main health outcomes being cholesterol level, kidney function and liver function. All papers were found to have a moderate or high risk of bias. The papers are summarised in Appendix 9, **Error! Reference source not found.** and **Error! Reference source not found.**. No metabolic outcomes were eligible for meta-analysis.

Cholesterol and triglycerides

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Total cholesterol level	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTEDA, PFBS, PFHxA, PFUdA. PFBA, PFHA	Sufficient evidence: PFOA, PFOS Inadequate evidence; PFHxS, PFNA, PFDA, PFDoA, PFTEDA, PFBS, PFHxA, PFUdA. PFBA, PFHA
HDL level	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTEDA, PFBS, PFHxA, PFUdA, PFHpS, PFBA, PFHA	Inadequate evidence
TC:HDL	PFOA, PFOS, PFHxS	Inadequate evidence
LDL level	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTEDA, PFBS, PFHxA, PFUdA, PFHpS, PFBA, PFHA	Inadequate evidence
VLDL level	PFOA, PFOS	Inadequate evidence
HDL:LDL	PFOA, PFOS	Inadequate evidence
Non-HDL level	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Triglyceride level	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTEDA, PFBS, PFHxA, PFUdA, PFHpS, PFBA, PFHA	Inadequate evidence

Total cholesterol level

Twenty-five papers investigated the effect of PFAS exposure on serum concentrations of cholesterol in children and adults. Primarily, the papers determined the relationship between PFAS and total cholesterol levels through cross-sectional or cohort studies of highly exposed communities, including residents of contaminated regions and employees of chemical production plants. PFOA and PFOS were the main exposures of interest in most papers, however the effects of 10 additional PFAS were investigated across the 25 evaluated papers.

Overall, the literature supports a positive association between PFAS exposures and cholesterol levels; elevated exposure levels relate to higher measurements of total cholesterol in the blood stream.

Of 22 papers that investigated the association between PFOA and total cholesterol level, 12 authors found a positive association between the exposure and health outcome. Seven papers (Costa et al., (122) Eriksen et al., (123) Sakr et al., (124, 125)Skuladottir et al., (126) Steenland et al., (127) and Winquist & Steenland (128)) identified this relationship in adults, three papers (Frisbee et al., (129) Geiger et al., (130) and Zeng et al.(131)) in children and two papers (Fu et al. (132) and Nelson et al.(133)) related to participants aged 12 to 80-years old and 0 to 88-years old, respectively. In contrast, the remaining 10 papers reported no association between PFOA and total cholesterol level in children and adults. (134-143)

Costa et al. (122) reported a positive association in a small cohort study of PFOA production workers (multiple regression β (95% CI); 21.7 (6.83, 36.6)), Sakr et al. (124) in a cohort study of 454 DuPont workers exposed to AFPO (increase in serum measure β (95 % CI); -1.06 (0.24, 1.88) and Sakr et al. (124) in a cross-sectional study of 1,025 DuPont workers exposed to Ammonium Perfluorooctanoate (APFO) (linear regression β (SE); 4.036 (1.284)). Eriksen et al. (123) (linear regression (95% CI); 4.4 (1.1, 7.8)) found a significant association between PFOA exposure and total cholesterol in a Danish cross-sectional study of 753 adult men and women enrolled in the Danish Diet, Cancer and Health cohort, and Steenland et al. (127) (OR Q4-Q1 (95% CI); 1.40 (1.29, 1.51)) and Winquist & Steenland (128) (HR (Q5-Q1) (95% CI); 1.19 (1.11, 1.28)) reported the association in a residential cohort of adults enrolled in the C8 Health Project. Skuladottir et al. (126) identified an association in 854 pregnant women enrolled in the Aarhus Birth Cohort study (mean change in serum measure (Q5-Q1) (95% CI); 0.45 (0.15, 0.75)).

Frisbee et al. (129) identified a significant association between PFOA and total cholesterol in 12,476 children in the C8 Health Project (estimated marginal means Q1-Q5 (β +/- SE for trend); 5.8 (1.3+/-0.3) for 1 to 11.9-year olds and 4.2 (1.1+/-0.3) for 12 to 17.9 year olds). Geiger et al. (130) found an association in approximately 800 child participants from NHANES (multivariate OR (Q4-Q1) (95% CI); 1.16 (1.05, 2.12)), while Zeng et al. (131) found a similar increase in Taiwanese children enrolled in the Genetic and Biomarkers study for childhood asthma (change in cholesterol (β) per µg/L increase in PFAS (95% CI); 6.57 (2.72, 10.42)). Fu et al. (132) reported a significant increase in total cholesterol in 33 randomly selected males and females who visited the Yuanyang Red Cross Hospital (regression β (95% CI); 0.054 (0.011, 0.098)), as did Nelson et al. (133) in over 2,000 NHANES participants (difference in cholesterol levels (95% CI); 9.8 (-0.2, 19.7)).

The evidence on the association between PFOA and total cholesterol level consistently shows a significant positive association between the exposure and health outcome, although, results are also presented that show PFOA has no effect on serum concentrations of cholesterol. Considering that four of the 10 papers reporting no association between elevated PFOA levels and increased total cholesterol were conducted by Olsen et al. (138-140, 144) and were analyses based on the same, or similar cohorts, there is more support for a positive association between PFOA and total cholesterol than there is for the alternative. However, many of the papers highlighted that even though the association was statistically significant between PFOA and total cholesterol level, it is unlikely to be a clinically significant relationship as the effect of the exposure was reported as minimal. In addition many of the papers were

considered to have high risk of bias. The magnitude of the effect of PFOA in the development of high total cholesterol levels remains unclear.

Eighteen papers investigated the relationship between PFOS and total cholesterol level. There were inconsistent results regarding the association between increases in PFOS exposure and serum cholesterol measurements. A significant association was reported by eight papers. (123, 126, 127, 129-131, 133, 142) PFOS was concluded to have no effect on total cholesterol levels in 10 additional papers. (132, 134-136, 138-141, 144, 145)

Level of PFOS exposure was positively associated with total cholesterol concentrations in papers of various populations and age groups. Eriksen et al. (123) found significant trends between increases in total cholesterol and high PFOS levels in men and women (linear regression β (95% CI); 4.6 (0.8, 8.5)) and Steenland et al. (127) further reported the effect in adults (OR (Q4-Q1) (95% CI); 1.51 (1.40, 1.64). Skuladottir et al. (126) reported the association in women (mean change in serum measure (Q5-Q1) (95% CI); 0.44 (0.15, 0.74)) and Starling et al. (142) concluded the positive association in 891 women enrolled in the Norwegian Mother and Child (MoBa) cohort study between 2003 and 2004 (linear regression β per ln-ng/mL PFAS (95% CI); 8.96 (1.70, 16.22). Frisbee et al. (129) reported a significant increase in cholesterol levels in children associated with elevated PFOS exposure (estimated marginal means (Q5-Q1) (β +/- SE for trend); 5.5 (1.3+/-0.3) for 1 to 11.9-year olds and 9.5 (2.1+/-0.3) for 12 to 17.9-year olds) and Geiger et al. (130) further found evidence of a significant effect in children (multivariate OR (Q4-Q1) (95% CI); 1.53 (1.11, 1.64)). Zeng et al. (131) further reported a significant positive association between PFOS and total cholesterol levels in adolescents (change in cholesterol (β) per µg/L increase in PFAS (95% CI); 0.31 (0.18, 0.45)) and Nelson et al. (133) reported the association in people aged 12 to 80-years old (difference in cholesterol levels (95% CI); 13.4 (3.8, 23.0)).

Similar to PFOA, there is strong evidence towards a positive association between PFOS exposure and elevated total cholesterol. Although, we consider the evidence to be inconsistent, three of the 10 papers reporting no association between PFOS and total cholesterol were conducted by Olsen et al. (138, 140, 144) on 3M employees. When considering these papers as updates, the association between elevated PFOS levels and increased serum cholesterol measurements is consistently reported in the literature. However, regardless of the level of evidence, the association is undermined by the high risk of bias determined for many of the papers.

The effect on total cholesterol level was further investigated for exposure to other PFAS, including PFNA and PFHxS. In total, eight papers investigated the relationship for several PFAS. Fisher et al. (134) reported a significant positive association between PFHxS and total cholesterol levels in women and men that participated in the Canadian Health Measures Survey between 2007 and 2009 (weighted β (95% CI); 0.03 (0.01, 0.05). Nelson et al. (133) found a positive association for PFNA (difference in cholesterol levels (95% CI); 13.9 (1.9, 25.9)) and a negative association for PFHxS (difference in cholesterol levels (95% CI); -7.0 (-13.2, -0.8)). Zeng et al. (131) instead stated a significant association between PFBS (change in cholesterol (β) per µg/L increase in PFAS (95% CI); 19.30 (0.60, 38.0)) and PFNA (change in cholesterol (β) per µg/L increase in PFAS (95% CI); 12.92 (0.7333, 25.10)) exposures and increased total cholesterol levels. Mundt et al. (146) Rotander et al. (141) and Starling et al. (142) reported no association between total cholesterol and PFNA or PFHxS. Skuladottir et al. (126) further concluded no association between 17 other PFAS, including PFNA and

PFHxS, and total cholesterol levels in women. Fu et al. (132) found no significant association between PFNA, PFDA and PFUdA and total cholesterol levels in people aged 0 to 88-years old.

Unlike PFOA and PFOS exposure, there is inadequate evidence in the literature to support an association between other PFAS, particularly PFHxS, and total cholesterol. As two papers suggest a positive association between PFNA and the health outcome, results are conflicting.

Total cholesterol meta-analysis for adults

Seven articles that reported the relationship between PFOA and total cholesterol were considered for meta-analysis. (122, 123, 127, 134, 139, 141, 143) Wang et al. (143) presented results for two papers (general population and occupational). Five papers (127, 134, 138, 141, 145) examined the association between PFOS and total cholesterol and were considered for meta-analysis. All papers were cross-sectional except for Costa et al. (122), which was a cohort design. However, there were an inadequate number of papers with comparable outcome measures for inclusion in a meta-analysis.

High-density lipoprotein (HDL) level

HDL molecules are comprised of fats and proteins and circulate in the blood stream. HDL support the transport of cholesterol from tissues to the liver, and therefore, facilitate the removal of cholesterol from the human body.

Twenty-two papers analysed the relationship between PFAS exposure and serum HDL concentration. Largely, the papers do not support an association between several PFAS exposures and HDL measurements, however, the results of the 22 papers are inconsistent, particularly in relation to PFOA and PFOS.

Nineteen of the papers investigated the effect of PFOA exposure, and predominantly, the authors reported no association between serum concentrations of PFOA and HDL measurements. In total, 18 papers reported no association between the exposure and health outcome. (122, 124, 125, 127, 129-142) Contrary to these results, Wang et al. (143) reported a negative association between PFOA levels and HDL measurements in a comparison of 55 employees of fluorochemical plants in Changshu City, China and 132 residents of the same region between May 2010 and October 2011 (linear multivariate regression β (95% CI); workers – -0.07 (-0.12, -0.01) and residents – 0.02 (-0.02, 0.05)). Therefore, these 19 papers do not support an association between PFOA and HDL levels in children and adults.

The effect of PFOS on HDL concentration was further examined in 16 papers. The results presented similarity to the reported associations for PFOA exposure, with 13 of the evaluated papers showing no significant effect of elevated PFOS exposure levels on HDL measurements. (127, 130-136, 138-141, 144) In contrast, three papers supported a significant association between PFOS and HDL levels.

Frisbee et al. (129) reported a significant negative association between PFOS exposure levels and HDL concentrations (estimated marginal means (Q5-Q1) (β +/- SE for trend); 1.6 (0.3+/-0.1) for 1 to 11.9-year olds and 1.5 (0.4+/-0.1) for 12 to 17.9-year olds). Starling et al. (142) identified a positive association between PFOS and HDL level in women (linear regression (Q4-Q1) (95% CI); 4.45 (2.04, 6.86)). Château-Degat et al. (145) found a significant positive association between PFOS levels and HDL concentrations in a cross-sectional study of 716

Inuit women and men from Canada aged 18 to 74-years old (multiple regression β (p); women: 0.0042 (0.001) and men: 0.0016 (<0.001)). While these three papers suggest PFOS may affect HDL levels, there is a larger body of evidence that reports no association.

Six papers further investigated the association between other PFAS exposures and HDL level. Of the six papers that assessed the effect of additional PFAS exposure, five papers reported no association with serum HDL concentration. (131-134, 146) However, Starling et al. (142) found a positive association for PFHxS (linear regression β (Q4-Q1) (95% CI); 3.21 (0.77, 5.65)), PFNA (linear regression β (Q4-Q1) (95% CI); 3.26 (0.47, 6.05)) and PFUnDA (linear regression β Q1 vs Q4 (95% CI); 7.61 (4.98, 10.25)) and HDL levels. The study further supported this trend for PFDA (linear regression β <median vs ≥median (95% CI); 2.72 (0.89, 4.55)), though stated no significant effects related to elevated PFHpS exposure levels. In summary, the evidence largely supports that these additional PFAS exposures have no association with HDL level.

HDL cholesterol meta-analysis for adults

There were six articles that presented results from seven papers and reported the association between HDL Cholesterol and PFOA exposure. (122, 127, 134, 139, 141, 143) Four articles presenting results from five papers on the association between HDL Cholesterol and PFOS were further considered for meta-analysis. (127, 134, 141, 145) Wang et al. (143) presented results for two cohorts (general population and occupational) and Château-Degat et al. (145) presented sex-specific results. However, there were an inadequate number of papers with comparable outcome measures for inclusion in a meta-analysis.

Total cholesterol: HDL

Three papers investigated the effect of elevated PFAS exposure on the ratio of total cholesterol and HDL measurements (total cholesterol: HDL). In a cohort study of 179 male 3M employees from Cottage Grove, USA, Olsen et al. (140) found a significant decrease in total cholesterol: HDL in relation to increased PFOA exposure levels (adjusted linear regression β (SE); -0.00239 (0.00083)). Study investigators did not find an association for increased PFOS exposure levels. In contrast, Fisher et al. (134) reported a significant positive association between PFHxS exposure levels and total cholesterol: HDL in women and men (weighted β (95% CI); 0.03 (0.011, 0.05)). The study investigators did not find an association for PFOA and PFOS exposures. Steenland et al. (127) found a positive relationship between PFOA and PFOS exposure and total cholesterol among C8 cohort study members. Therefore, the results are conflicting for the association between PFAS and total cholesterol: HDL measurements, with all three papers reporting either significant positive or negative results.

Low-density lipoprotein (LDL) level

In contrast to HDL, LDL bind to cholesterol in the liver and facilitate transport of the molecules to the tissues of the human body. As LDL support the distribution of cholesterol to cells, rather than the removal, high concentrations of LDL are linked with the development of cardiovascular disease outcomes, including atherosclerosis.

Eighteen papers investigated the association between PFAS exposure and serum LDL concentrations. In summary, the literature does not suggest an association between PFAS and LDL levels in adults and children, however, results are inconsistent for the effects of PFOA, PFOS, PFHxS and PFNA exposures.

Frisbee et al. (129) stated a significant positive association between PFOA (estimated marginal means (Q5-Q1) (β +/- SE for trend); 1 to 11.9-year olds: 4.9 (1.0+/-0.3) and 12 to 17.9-year olds: 3.2 (0.7+/-0.2)) and PFOS (estimated marginal means (Q5-Q1) (β +/- SE for trend); 1 to 11.9-year olds:3.4 (0.9+/-0.3) and 12 to 17.9-year olds: 7.5 (1.7+/-0.2)) and LDL measurements in children. Geiger et al. (130) also found a significant increase in LDL levels related to elevated PFOS concentrations (multivariate OR (T3-T1) (95% CI); 1.76 (1.10, 2.82)), however stated no significant association for PFOA exposure. In addition, Zeng et al. (131) reported a significant positive association between PFOA (change in LDL (β) per µg/L increase in PFOA (95% CI); 4.66 (1.67, 7.65)) and PFOS (change in LDL (β) per µg/L increase in PFOS (95% CI); 0.28 (0.18, 0.38)) and LDL levels in children. Fitz-Simon et al. (135) further reported a positive trend for PFOA and PFOS in adults, however, the significance level of the association was not reported in the study. Steenland et al. (127) further supported a positive association between PFOA and PFOS exposure and LDL levels in adults. In contrast, 11 papers reported no association between PFOA and LDL concentrations (124, 125, 130, 132, 133, 136, 137, 139, 141-143) and six papers found no significant trends relating to PFOS exposure levels (132, 133, 136, 141, 142, 145)

While results were largely conflicting in relation to the effects of elevated PFOA exposure levels, there is a similar number of papers that support a significant and non-significant association related to PFOS. Five papers stated a significant positive association and six papers stated no association for PFOS and LDL. This suggests that the effect of PFOS exposure is unclear, and may require further investigation, particularly as papers have consistently reported an association between PFOS and total cholesterol, which may be due to the increase in HDL levels. However, many of the papers evaluated were considered to be moderate to high risk of bias.

In relation to the effects of other PFAS exposures, results are also inconsistent. Fisher et al. (134) concluded a significant positive association between PFHxS and LDL measurements in adults (weighted β (95% CI); 0.06 (0.01, 0.11)). Zeng et al. (131) also found a positive association in relation to PFNA exposure levels in children (change in LDL (β) per µg/L increase in PFNA (95% CI); 9.63 (0.20, 19.06)); however, they did not find associations with PFBS, PFDA, PFDoA, PFHxA, PFHxS and PFTEDA exposures. Nelson et al. (133) identified a positive association between PFHxS exposure and LDL levels (difference in LDL levels per µg/L increase in PFHxS (95% CI); -2.06 (-3.54, -0.58)), and no consistent results related to PFNA exposure levels. Mundt et al. (146) found no association between PFNA and LDL level and Fu et al. (132) did not identify any association for PFNA, PFDA and PFUdA. Starling et al. (142) did not find an association for PFHxS, PFNA, PFDA, PFUdA and PFHpS and Rotander et al. (141) found no effects related to PFHxS. Largely, the results show that PFAS exposures are not associated with LDL level; however, there are discrepancies with some papers reporting significant results.

LDL cholesterol meta-analysis for adults

We considered five articles presenting results for six papers (two cohorts: general population and occupational) for meta-analysis of the relationship between LDL Cholesterol and PFOA. (127, 134, 139, 141, 143) Four articles that further presented results on the association between HDL cholesterol and PFOS were also considered for meta-analysis. (127, 134, 141, 145) However, there were an inadequate number of papers which with comparable outcome measures for inclusion in a meta-analysis

Very low density lipoprotein (VLDL) level

Two papers investigated the effect of PFAS exposure on VLDL levels: Sakr et al. (124) reported no significant relationship between PFOA and VLDL measurements and Mundt et al. (146) found no association between PFNA and VLDL level.

HDL: LDL

Two papers investigated the relationship between elevated PFAS levels and HDL to LDL ratio measurements (HDL: LDL). Wang et al. (143) stated a negative association between PFOA exposure levels and HDL: LDL in adults (linear multivariate regression β (95% CI); workers: -0.09 (-0.16, -0.02) and residents: 0.02 (-0.04, 0.08)). In contrast, Steenland et al. (127) found a positive association between PFOA (linear regression β (SD); 0.0081 (0.00110)) and PFOS (linear regression β (SD); 0.02290 (0.00202)) exposures and VLDL levels in adults. Therefore, results are conflicting for this health outcome.

Non-HDL level

The measurement of non-HDL is the concentration of LDL and triglycerides in the blood stream, and is equal to the concentration of total cholesterol level minus the concentration of HDL.

Four papers investigated the association between PFAS levels and non-HDL measurements. Nelson et al. (133) stated an increase in non-HDL in relation to increases levels if PFOA (difference in non-HDL levels per µg/L increase in PFOA (95% CI); 1.38 (0.12, 2.65)) and PFOS (difference in non-HDL levels per µg/L increase in PFOS (95% CI); 0.25 (0.00, 0.50)) in children and adults, however the study did not present results on the significance levels of the association. The study further stated a negative association between PFHxS and non-HDL measurements (difference in non-HDL levels per µg/L increase in PFHxS (95% CI); -1.13 (-1.90, -0.35)), and no consistent results related to PFNA exposure levels. In contrast, Fisher et al. (134) stated a significant positive association between PFHxS and non-HDL levels in adults (weighted β (95% CI); 0.04 (0.018, 0.06)); however found no significant results related to elevated PFOA and PFOS exposures. Château-Degat et al. (145) further reported no significant findings related to PFOS exposure and non-HDL measurements, and Olsen et al. (140) stated no significant associations for PFOA and PFOS. In summary, these findings suggest there is inconsistent evidence to support a significant association between PFAS exposures and non-HDL measurements.

Triglyceride (TG) level

TG are fat molecules that are created from the breakdown of dietary carbohydrates during digestion. The molecules travel through the blood stream to tissues and function to store fat that the human body does not immediately require for energy. As increased levels of TG are associated with increased fat storage, a high concentration of the molecules is related to the development of metabolic syndrome and cardiovascular diseases.

In total, 19 papers all reported no association of PFAS exposure on serum TG concentration. As for the trends in cholesterol measurements, there were discrepancies in the results presented across the literature and the 19 evaluated papers did not support an association between PFAS and TG levels in adults and children.

The relationship was reported for PFOA exposure in 17 papers. Olsen et al. (139) found a significant association between increased PFOA exposure levels and TG measurements in a cross-sectional study of 552 male employees from 3 3M plants in Antwerp, Belgium, Decatur, USA and Cottage Grove, USA. However, these positive results only related to employees from Antwerp (adjusted β (SE); 0.0169 (0.0270)), and not males from the USA. A significant association between PFOA and TG levels was also reported in children by Zeng et al. (131) (change in TG (β) per µg/L increase in PFOA (95% CI); 19.63 (14.82, 24.34)). Steenland et al. (127) further reported a significant positive association between PFOA and TG measurements in adults (linear regression β (SD); 0.00169 (0.00219)). Non-significant results related to the effects of PFOA on TG levels were reported by 14 papers. (122, 125, 129, 130, 132, 134-139, 141-143) Therefore, there is more evidence in the literature to suggest PFOA does not affect changes in TG levels in adults and children.

Thirteen papers examined the effect of PFOS exposure on TG measurements. Château-Degat et al. (145) reported a significant negative association between PFOS exposure levels and TG in women (multiple regression β (p); -0.0014 (0.040)); however this trend was not significant for men. In contrast, Steenland et al. (127) reported a positive association between PFOS and TG measurements in adults (linear regression β (SD); 0.01998 (0.00402)). Zeng et al. (131) reported a significant positive association between PFOS and TG level in children (change in TG (β) per µg/L increase in PFOS (95% CI); 0.19 (0.00, 0.38)). As for PFOA, the effects of PFOS exposure were reported to be non-significant by many papers; 10 papers stated no relationship between the exposure and health outcome. (129, 130, 132, 134-136, 138, 141, 142, 144)

Three of the 19 papers on TG levels stated the effects of additional PFAS exposures. Zeng et al. (131) reported a significant positive association between PFNA exposure and TG measurements in children (change in TG (β) per µg/L increase in PFNA (95% CI); 23.01 (6.49, 39.52), however stated no significant effects related to PFBS, PFDA, PFDoA, PFHxA, PFHxS and PFTEDA exposures. Fu et al. (132) further reported no association between TG concentration and several PFAS exposures, including PFBA, PFHA, PFHpA, PFNA, PFDA and PFUdA. Starling et al. (142) confirmed the same finding for PFHpS, PFHxS, PFNA, PFDA and PFUdA, and Mundt et al. (146) found no association between PFNA and TG levels. Therefore, there is also insufficient evidence to state a positive association between PFNA and other PFAS exposures and TG concentrations in serum.

Triglyceride level meta-analysis for adults

Six articles that presented results from six papers, and reported the association between triglyceride levels and PFOA were considered for meta-analysis. (122, 127, 134, 139, 141, 143) Five articles that presented results from six papers and determined the association between Triglycerides and PFOS were also considered for meta-analysis. (127, 134, 138, 141, 145) Wang et al. (143) presented results for two cohorts (general population and occupational) and Château-Degat et al. (145) presented sex-specific results. However, there were an inadequate number of papers which with comparable outcome measures for inclusion in a meta-analysis.

Kidney function

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Glomerular filtration rate	PFOA, PFOS, PFHxS, PFNA	Limited evidence for a negative association; PFOA, PFOS Inadequate evidence; PFHxS, PFNA
Hyperuricemia	PFOA, PFOS, PFHxS, PFNA, PFDA, PFBS	Limited evidence for a positive association; PFOA, PFOS Inadequate evidence; PFHxS, PFNA, PFBS, PFDA
Chronic kidney disease	PFOA, PFOS	Limited evidence for a positive association; PFOA, PFOS
Blood urea nitrogen level	PFOA	Inadequate evidence
Creatinine level	PFOA	Inadequate evidence

Twelve papers investigated the relationship between PFAS exposure and kidney function (Appendix 9). No health outcomes related to kidney function were eligible for meta-analysis. Primarily, the papers used uric acid concentrations as a biomarker of kidney function. Elevated levels of uric acid (hyperuricemia) are associated with risk of hypertension, diabetes mellitus, cardiovascular disease, and kidney disease. Concentration of serum creatinine was further used as a biomarker for kidney function in several papers. Creatinine is a chemical waste product carried in the blood until it is filtered by the kidneys and eliminated through urine. A high serum creatinine is suggestive of poorer kidney function. From the papers, it is unclear how PFAS exposure and uric acid are connected, although there are several hypotheses. When kidney function is reduced, creatinine and uric acid can accumulate in the blood. The study by Qin et al. (147) provides an overview of possible connections between serum uric acid levels and PFAS exposure.

In a study of children enrolled in the C8 Health Project, Watkins et al. (148) found a positive association between reduced kidney function (indicated by the kidney glomerular filtration rate, GFR) and PFOA (change in eGFR (95% CI); -0.73 (-1.38, -0.08)), PFOS (change in eGFR (95% CI); -1.34 (-1.91, -0.77)), PFHxS (change in eGFR (95% CI); -0.88 (-1.41, -0.36)) and PFNA (change in eGFR (95% CI); -1.02 (-1.64, -0.40)). Kataria et al. (149) further investigated reduced kidney function in children aged 12 to 19-years old that participated in the NHANES study and found a significant positive association between decrements of estimated GFR and increased concentrations of uric acid and PFOA (eGFR (95% CI) and uric acid concentration (95% CI); 6.84 mL/min/1.73 m^2 (2.19, 11.48) and 0.21 mg/dL (0.056, 0.37)) and PFOS (eGFR (95% CI) and uric acid concentration (95% CI); 9.69 mL/min/1.73 m^2 (4.59, 14.78) and 0.19 mg/dL (0.032, 0.34)). In contrast, Kataria et al. (149) found no significant association for PFHxS and PFNA. Although results are conflicting for PFHxS and PFNA, these papers suggests that PFAS may influence the uric acid level by reducing kidney function. Increased investigational attention into these mechanisms appears warranted in order to clarify the biological mechanisms underlying our observations, however it is noted that both papers were evaluated to have a high risk of bias.

The association between PFAS exposure and uric acid levels was assessed by six papers. Costa et al. (122) found a positive association between PFOA exposure and elevated uric acid levels (multivariate analysis coefficient (95% CI); 0.026 (0.001, 0.053)) in a cohort study of workers from a PFOA production plant in Italy. The same significant trend was reported by Steenland et al. (150) in adults enrolled in the C8 Health Project between increased risk of hyperuricemia and PFOA (excess uric acid in the blood OR Q1 vs Q4 (95% CI); 1.47 (1.37, 1.58)) and PFOS (excess uric acid in the blood OR Q1 vs Q4 (95% CI); 1.26 (1.17, 1.35)) exposure.

All other papers that investigated the effect of the exposure on uric acid levels were cross-sectional in design. Primarily, the papers found a positive association between serum PFOA and increased uric acid levels, except Rotander et al. (141) who investigated exposed Australian fire-fighters. Rotander et al. (141) stated a non-significant association for PFOS and PFHxS exposures. Shankar et al. (151) found a positive relationship between serum uric acid and PFOS (multivariate OR (95% CI); 1.97 (1.44, 2.70)) and PFOA (multivariate OR (95% CI); 1.48 (0.99, 2.22)), in a survey of the general USA population using NHANES data for the years 1999 to 2006. Geiger et al. (152) and Qin et al. (147) further evaluated the association between serum PFAS and uric acid levels in children. The papers reported a positive association between PFOA and hyperuricemia in general populations of the USA (multivariate OR (95% CI); 1.62 (1.10, 2.37)) (152) and Taiwan (OR (95% CI); 2.16 (1.29, 3.61)). (147) Geiger et al. (152) also found a positive association between PFOS levels and hyperuricemia (multivariate OR (95% CI); 1.65 (1.10, 2.49), however, Qin et al. (147) did not confirm this association. In summary, there is limited evidence to support a positive association between PFOA levels and hyperuricemia; however, many of the papers were cross-sectional in nature making it difficult to assess potential for causality.

Dhingra et al. (153), Shankar et al. (154) and Steenland & Woskie (155) investigated the association between PFAS exposure and chronic kidney disease in adults living in the USA. Dhingra et al. (153) found no association between modelled PFOA exposure and kidney disease in adults enrolled in the C8 Health Project. However, Steenland & Woskie (155) reported an elevated mortality from chronic kidney disease (SMR (95% CI); 3.11 (1.66, 5.32)) in DuPont workers exposed to PFOA from the West Virginia production plant. Shankar et al. (154) also identified a significant positive association between PFOA (multivariate OR Q1 vs Q4 (95% CI); 1.73 (1.04, 2.88)) and PFOS (multivariate OR Q1 vs Q4 (95% CI); 1.82 (1.01, 3.27)) exposure and chronic kidney disease in a cross-sectional study of adults enrolled in the NHANES study. Therefore, these papers present limited evidence to support a positive association between PFAS and chronic kidney disease. However, it is important to consider that the papers by Dhingra et al. (153) and Shankar et al. (154) were evaluated to have a high risk of bias.

Emmett et al. (18) reported on the relationship between serum PFAS levels and blood urea nitrogen and creatinine levels in adults who lived in the Little Hocking Water Association district, USA. The study investigators did not identify a significant association between PFOA exposure and the two biomarkers of kidney function.

Liver function

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
ALT level	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHxA, PFUdA	Inadequate evidence
GGT level	PFOA, PFOS	Inadequate evidence
Bilirubin level	PFOA, PFOS	Inadequate evidence
Albumin level	PFOA	Inadequate evidence
ALP level	PFOA	Inadequate evidence
α2-globulin level	PFOA	Inadequate evidence
AST level	PFOA	Inadequate evidence
Liver disease	PFOA	Inadequate evidence
Gilbert syndrome	PFOA, PFOA, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFPeA, PFHxA, PFHpA	Inadequate evidence
Lobular inflammation	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHxA	Inadequate evidence

Seven papers investigated the effect of PFAS exposure on liver function in humans. Only one study was conducted in the general population (156), where all other papers investigated either exposed residential populations (such as in the C8 Health Project) or occupationally exposed cohorts. Most of the papers investigated the effect of PFOA on liver enzymes, including Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma Glutamyl Transferase (GGT) and bilirubin, although some papers investigated liver disease endpoints.

A cross-sectional study by Darrow et al. (157) reported a significant positive association between the liver biomarker ALT and modelled PFOA exposure (increase in ALT% (95% CI); 6 (4, 8), no association with GGT and a negative association with direct bilirubin (0.5% decrease in direct bilirubin per log increase in PFOA) for adults enrolled in the C8 Health Project. A positive association with ALT and negative association with direct bilirubin was also found by Costa et al. (122) (multivariate analysis coefficient for ALT (95% CI); 0.116 (0.054, 0.177) and multivariate analysis coefficient for direct bilirubin (95% CI); -0.080 (-0.137, -0.024)) in male employees for an Italian PFOA production plant. Among C8 study participants, Gallo et al. (158) reported a significant positive association between PFOA (OR for high ALT levels for In-unit (95% CI); 1.10 (1.07, 1.13)) and PFOS (OR for high ALT levels for In-unit (95% CI); 1.13 (1.07, 1.18)) and ALT, while direct bilirubin was not associated with PFAS. Gallo et al. (158) further reported a non-significant association between PFOA and PFOS and GGT levels. In contrast to the results presented by Darrow et al. (157), Costa et al. (122) and Gallo et al. (158), Rantakokko et al. (156) reported no significant association between ALT and PFOA and PFOS exposure. The study investigators only identified a significant association for PFHxA (p=0.011), and did not find associations with PFHxS, PFNA, PFDA and PFUdA.

In addition to ALT and direct bilirubin, Costa et al. (122) reported a significant positive association between PFOA exposure and GGT (multivariate analysis coefficient (95% CI); 0.177 (0.076, 0.278)), Alkaline Phosphatase (ALP) (multivariate analysis coefficient (95% CI);

0.057 (0.007, 0.107)), and α2-globulin (multivariate analysis coefficient (95% CI); 0.026 (0.007, 0.045)). The study found no significant association between PFOA and albumin and AST. Emmett et al. (18) also concluded no significant association between PFOA and albumin and bilirubin for residents living in the Little Hocking Water Association district, USA.

All papers investigating liver disease endpoints reported that there was no evidence that exposure to PFOA increases the risk of clinically diagnosed liver disease. (18, 157, 159) Fan et al. (160) investigated the association between PFAS and Gilbert syndrome in adults enrolled in the C8 Health Project. The study reported a significant positive association between the syndrome and exposure to PFHxA (geometric mean (95% CI); 1.81 (1.72, 1.89) compared to control geometric mean (95% CI); 1.12 (1.11, 1.13)) and PFDA in men only (geometric mean (95% CI); 0.75 (0.73, 0.77) compared to control geometric mean (95% CI); 0.72 (0.71, 0.72)). Fan et al. (160) reported non-significant associations for eight PFAS, including PFOA, PFOS, PFHxS, PFNA, PFUdA, PFDoA, PFPeA and PFHpA.

The effect of PFOA exposure on liver histology was further assessed by Rantakokko et al. (156). The maximally adjusted model showed a significant negative association at baseline between PFOA (OR for lobular inflammation (95% CI); 0.02 (<0.01, 0.66)), PFNA (OR for lobular inflammation (95% CI); 0.02 (<0.01, 0.53)), PFDA (OR for lobular inflammation (95% CI); 0.05 (<0.01, 0.83)), and PFHxS (OR for lobular inflammation (95% CI); 0.02 (<0.01, 0.53)) and lobular inflammation in the liver, and no association for PFOS, PFUdA and PFHxA. No associations were found with other liver histology parameter at baseline.

Overall, there are several reported positive associations between markers of liver function and exposure to PFOA; however, results are inconsistent. While there is evidence to suggest a positive association between PFAS and markers of liver inflammation—PFOA and α2-globulin and ALP levels, and PFHxA and ALT levels—the results from Rantakokko et al. (156) finding increased PFOA and PFHxA levels are associated with decreased lobular inflammation contrast these findings. Further, due to the cross-sectional design of the majority of the papers, the temporality and causality of the exposure is unknown, and therefore, most of these significant findings were considered to be at high risk of bias. Thus, we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Other metabolic outcomes

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Metabolic syndrome	PFOA, PFOS, PFNA, PFHxS, PFHS	Inadequate evidence
Glycaemic control	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
Metabolic function (unspecified)	PFOA, PFOS, PFNA, PFHxS, PFDA	Inadequate evidence

Metabolic syndrome

Metabolic syndrome is defined as at least three of the following criteria; Waist measurement ≥ 80 cm for women and ≥ 90 cm for men; Serum triglyceride ≥ 1.7 mmol/L; Serum HDL-C < 1.03 mmol/L in men and < 1.29 mmol/L in women; Systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or a self-report of taking antihypertensive medications; Fasting glucose ≥5.6 mmol/L or a self-report of taking antihyperglycemic medications.

Lin et al. (161) and Fisher et al. (134) investigated the relationship between PFAS exposure and metabolic syndrome in adolescents and adults using data from the 1999-2004 NHANES surveys. Overall, the reported associations between PFAS exposure and the development of metabolic syndrome are conflicting. Lin et al. (161) found an inverse relationship between serum PFNA serum levels and the prevalence of metabolic syndrome in 474 adolescents that participated in the 1999–2000 or 2003–2004 NHANES waves (OR per 1-unit increase in PFNA (95% CI); 0.37 (0.21–0.64)); however, the effect was not observed in adults, additionally the inverse relationship was not significant for PFHxS, PFOA or PFOS. In contrast, Fisher et al. (134) found no association between serum PFOA, PFOS and PFHxS levels and metabolic syndrome or glucose homeostasis using cross-sectional data from the Canadian Health Measures Survey. Overall, the available studies are conflicting regarding the relationship between PFAS exposure and metabolic syndrome. While there is evidence to suggest PFNA is inversely associated with metabolic syndrome, it is important to interpret the results of this single study with caution, as it was assessed to have a high risk of bias, due to the inability to assess temporality of the exposure and outcome. As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Glycaemic control

Glycaemic control refers to the regulation of blood glucose levels in the human body. The process allows blood glucose levels to stay within the normal range in healthy individuals. Timmerman et al. (162) investigated the association between PFAS exposure and glycaemic control in children aged between 8 to 10-years old. The study reported no association between PFOA and PFOS and biomarkers of glycaemic control, and concluded there was no increased risk of adiposity in children in the age range. The effect of PFAS exposure on overweight and obesity, and diabetes, is discussed under specific health outcomes.

Adiponectin is a specific protein in the human body that is involved in the regulation of blood glucose levels and the breakdown of fatty acids. In a separate study, Lin et al. (163) evaluated the association between PFNA exposure and adiponectin concentration in 287 Taiwanese

children and young adults aged 12 to 30-years old through a prospective cohort study. Lin et al. (163) reported that the log transformed mean adiponectin level (ng/mL) was significantly increased across quartiles of PFNA concentration (8.78, 8.73, 9.06, and 9.36; P for trend = 0.010). Lin et al. (163) found no significant effects related to PFOA, PFOS and PFUdA. The evidence presented for a significant association between increased PFNA levels and increased serum adiponectin concentration is limited, and the study was assessed as being at high risk of bias. As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Metabolic function (unspecified)

Fleisch et al. (164) investigated the associations between serum PFAS levels and several metabolic outcomes (unspecified) through a prospective birth cohort in the United States. Plasma concentrations of PFAS were measured in the mother in their first trimester and attributed to the prenatal exposure of the child. Exposure was further measured in mid-childhood for participants during the follow-up period. Fleisch et al. (164) reported no evidence for adverse effects of prenatal and early-life PFAS exposure on metabolic functions in children.

Thyroid outcomes

The thyroid gland stores and produces hormones that affect the function of most organs in the body; particularly regulating metabolic rate, weight and energy levels. Thyroid Stimulating Hormone (TSH) controls the thyroid hormone levels and subsequent function.

The effect of PFAS exposure on the functioning of the thyroid gland in neonates, children, adults and pregnant women was investigated in 25 papers included in this review. The studies, primarily examined prenatal exposure to PFOA and PFOS and the concentration of thyroid hormones in neonates and their mothers. Several studies also focused on the effect of PFAS exposure on the adult thyroid gland. All papers were found to have a moderate or high risk of bias. A summary of the papers is provided in Appendix 10. Studies that provided quantitative evidence for TSH and Thyroxine (T_4) concentrations in infants (children <5 years old), and TSH concentrations in pregnant women, were considered for meta-analysis.

Thyroid Stimulating Hormone (TSH) level

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
TSH in infants		
	Umbilical cord; PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTrDA, PFUdA, PFPeA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFHpS, PFHpA, PFHxA	Inadequate evidence
	During infancy; PFOA, PFOS, PFNA	Inadequate evidence
TSH in children	PFOA, PFOS, PFNA	Inadequate evidence
TSH in pregnancy	PFOA, PFOS, PFHxS, PFNA PFDA, PFUdA, PFHpS, PFHpA, PFHxA, PFDoA	Inadequate evidence
TSH in adults	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnDA	Inadequate evidence

Acting as a control mechanism for thyroid hormones, TSH is released from the pituitary gland in the brain to stimulate the production of Thyroxine (T_4) and Triiodothyronine (T_3) in the thyroid gland. T_4 and T_3 increase the body's metabolic rate. The measurement of blood concentration of TSH is used as a biological marker of thyroid function; high concentrations of TSH can be used to define an underactive thyroid gland, and in contrast, low concentrations of TSH can indicate an overactive thyroid gland.

TSH in infants

Nine studies examined the association between prenatal and early infancy exposure to PFAS and TSH levels in neonates and infants (0 to 5-years old). (18, 63, 165-171) Overall, the studies presented no significant association between PFAS exposure and TSH measurements; however, conflicting results were reported for PFOA, PFOS and PFNA. In

addition, there were clear differences between the results reported for maternal and umbilical cord measurements of PFAS.

Umbilical cord blood studies

The association between umbilical cord blood measurements of PFAS and TSH levels in neonates was reported in four studies. (63, 168-170) Shah-Kulharni et al. (168) reported a significant negative association between PFNA and TSH concentrations in umbilical cord blood of Korean girls enrolled in the Ewha Birth and Growth Retrospective Cohort between 2006 and 2010 (adjusted regression coefficient β (CI); -1.69 (-3.31, -0.08)), and no association for PFOA, PFOS, PFHxS, PFDA, PFDoA, PFTrDA, PFUdA and PFPeA. In contrast, Tsai et al. (169) identified a significant positive association between umbilical cord measurements of PFOS and TSH at birth in 118 infants enrolled in the Taiwan Birth Panel Study (adjusted regression coefficient β (CI); 0.346 (0.101, 0.591)), and no significant relationship for PFOA, PFDA and PFUnDA. However, when the results were stratified by sex, Tsai et al. (169) only found a significant association for boys (adjusted regression coefficient β (CI); 0.333 (0.012, 0.678)). Kim et al. (63) and Yang et al. (170) further reported no significant associations between PFOA, PFOS and PFHxS and cord blood TSH levels. Yang et al. (170), Shah-Kulharni et al. (168) and Tsai et al. (169) also reported no association for PFNA, PFDA, PFUdA and PFDoA. Yang et al. (170) further reported no significant association for PFTrDA.

Maternal blood studies

Five studies investigated the effect of maternal concentrations of PFAS on TSH levels in infants. (63, 165, 166, 170, 171) Kim et al. (63) conducted a cohort study of pregnant mothers presenting to three hospitals in Seoul, South Korea and reported a significant positive association between maternal levels of PFOA during pregnancy and TSH concentrations in the umbilical cord of infants at birth (adjusted Pearson correlation test (*p*); 0.443 (<0.05)). The investigators did not identify an association between TSH concentration and levels of PFOS, PFHxS and PFTrDA. In contrast, using data from the Hokkaido Study on the Environment and Children's Health study, Kato et al. (166) found a significant positive association between maternal PFOS concentration and TSH levels in 392 infants at 4-days old (regression coefficient β (*p*); 0.177 (0.001)), and no significant results for PFOA exposure. Berg et al. (165), Wang et al. (171) and Yang et al. (170) reported no significant associations between TSH levels in neonates and a range of PFAS exposures, including PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFHpS, PFHpA and PFHxA, although Wang et al. (171) did find a significant positive association between PFHxS and TSH.

Emmett et al. (18) and Lopez-Espinosa et al. (167) investigated the association between PFAS exposure and TSH levels later in infancy, at 2.5-years old (and above) and for children aged 1 to 5-years old, respectively. Using the C8 Health Project cohort, Lopez-Espinosa et al. (167) found a negative association among children aged 1 to 5-years old for measured PFOA exposure and TSH (TSH change with IQR shift in PFOA (CI); -4.3 (-8.2, -0.3)). The study further reported no association between PFOS and PFNA in children of the same age. In contrast, Emmett et al. (18) reported no association between PFOA, PFOS and PFNA concentrations and TSH levels in infants aged ≥2.5 years.

The nine studies investigating TSH levels during infancy are largely consistent in demonstrating no significant association between elevated PFAS and TSH. However, inconsistent results in the literature were found for umbilical cord measurements of PFNA and

PFOS, maternal concentrations of PFOA and PFOS, and early infancy measurements of PFOA. These results identify both significant negative (umbilical cord PFNA and early infancy PFOA exposures) and significant positive (umbilical cord PFOA, maternal PFOA and maternal PFOS exposures) associations for the relationship between PFAS and TSH levels during infancy. There were differences between the associations reported for umbilical cord and maternal concentrations of PFAS similar to other prenatal health outcomes. The investigation between PFAS and TSH in infants provide largely inconsistent or no evidence of an association.

TSH meta-analysis for infants

Five studies investigating TSH levels and PFOA and PFOS in infants were considered for meta-analysis. (63, 167-170) All studies were conducted through cross-sectional designs, except for Kim et al. (63), which was a cohort study. As two studies used log transformed TSH as the outcome and three used non-transformed TSH, there were an inadequate number of comparable studies for inclusion in a meta-analysis.

TSH in children

Emmett et al. (18) and Lopez-Espinosa et al. (167) reported on the association between PFAS exposure and TSH levels during childhood (6–17-years). Emmet et al. (18) conducted a cross-sectional study of the residents of a community supplied with water containing elevated levels of PFOA in West Virginia, US and found no association between PFOA exposure and TSH levels in children aged 2.5 to 10-years old and 11 to 20-years old. Information was not available specifically for children aged 6 to 17-years old. Using data from the C8 Health Project, Lopez-Espinosa et al. (167) further found no significant association between PFOA, PFOS and PFNA and TSH concentrations in children aged 6 to 10-years and 10 to 17-years. Neither study suggested a potential association between exposure to PFAS and concentrations of TSH in children.

TSH in pregnant women

Seven studies investigated PFAS exposure and TSH concentrations in women during pregnancy. (165, 166, 170-174) The studies presented inconclusive results for the relationship between PFOS exposure levels during pregnancy and TSH levels.

Berg et al. (173), Wang et al. (172) and Webster et al. (174) reported a significant positive association between PFOS and TSH concentrations during pregnancy. Berg et al. (173) investigated TSH levels in pregnant women enrolled in the Northern Norway Mother-and-Child Contaminant Cohort Study between 2007 and 2009 that participated in blood testing during their second trimester and at 3 and 6-weeks postpartum. Berg et al. (173) identified a significant mean difference in TSH concentration for PFOS (Q1 vs Q4 (CI); 0.35 (0.21, 0.50). Wang et al. (172) tested 903 pregnant women enrolled in the Norwegian Mother and Child Cohort Study between the years 2003 and 2004 for TSH levels at 18-weeks' gestation. Wang et al. (172) found that increased PFOS concentration was associated with an increase in TSH concentration (percentage increase per 1 ng/mL increase PFAS (CI); 0.8 (0.1, 1.6)). Among 152 pregnant women in their second trimester enrolled in the Canadian Chemicals, Health and Pregnancy Study, Webster et al. (174) found higher TSH levels in women with higher PFOS levels (adjusted regression coefficient β (CI); 0.9 (0.2, 2.0)). In contrast, both Kato et al. (166) and Yang et al. (170) reported a significant negative relationship between the

exposure and health outcome (adjusted regression coefficient β (*p*); -0.214 (<0.001) and adjusted Spearmen correlation test (p); -0.261 (<0.01), respectively). The study by Yang et al. (170) was a cross-sectional study of 157 Chinese mothers from Beijing. Berg et al. (165) and Wang et al. (171) found no association between PFOS and TSH levels in pregnant women.

In relation to the effects of other PFAS exposures, Wang et al. (171) reported a significant positive association between PFHxS and TSH concentrations during the third trimester in a cohort of 285 Taiwanese women enrolled in the Taiwan Maternal and Infant Cohort Study between 2000 and 2001 (adjusted regression coefficient β (CI); 0.105 (0.002, 0.207)). Yang et al. (170) also found a significant negative association for PFNA, PFDA, PFDoA and PFUnDA (adjusted Spearmen correlation test (*p*); -0.170 (<0.05), -0.216 (<0.01), -0.231 (<0.01) and 0.202 (<0.05), respectively). In agreement, Berg et al., (165) Wang et al. (172) and Yang et al. (170) reported no significant association for PFHxS. Berg et al. (165, 173) and Wang et al. (171, 172) further reported no association for PFDA. Berg et al. (165, 173) and Wang et al. (171) found no association for PFUdA. Apart from Webster et al. (174) who noted a positive finding, no studies reported significant findings for PFOA and TSH in pregnant women, nor did they find a significant association between PFHpS, PFHpA, PFHxA and PFDoA. Webster et al. (174) was the only study to identify a significant association between TSH and PFNA.

Webster et al. (174) reported significant associations between several PFAS and TSH levels in pregnant women. However, the results related only to women in the cohort with high thyroid peroxidase antibody (TPOAb) levels, which are an indicator of thyroid disease caused by autoimmune responses (Hashimoto's disease). In contrast to the results presented by the other six studies for TSH, Webster et al. (174) found a significant positive association between PFOA, PFOS and PFNA and TSH concentrations in pregnant women. The results suggest that the association between elevated PFAS exposure and TSH is modified by high concentrations of TPOAb in pregnant women. As TSH levels in a sub-population of individuals with high TPOAb were significantly associated with increased levels of PFOA, PFOS and PFNA, the study's findings suggest effect modification of the association between PFAS and TSH by TPOAb. Webster et al. (174) hypothesised that the functioning of the thyroid gland and the production of related hormones is only altered by PFAS when the human body is simultaneously affected by other stressors, including pregnancy and high levels of TPOAb.

Overall, the seven studies are inconsistent regarding the association between PFAS and TSH concentrations in pregnant women, particularly in relation to the effect of PFOS exposure. Of the 6 studies investigating PFOS, four showed no association, one a positive effect and one a negative effect. The results for Webster et al. (174) relating only to women with high TPOAb concentrations makes it difficult to compare to the significant positive findings reported by Berg et al. (173) and Wang et al. (172). Although Wang et al. (171) and Yang et al. (170) reported several other significant findings, non-significant associations were consistently reported for PFHxS, PFNA and PFUdA across the other studies. Most studies were evaluated to have a high risk of bias.

TSH meta-analysis for pregnant women

Five studies that reported on the relationship between PFOS and maternal TSH were considered for meta-analysis. (165, 166, 170, 171, 174) The study by Yang et al. (170) was a cross-sectional study and the other four all other studies were a cohort design; however cross-

sectional analyses were undertaken on outcomes and exposures from blood samples obtained during pregnancy. Webster et al (174) collected two samples of blood at both 15 and 18 weeks gestation and included data from both samples in in the analysis. Of the five relevant studies, three used log-transformed TSH as the outcome and two used untransformed values. Thus, there were an inadequate number of studies which with comparable outcome and exposure measures for inclusion in a meta-analysis.

TSH in adults

Seven studies considered the association between PFAS and TSH concentrations in adults. (18, 175-180) The studies reported no significant association between exposure to PFOA, PFOS, PFHxS, PFNA, PFDA and PFUdA and TSH levels in adults. However, in a sub-analysis of 26 adults with high TPOAb and low urinary iodine concentrations, Webster et al. (180) found a significant positive association for increasing interquartile range of PFOA, PFOS, PFHxS and PFNA and TSH levels (estimated difference (%) in TSH (CI); 16.2 (5.1, 28.5), 17.1 (6.6, 28.7), 27.3 (0.7, 60.9) and 20.5 (4.3, 39.1), respectively). Webster et al. (180) used cross-sectional data from 1,525 adults who participated in the 2007–2008 wave of the NHANES study, and reported no association between PFAS and TSH concentrations in the general North American population, but found significant results when considering a sub-population of adults who had several other stressors affecting their thyroid gland and impairing its production of T_3 and T_4 hormones.

Similarly to their reports on pregnant women in 2014, Webster et al. (180) hypothesised that high PFAS concentrations only caused disruptions in the thyroid gland (and the production of related hormones) when the human body was subjected to other biological or chemical agents, in this case high TPOAb and low iodine, both of which are associated with the development of hypothyroidism. Webster et al. (180) proposes a *multiple hit hypothesis* to describe the effect modification of PFAS and TSH by high TPOAb and low iodine in adults. The findings of six of seven studies suggest that there is no association between PFAS and TSH levels in adults. Webster et al. (180) used cross-sectional data, and PFAS and TSH concentrations were measured concurrently, making it difficult to assess temporality of exposure. For this reason, the study was determined to have a high risk of bias and the results should be interpreted with caution.

Thyroxine (T_4) level

Following signalling from the pituitary gland via TSH, the thyroid gland produces T_4 hormones. T_4 are transported through the body in the blood stream to control the conversion of oxygen and kilojoules to energy, thereby influencing the body's rate of metabolism. Circulation of T_4 in the human body is crucial to many physiological processes throughout the lifespan, including the development of the foetal brain and nervous system.

T_4 exists in two states; T_4 bound to carrier-proteins in the blood or free T_4. Carrier-proteins facilitate the transport of T_4 around the human body; however, prevent T_4 from entering tissues from the blood stream. Free T_4 instead move into tissues without restriction and have the capacity to change the metabolic rate of a cell. Testing the concentration of free and total T_4 hormones in the blood is used as an indicator of the thyroid gland function in the human body.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Total T_4		
Total T_4 in infants		
	Umbilical cord; PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFUdA, PFTrDA, PFPeA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS, PFNA, PFDA, PFTrDA, PFUdA, PFDoA, PFHpA, PFHxA	Inadequate evidence
	During infancy; PFOA, PFOS, PFHxS, PFTrDA	Inadequate evidence
Total T_4 in children	PFOA, PFOS, PFNA	Inadequate evidence
Total T_4 in pregnancy	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFHpS, PFHpA, PFHxA	Inadequate evidence
Total T_4 in adults	PFOA, PFOS, PFHxS, PFNA, PFDA	Inadequate evidence
Free T_4		
Free T_4 in infants		
	Umbilical cord; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFHpA, PFHxA	Inadequate evidence
Free T_4 in pregnancy	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFHpA, PFHpS, PFHxA	Inadequate evidence
Free T_4 in adults	PFOA, PFOS, PFHxS, PFNA, PFDA PFUdA	Inadequate evidence

Total T_4 level in infants

Seven studies reported on the effect of prenatal PFAS exposure on concentrations of total T_4 in neonates and infants (age range; 0 to 5-years old). (63, 167-171, 181) Four studies (167-169, 181) investigated the association between umbilical cord exposure levels of PFAS and total T_4 and one study (171) investigated the relationship for maternal concentrations of PFAS. Two studies reported on both umbilical cord and maternal measurements. (63, 170) Results were conflicting across the seven studies, with authors reporting both significant positive and negative trends for PFAS exposure and total T_4 in infants. Largely, significant positive results for total T_4 related to umbilical cord measurements of PFAS and significant negative results were associated with maternal measurements of PFAS, however, studies also reported many non-significant findings across both exposures.

Umbilical cord blood studies

De Cock et al. (181), Shah-Kulharni et al. (168) and Yang et al. (170) each concluded at least one significant positive association between PFAS and total T_4 concentrations in the umbilical cord of neonates at birth, however there was no overlap between the findings reported by the 3 studies. De Cock et al. (181) found a significant positive association between PFOA and total T_4 levels in the umbilical cord for female neonates enrolled in the Linking endocrine disrupting hormones in maternal nutrition to child health (LINC) study (adjusted regression coefficient β Q1 vs Q4 (CI); 38.6 (13.34, 63.83)), and reported no association for PFOS for either females or males. Shah-Kulharni et al. (168) instead concluded a significant positive association between PFPeA and total T_4 levels in the umbilical cord for neonates (adjusted regression coefficient β (CI); 0.27 (0.04–0.49)), and Yang et al. (170) reported a significant positive trend for PFOS, PFDA and PFUdA (adjusted Spearmen correlation test (p); 0.172 (<0.05), 0.181 (<0.05) and 0.172 (<0.05), respectively). Both Shah-Kulharni et al. (168) and Yang et al. (170) found no association between umbilical cord levels of PFOA, PFHxS and PFDoA and total T_4. Shah-Kulharni et al. (168) also reported non-significant findings for PFOS, PFNA, PFDA, PFUdA and PFTrDA, conflicting with the significant results reported by both de Cock et al. (181) and Yang et al. (170). Yang et al. (170) further concluded no significant association between umbilical cord measurements of PFNA and total T_4.

In contrast, Tsai et al. (169) found a significant negative association between PFOS and total T_4 concentrations in umbilical cord blood for male infants (adjusted regression coefficient β (CI); -0.667 (-1.283, -0.05)). Tsai et al. (169) also reported non-significant results for PFOA, PFDA and PFUdA levels and total T_4. Kim et al. (63) and Lopez-Espinosa et al. (167) found no significant association between all investigated PFAS exposures and total T_4 in infants, including PFOA, PFOS, PFHxS and PFTrDA. (63) Lopez-Espinosa et al. (167) included total T_4 concentrations for infants up to 5-years old in their analyses, which should be considered in the interpretation of their findings.

Maternal blood studies

Kim et al. (63) and Yang et al. (170) investigated the association between maternal concentrations of PFAS and umbilical cord blood measurements of total T_4. As found for umbilical cord measurements of PFAS exposure, Kim et al. (63) concluded a non-significant relationship between maternal concentrations of PFOA, PFOS and PFHxS and total T_4 levels in South Korean infants. However, Kim et al. (63) found a significant negative association for PFTrDA (Pearson correlation (*p*); -0.441 (<0.05)), which was not reported for umbilical cord

blood. Similarly, Yang et al. (170) investigated differences in the effect of maternal and umbilical cord blood concentration of PFAS on total T_4 concentrations in infants and found no significant associations between maternal concentrations of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA and PFDoA and total T_4 levels. Wang et al. (171) only reported on the relationship between maternal measurements of PFAS and total T_4 levels. The study found a significant negative association between maternal concentrations of PFNA, PFUdA and PFDoA women and total T_4 levels in mother-infant pairs (adjusted regression coefficient β (CI); -0.213 (-0.384, -0.042), -0.052 (-0.095, -0.010) and -1.920 (-3.345, -0.495), respectively), and also found no significant associations for PFOA, PFOS, PFHxS, PFDA, PFHpA and PFHxA and total T_4 levels.

The association between prenatal PFAS exposures and total T_4 in neonates and infants across the seven studies was conflicting, and there were clear differences in T_4 hormones when considering PFAS measurements in umbilical and maternal bloods. Although significant results were reported in evaluated papers, there was insufficient evidence to conclude a potential relationship between PFAS and total T_4 in neonates and infants.

T_4 meta-analysis for infants

We considered six papers investigating PFOA and PFOS and T_4 in infants for meta-analysis. (63, 167-170, 181) De Cock et al. (181) presented results separately by sex of the child, thus there were potentially seven sets of results. De Cock et al. (181) and Kim et al. (63) were cohort studies, and the other four studies were cross-sectional designs. Of the seven sets of results, two reported correlation coefficients as measures of association (one of which used log transformed T_4) and five reported coefficients from linear regression (two using quartiles for PFAS, two using log transformed PFAS, one using PFAS log transformed and as quartiles, and one using log transformed outcome and exposures). Thus, there were an inadequate number of studies which with comparable outcome and exposure measures for inclusion in a meta-analysis.

Total T_4 level in children

In addition to investigating total T_4 levels in infants, Lopez-Espinosa et al. (167) reported on the association between PFAS exposure concentrations and total T_4 in children (age range 6 to 17-years old). The study found a significant positive association between PFOA, PFOS and PFNA and total T_4 concentrations (TSH change with IQR shift in PFAS (CI); PFOA (6–10-years old): 0.9 (0.0, 1.8); PFOS (6 to 10-years old): 0.0 (0.2, 1.7) and (>10-years old): 1.2 (0.6, 1.9); PFNA (6 to 10-years old): 1.0 (0.3, 1.7) and (>10-years old): 1.3 (0.7, 1.9)). However, the finding for PFOA was specific to children aged 6 to 10-years old only. These significant trends between childhood exposure to PFAS and total T_4 levels have not been investigated in other studies to date, and would benefit from further research.

Total T_4 level in pregnant women

Four studies examined the association between PFAS exposure and concentrations of total T_4 in pregnant women. (165, 170, 171, 174) Overall, the studies reported non-significant findings related to PFAS and total T_4 levels in women. Berg et al. (165) and Yang et al. (170) concluded no significant association between PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA (170) and PFHpS (165) and total T_4. Wang et al. (171) found no relationship for PFOA, PFOS, PFHxS, PFDA, PFHpA and PFHxA and total T_4, but identified a significant negative

association between PFNA, PFUdA and PFDoA and total T_4 concentrations in pregnant women (adjusted regression coefficient β (CI); -0.189 (-0.333, -0.046), -0.062 (-0.097, -0.026) and -1.742 (-2.785, -0.700), respectively). Non-significant findings were reported by Webster et al. (174) for all women with normal TPOAb, and further, for the association between PFOA, PFOS, PFHxS and PFNA in women with high TPOAb. Overall, the 4 studies do not support an association between PFAS exposure and concentrations of total T_4 in pregnant women; however, there is conflicting evidence for PFNA, PFUdA and PFDoA exposures.

Total T_4 level in adults

Five studies reported on the association between elevated PFAS levels and T_4 concentrations in adults. (176, 177, 179, 180, 182) In summary, there is no clear and consistent evidence to suggest an association between PFAS and T_4 serum levels in adults. Across the five evaluated studies the results are conflicting for PFOA, PFOS and PFHxS exposures.

In a cross-sectional study of 52,296 adults enrolled in the C8 Health Project, Knox et al. (177) reported a significant increase in T_4 measurements related to higher PFOA and PFOS exposure levels (regression coefficient β for PFOA (p); women 20 to 50-years old: 0.5 (<0.0001), women >50-years old: 0.08 (<0.0001), men >50-years old: 0.06 (0.001); β for PFOS (*p*); women 20 to 50-years old: 0.14 (<0.0001), women >50-years old: 0.08 (<0.0001), men 20 to 50-years old: 0.05 (0.0001) and men >50-years old: 0.05 (0.0001)). The study did not report a significant association between PFOA and T_4 levels in men aged 20 to 50-years old. In a cross-sectional study of 87 men and women aged 55 to 74-years old, living near the Hudson River, New York, US, Shrestha et al. (179) found a positive association between PFOS and T_4 (Pearson correlation coefficient (p); 0.39 (<0.001)), but reported no effects related to PFOA exposure levels. Wen et al. (182) found positive findings related to PFHxS and T_4 levels in adult NHANES study participants between 2007 and 2010, although results were specific to women (adjusted regression coefficient β (CI); 0.260 (0.108, 0.413)). Wen et al. (182) found no significant results related to PFOA, PFOS and PFNA in men and women. Jain et al. (176) found no significant association between PFOA, PFOS, PFHxS, PFNA and PFDA exposure levels and serum concentrations of T_4 in adults. Webster et al. (180) found no changes in serum T_4 in relation to increased PFOA, PFOS, PFHxS and PFNA levels in adults with normal iodine and TPOAb levels, and in a sub-analysis of men and women with low iodine and high TPOAb levels. Overall, there is conflicting evidence for the association between PFAS and T_4 levels in adults, specifically in relation to the effects of PFOA, PFOS and PFHxS.

Free T_4 level in infants

Three studies investigated the effect of PFAS exposure on free T_4 levels in infants. (166, 170, 171) All three studies reported no significant association between maternal concentrations of PFAS and free T_4 levels in infants at birth, including PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFHpA and PFHxA. Yang et al. (170) further found no significant relationship between umbilical cord concentrations of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA and PFDoA and free T_4 in neonates.

Free T_4 level in pregnant women

Five studies reported on the association between PFAS exposure and free T_4 concentrations in pregnant women. (165, 166, 170, 171, 174) Overall, the findings reported for free T_4 were similar to those reported for total T_4. Wang et al. (171) reported a significant negative

association for PFNA, PFUdA and PFDoA in women (adjusted regression coefficient β (CI); -0.019 (-0.028, -0.009), -0.004 (-0.007, -0.002) and -0.132 (-0.204, -0.059), respectively) and non-significant findings for PFOA, PFOS, PFHxS, PFNA, PFDA, PFHpA and PFHxA. Berg et al. (165) found no significant findings for PFOS, PFNA, PFHxS, PFUdA, PFDA and PFHpS and free T_4, which was also reported for total T_4 concentrations in pregnant women. In contrast, Yang et al. (170) found a significant negative relationship between PFDoA and free T_4 levels in pregnant women (adjusted Spearmen correlation test (p); -1.60 (<0.05)), which was not found for total T_4. The study further reported non-significant findings for PFOA, PFOS, PFHxS, PFNA, PFDA and PFUnDA. Kato et al. (166) reported no significant association between PFOA and PFOS and total T_4 and Webster et al. (174) reported no significant relationship between PFAS and free T_4 in pregnant women with normal or high TPOAb levels. The negative association between PFDoA and free T_4 in pregnant women may warrant further investigation, as Wang et al. (171) and Yang et al. (170) both reported this finding. Both studies were evaluated to have high risk of bias, which suggests that results should be interpreted with caution.

Free T_4 level in adults

Six studies further determined the effected of elevated PFAS exposure levels on serum concentrations of free T_4 in adults. (175, 176, 178-180, 182) As stated for changes in T_4 related to PFAS levels in adults, the literature reports conflicting results for PFOS and PFHxS. The association between T_4 levels and PFNA is further unclear.

In 144 males aged 20 to 30-years old enrolled in a cohort study in Taiwan, Lin et al. (178) reported a significant increase in serum free T_4 levels across increasing tertiles of PFNA measurements (mean free T_4 (SE); T1: 1.11 (0.02) and T3: 1.17 (0.03)). The investigators concluded no significant effects related to PFOA, PFOS and PFUdA exposure, and no association between PFAS and free T_4 levels in females. Shrestha et al. (179) reported no significant change in free T_4 levels in adults with elevated PFOA exposure levels. They did identify a significant positive association between PFOS and free T_4 serum concentrations (Pearson correlation coefficient (p); 0.23 (0.03)). Wen et al. (182) reported a significant decrease in free T_4 levels in relation to elevated PFHxS levels in adults (adjusted regression coefficient β (CI); -0.016 (-0.029, -0.003)), though stated no significant findings for PFOA, PFOS and PFNA, or for females across all exposures. Webster et al. (180) found a significant negative relationship between PFHxS and free T_4 levels in adults, and negative association related to interquartile increases in PFOS serum concentration (estimated difference (%) in TSH (CI); -8.3 (-15.8, -0.2) and -4.4 (-7.6, -1.1), respectively)). However, the results reported by Webster et al. (180) were reported only for adults with low iodine levels and high TPOAb levels. No significant results were found in an analysis of adults with normal iodine and TPOAb levels for PFOA, PFOS, PFHxS and PFNA. Similarly, Bloom et al. (175) and Jain et al. (176) found no significant association between free T_4 levels and several PFAS exposures in adults including PFOA, PFOS, PFHxS, PFNA, PFDA (Bloom et al., 2010 only), PFDA (Jain et al. (176) only) and PFUdA (Bloom et al. (175) only). Therefore, there is inconsistent evidence present across the six studies to suggest a significant increase in free T_4 levels in adults who have had elevated exposure to PFNA and PFOS, and further conflicting results related to a decrease in serum free T_4 levels related to increased exposure levels of PFHxS.

Triiodothyronine (T_3) level

Triiodothyronine hormones are produced by the thyroid gland through the same TSH signalling pathway as T_4, however, this occurs to a lesser degree. Triiodothyronine are primarily produced by the breakdown of T_4 in tissues of the human body, particularly in the liver. Like T_4, T_3 function to control metabolism. Although the concentration of T_3 in the human is less than T_4, T_3 is considered to be more biologically active, with an increased effect on metabolic rate.

Triiodothyronine also exists bound and unbound to carrier-proteins. Testing of free and total T_3 hormones in the blood stream is another method used by medical practitioners to assess thyroid function in the human body.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Total T_3		
Total T_3 in infants		
	Umbilical cord; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFPeA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFHpA, PFHxA, PFTrDA	Inadequate evidence
Total T_3 in pregnancy	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFUdA, PFHpS, PFHpA, PFHxA	Inadequate evidence
Total T_3 in adults	PFOA, PFOS, PFHxS, PFNA, PFDA	Inadequate evidence
Free T_3		
Free T_3 in infants		
	Umbilical cord; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFPeA	Inadequate evidence
	Maternal; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFTrDA	Inadequate evidence
Free T_3 in pregnancy	PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFHpS, Me-PFOSA-AcOH	Inadequate evidence
Free T_3 in adults	PFOA, PFOS, PFHxS, PFNA, PFDA	Inadequate evidence

Total T_3 level in infants

Five studies investigated the association between prenatal exposure to PFAS and total T_3 concentrations in infants. (63, 168-171) Of the five studies, two reported on umbilical cord blood measurements of PFAS (168, 169), one reported on maternal concentrations of PFAS (171) and a further two reported on both exposure measurements. (63, 170) The studies presented conflicting findings relating to the effect of several PFAS exposures, including PFOS, PFHxS and PFNA, and showed differences between the associations reported for

umbilical cord and maternal concentrations of PFAS. All studies reported no significant association between both maternal and umbilical measurements of PFOA and total T_3 levels in infants.

Umbilical cord blood studies

Yang et al. (170) found a significant positive association between umbilical cord measurements of PFOS and PFNA and total T_3 (adjusted regression coefficient β (*p*); 0.188 (<0.05) and adjusted Spearmen correlation test (p); 0.170 (<0.05), respectively), and no associations for PFOA, PFHxS, PFDA, PFUdA and PFDoA exposures. In contrast, Shah-Kulharni et al. (168) reported a significant association between PFHxS, PFDA and PFPeA and total T_3 levels in the umbilical cord blood of infants at birth. However, results were sex-specific, with PFHxS and PFDA positively associated with total T_3 in girls (adjusted regression coefficient β (CI); 4.28 (0.39, 8.17) and 3.93 (-0.07, 7.93), respectively)) and PFPeA was negatively associated with total T_3 in boys (adjusted regression coefficient β (CI); -2.69 (-5.16, -0.23)). The study reported non-significant findings for PFOA, PFOS, PFNA, PFDoA, PFTrDA and PFUdA exposures. Kim et al. (63) and Tsai et al. (169) further reported non-significant associations between umbilical cord measurements of PFOA and PFOS, PFHxS (Kim et al. (63) only), PFDA (Tsai et al. (169) only), PFTrDA (Kim et al. (63) only) and PFUdA (Tsai et al. (169) only) and total T_3 concentrations in infants at birth.

Maternal blood studies

Kim et al. (63) and Wang et al. (171) both concluded a significant negative association between maternal PFAS exposure levels and total T_3 levels in infants. Kim et al. (63) reported a significant negative association for PFOS and PFTrDA (adjusted Pearson correlation (p); -0.414 (<0.05) and -3.80 (<0.05), respectively). In contrast, Wang et al. (171) found a significant negative relationship between PFNA, PFDA, PFUdA and PFDoA ((adjusted regression coefficient β (CI); -0.002 (-0.004, -0.001), -0.017 (-0.028, -0.005), -0.001 (-0.001, -0.0002) and -0.022 (-0.035, -0.009), respectively). The studies both reported non-significant findings for PFOA and PFHxS. Wang et al. (171) found no significant association between PFHpA and PFHxA and total T_3 levels. In contrast to the findings for umbilical cord PFAS levels, Yang et al. (170) reported no significant associations between maternal concentrations of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA and PFDoA and total T_3 levels in infants.

In summary, across the five studies that reported on the association between prenatal PFAS exposure levels and total T_3 levels in infants there is evidence to support a negative association between umbilical cord levels of PFPeA and T_3 levels and between maternal levels of PFTrDA during pregnancy and T_3 levels. However, as the two studies showing this association were evaluated to have a high risk of bias, these findings should be interpreted with care. Thus, this association is considered to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias. Across the evaluated literature, there is also inconsistent evidence presented in relation to the effects of PFOS, PFNA, PFHxS, PFDA, PFUdA and PFDoA.

Total T_3 level in pregnant women

Four studies investigated the effect of PFAS exposure on total T_3 levels in pregnant women. (165, 170, 171, 173) Berg et al. (173) found a significant negative association between PFDA concentrations and total T_3 levels in pregnant women (mean difference in T_3 concentration Q1

vs Q4 (CI); -0.1 (-0.14, -0.06)). Berg et al. (173) reported non-significant results for PFOS and PFUdA exposures. In a replica study of the same 391 pregnant women from the Northern Norway Mother-and-Child Contaminant Cohort Study (MISA) over the same time period, Berg et al. (165) found an association between additional PFAS and total T_3 levels in pregnancy women during their second trimester. The study also reported a significant negative relationship between PFDA exposure and total T_3 levels (regression coefficient β Q1 vs Q4 (CI); 0.02 (-0.044, -0.005)), though found no association for PFOS, PFNA, PFHxS, PFUdA and PFHpS. Wang et al. (171) reported findings which conflicted with the Berg et al. (165, 173) studies, finding a significant positive association between PFDA and total T_3 concentrations (adjusted regression coefficient β (CI); 0.002 (0.000, 0.003)). Wang et al. (171) also reported no significant association for eight additional PFAS, including PFOA, PFOS, PFHxS, PFNA, PFUdA, PFDoA, PFHpA and PFHxA. Further contributing to inconsistencies in the results across the four studies, Yang et al. (170) found no significant association between PFDA and total T_3 levels, and a significant negative association for PFDoA (adjusted Spearmen correlation test (p); -0.301(<0.01)). Yang et al. (170) found no associations for PFOA, PFOS, PFHxS, PFNA and PFUdA in agreement with Berg et al. (165, 173) and Wang et al. (171). Thus, there is inconsistent evidence presented for a negative association between PFDoA exposure levels during pregnancy and total T_3 levels, and further evidence of increased, decreased and unchanged levels of total T_3 in relation to elevated PFDA exposure levels during pregnancy.

Total T_3 level in adults

Five studies investigated the effect of PFAS exposure on total T_3 levels in adults. (176, 177, 179, 180, 182) Jain et al. (176), Webster et al. (180) and Wen et al. (182) all reported on findings from the 2007–2008 waves of the NHANES study. However, Wen et al. (182) further incorporated findings from the 2009–2010 NHANES wave. Jain et al. (176) and Wen et al. (182) reported a significant positive association between PFOA and total T_3 concentrations in adults (adjusted regression coefficient for change in serum T_3 relative to a unit increase in log-transformed PFOA (CI) 6.628 (0.545, 12.712)), although the findings reported by Wen et al. (182) were specific to women. Both Jain et al. (176) and Wen et al. (182) reported non-significant findings for PFOS and PFNA, and Jain et al. (176) also reported no significant association between PFDA exposure and total T_3 concentrations in adults. Wen et al. (182) concluded a significant positive relationship between PFHxS and total T_3 levels in women (adjusted regression coefficient for change in serum T_3 relative to a unit increase in log-transformed PFHxS (CI) 4.074 (2.232, 5.916)), which was not a significant association reported by Jain et al. (176) for the NHANES participants.

While the results reported by Webster et al. (180) also related to adults who participated in the 2007–2008 NHANES wave, the findings were specific to a sub-analysis of women and men with high TPOAb and low iodine levels. Webster et al. (180) concluded a significant positive association between PFOA, PFOS, PFHxS and PFNA and total T_3 concentrations in women with high TPOAb and low iodine levels (estimated difference (%) T_3 in (CI) per IQR of PFAS; 12.4 (7.0, 18.1), 12.0 (6.7, 17.7), 13.8 (6.0, 22.1) and 15.4 (6.3, 25.3), respectively)). As stated previously, Webster et al. (180) hypothesised that PFAS influenced the functioning of the thyroid gland, including the production of T_3 hormones, only when the human body was subjected to additional stressors, which in this case was high TPOAb levels and low iodine levels. The study, therefore, suggests that the effect of PFAS on total T_3 levels is modified by high TPOAb and low iodine levels.

In contrast to the NHANES findings, Knox et al. (177) reported a significant negative association between PFOA and PFOS exposure and total T_3 uptake in adults (regression coefficient β for PFOA (p); women 20 to 50-years old: -0.08 (0.0001), women >50-years old: -0.07 (<0.005) and men >50-years old: -0.04 (0.037)); (regression coefficient β for PFOS (p); women 20 to 50-years old: -0.21 (<0.0001), women >50-years old: -0.17 (0.0001), men 20 to 50-years old: -0.05 (0.009) and men >50-years old: -0.09 (<0.0001)). Knox et al. (177) found no significant effects related to PFOA levels in males aged 20 to 50-years old. Shrestha et al. (179) reported no significant association for PFOA and PFOS exposures and total T_3 levels in adults, which further contributed to the conflicting results reported across the five studies. Despite the inconsistent evidence stated for PFOA, PFOS and PFNA, there is evidence to suggest that elevated PFHxS exposure levels are associated with increased total T_3 levels in adults. However, due to the cross-sectional design of the studies conducted by Wen et al. (182) and Webster et al. (180), the results should be interpreted with caution. We considered these studies to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Free T_3 level in infants

Yang et al. (170) investigated the association between prenatal exposure to PFAS and umbilical cord measurements of free T_3 levels in infants. Yang et al. (170) reported a significant positive association between umbilical cord concentrations of PFOS and free T_3 (adjusted Spearmen correlation test (p); 0.191 (<0.05)) and a significant negative association for maternal concentrations of PFOA (adjusted Spearmen correlation test (p); -1.69 (<0.05)). The study reported non-significant findings for both umbilical cord and maternal levels of PFHxS, PFNA, PFDA, PFUdA and PFDoA. In contrast, the study stated no significant associations for maternal PFOS and umbilical cord PFOA and free T_3 levels in infants. Thus, there is evidence to support an increase in free T_3 levels in infants related to elevated PFOS levels in the umbilical cord, and further evidence for a decrease in free T_3 levels in infants born to mothers with increased levels of PFOA during pregnancy, however, the cross-sectional design of this study contributed to a high risk of bias associated with these results, due to the unknown temporality of exposure. As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Free T_3 level in pregnant women

Three studies determined the association between concentrations of PFAS and free T_3 in pregnant women. (165, 170, 173) The two studies by Berg et al. (165, 173) reported on free T_3 levels in pregnant women enrolled in the same cohort, and therefore, each study concluded similar results, stating a significant negative association between PFUdA and free T_3 concentrations (Berg et al., (173); estimated mean difference in free T_3 concentration Q1 vs Q4 (CI); -0.18 (-0.25, -0.12) and Berg et al.,(165); β Q1 vs Q4 (CI); -0.02 (-0.033, -0.003)) and no significant association for PFDA. Berg et al. (165) further conducted analyses into the effect of additional PFAS on free T_3 concentrations during pregnancy, and found non-significant associations for PFNA, PFHxS and PFHpS. Yang et al. (170) reported a significant negative relationship between PFDoA and free T_3 levels (adjusted Spearmen correlation test (p); -0.268 (<0.01)), a significant positive association for Me-PFOSA-AcOH (adjusted Spearmen's correlation test (p); 0.163 (<0.05)) and no significant association for PFOS, PFHxS, PFNA, PFDA and PFUdA. Thus, the results presented by the Berg et al. (165, 173) studies and Yang

et al. (170) are conflicting, specifically related to PFUdA exposure levels. As Yang et al. (170) was the only study to report on the effects of PFDoA and ME-PFOSA-ACOH, the significant associations between the PFAS exposure and free T_3 concentrations in pregnant women are considered as evidence for an association; however, the high risk of bias assessment for the study justifies further investigation into the exposure-effect associations. We considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Free T_3 level in adults

Three studies investigated the effect of PFAS exposure on free T_3 concentrations in adults. (176, 180, 182) Jain et al. (176) and Wen et al. (182) found no significant association for PFOA, PFOS, PFHxS and PFNA and free T_3 concentrations in adults, and Jain et al. (176) further reported no association for PFDA. In contrast, Webster et al. (180) stated a significant increase in free T_3 levels in adults related to elevated exposure to PFOA, PFOS, PFHxS and PFNA (estimated difference (%) in free T_3 (CI) per IQR of PFAS; 4.8 (3.7, 5.8), 4.7 (3.9, 5.5), 3.9 (2.3, 5.5) and 6.3 (5.0, 7.5), respectively); however, results related to adults with low iodine levels and high TPOAb levels. Thus, there is inconsistent evidence to support a positive association between PFOA, PFOS, PFHxS and PFNA and changes in free T_3 levels in adults.

Thyroid disease

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Thyroid disease	PFOA, PFOS	Inadequate evidence
Congenital hypothyroidism	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFPeA, PFHxA, PFDoA, PFTrDA, PFBS, PFHpA, PFHpS, PFDS, PFBA	Inadequate evidence
Hypothyroxinemia in pregnancy	PFOA, PFOS, PFHxS	Inadequate evidence

Thyroid disease

The effect of elevated PFAS exposure levels on the development of thyroid disease in adults was reported by three studies. Melzer et al. (183) reported on the association between PFAS and thyroid disease in 3,974 adults that participated in the 1999–2000, 2003–2004 or 2005–2006 waves of the NHANES study. The study reported increased odds of current thyroid disease related to elevated PFOA exposure levels in women (adjusted OR Q4-Q1 (CI); 2.24 (1.38, 3.65)), however stated no significant effects related to PFOS exposure in women. Melzer et al. (183) further stated a significant association between elevated PFOS levels and current thyroid disease in men, however the result was only significant when comparing males in the highest PFOS exposure quartile (Q4) with those in both the lowest quartile (Q1) and second lowest quartiles (Q2), and not when comparing males in Q4 and Q1. Thus, the study does not support a significant association between PFOA and PFOS exposure levels and thyroid disease in men. Further, Melzer et al. (183) stated no significant results related to PFAS exposure levels and a previous diagnosis of thyroid disease (ever and current diagnosis of thyroid disease). In a study of 32,254 adults enrolled in the C8 Health Project or in a cohort of previous employees of a chemical plant between 1948 and 2002, Winquist et al. (184) reported a significant association between increased PFOA exposure levels and thyroid disease in women (adjusted HR Q5-Q1 (p); 1.37 (0.03)), though they concluded that there were no significant effects in men. Steenland et al. (185) found no significant association between thyroid disease and PFAS exposure in adults. Thus, although evidence is inconsistent, the evaluated literature suggests a positive association between PFOA and thyroid disease in women, and no effects related to PFOS exposure levels or thyroid disease in men.

Congenital hypothyroidism

Congenital hypothyroidism is a condition caused by inadequate thyroid hormone production in newborn infants. In a case-cohort study of 40 newborn infants that visited one hospital in Seoul, South Korea between July 2009 and February 2010, Kim et al. (186) found a significant association between congenital hypothyroidism and PFOA, PFNA, PFDA and PFUdA exposure levels (average PFAS level (ng/mL) for controls and cases (p); PFOA: 2.12 and 5.39 (<0.01), PFNA: 0.633 and 1.931 (<0.001), PFDA: 0.298 and 0.523 (<0.005) and PFUnDA: 0.438 and 0.982 (<0.005)). The study stated no association between 11 other PFAS and congenital hypothyroidism including PFOS, PFHxS, PFPeA, PFHxA, PFDoA, PFTrDA, PFBS, PFHpA, PFHpS, PFDS and PFBA. Therefore, there is evidence for a positive association

between elevated PFOA, PFNA, PFDA and PFUdA exposure levels and congenital hypothyroidism. As no study has evaluated these exposure-effect associations other than Kim et al. (186), and this study was evaluated as having a high risk of bias, the evidence reported for congenital hypothyroidism should be considered with caution. Thus, we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Hypothyroxinemia

Hypothyroxinemia is a condition in pregnancy where the T4 levels in the mother are low but the TSH levels are normal. Chan et al. (187) reported on the effect of PFAS exposure on the development of hypothyroxinemia in pregnant women, and found no significant association between concentrations of PFOA, PFOS and PFHxS and the health outcome.

Thyroglobulin levels

Associations a glance

Health outcome	PFAS exposure	Evaluation of evidence
Thyroglobulin in adults	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence

Thyroglobulin is the protein precursor of thyroid hormone. Wen et al. (182) investigated the association between PFAS exposure and thyroglobulin levels in adults, and reported no significant association for PFOA, PFOS, PFNA and PFHxS.

Neurodevelopmental and neurophysiological outcomes

We evaluated 23 papers investigating the effect of PFAS exposure on neurodevelopmental and neurophysiological outcomes in children and adults. The majority of studies centred on prenatal exposure to PFOA, PFOS, PFHxS and PFNA but the main health outcome discussed was childhood neurodevelopment. All papers were found to have a moderate or high risk of bias. The papers are summarised in Appendix 11.

Neurodevelopmental outcomes

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Infant neurodevelopment	PFOA, PFOS	Inadequate evidence
Childhood neurodevelopment	PFOA, PFOS, PFNA, PFDA, PFHxS, PFOSA	Inadequate evidence
ADHD	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpS	Inadequate evidence
Autism	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpS	Inadequate evidence
Behavioural problems	PFOA, PFOS, PFDA, PFHxS, PFNA	Inadequate evidence
Learning problems	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence

Infant neurodevelopment

Five studies reported on the association between PFAS exposure levels and neurodevelopmental outcomes in infants <5 years-old. (188-192) The studies examined the effect of PFOA and PFOS exposures on several neurodevelopmental outcomes, including cognitive and motor measurements. While there were clear significant findings presented across the five studies, the methods used to assess neurodevelopment in infants were heterogeneous and therefore, the outcomes of the studies are largely incomparable.

Using data on 349 mother-infant pairs enrolled in the HOME cohort study, Donauer et al. (189) found a significant positive association between hypotonic behaviour (a negative association with infant neurodevelopment) in children at 5 weeks old and elevated prenatal PFOA levels (OR 10-fold increase PFAS levels (CI); 3.79 (2.2, 12.8)), but did not find any associations related to 12 other neurodevelopmental outcomes including attention, quality of movement and reflexes. Goudarzi et al. (192) reported a significant negative association between PFOA levels and Mental and Psychomotor Developmental Indices (MDI and PDI, respectively) in female infants at 6-months old enrolled in the Hokkaido Study on Environment and Children's Health (adjusted β (CI); -0.296 (-11.96, -0.682)). However, results were not significant in

females at 18-months old, or in males at both ages. Chen et al. (188) reported no association between PFOA and neurodevelopmental outcomes in 2-year old children, and Fei et al. (190) further stated no significant results related to neurodevelopment measures at 18-months old. Forns et al. (191) reported no association between PFOA and neurodevelopment in children at 6-months and 2-years. Thus, there is inconsistent evidence regarding a negative association between PFOA exposure levels and neurodevelopmental outcomes in infants.

In 239 children aged 2-years old enrolled in the Taiwan Birth Panel Study, Chen et al. (188) found a significant decrease in gross-motor neurodevelopment associated with increased prenatal PFOS levels (multivariate regression β for PFAS exposure ≥90th percentile (CI); -8.8 (-1.5, -2.0)). However, a range of additional neurodevelopmental outcomes were not associated with PFOS exposure, including cognitive, language and fine-motor development. Donauer et al. (189) reported no association between PFOS exposure and neurodevelopment in children at 5-weeks of age. Fei et al. (190) concluded no significant effects related to PFOS exposure and neurodevelopmental outcomes at 18-months old and Forns et al. (191) also concluded no associations between PFOS and neurodevelopment in children at 6-months old and 2-years old. Goudarzi et al. (192) found no significant results for PFOS and neurodevelopmental measures in children at 6 and 18-months old. The evaluated literature does not suggest a decrease in infant neurodevelopment related to elevated PFOS levels.

Childhood neurodevelopment

The effect of PFAS exposure levels on neurodevelopment in children (≥5 years old) was further investigated in four studies. (193-196) As stated for infant neurodevelopmental outcomes, there was little consistency in the measures used to determine changes in childhood neurodevelopment related to PFAS exposure, making it difficult to compare study findings.

In a cohort of 79 children aged 9 to 11-years old, Gump et al. (193) reported a significant decrease in response inhibition times related to increased prenatal exposure to PFOS, PFNA, PFDA, PFHxS, and PFOSA (β for time-periods of the differential reinforcement of low rates of responding task (CI); (PFOS) 11–15 minutes: -0.25 (-0.46, -0.04); (PFNA) 6–10 minutes: -0.24 (-0.46, -0.02); (PFDA) 6–10 minutes: -0.24 (-0.45, -0.02) and 11–15 minutes: -0.23 (-0.45, -0.02); (PFHxS) 6–10 minutes: -0.31 (-0.53, -0.10); (PFOSA) 6–10 minutes: -0.25 (-0.46, -0.04)). The authors concluded that there was an association between these PFAS exposures and children's Impulsivity, but that the results require further investigation. Gump et al. (193) stated no significant results related to PFOA exposure levels. Vuong et al. (196) found a significant positive association between prenatal PFOS and PFHxS exposure levels and adverse neurodevelopmental outcomes in 256 children aged 5 to 8-years old enrolled in the HOME study. The results showed a higher odds of lower global executive functioning related to increased PFOS and PFHxS exposure levels (OR global executive composite scores ≥60 per 1-unit increase PFAS (CI); 2.19 (1.03, 4.66) and 1.71 (1.05, 2.77), respectively). However, the study did not report significant findings for PFOA, PFNA or PFDA exposure levels. In contrast, Høyer et al. (194) found a significant increase in hyperactivity (not specific to Attention Deficit Hyperactivity Disorder (ADHD)) related to elevated prenatal PFOA exposure levels in 1,113 children aged 5 to 9-years old enrolled in the INUENDO birth cohort (OR T3-T1 (CI); 3.1 (1.3–7.2)). Høyer et al. (194) reported no significant association between PFOA exposure and other neurodevelopmental outcomes. Stein et al. (197) found no association between increased prenatal PFOA exposure levels and neurodevelopmental measures in

children aged 6 to 12-years old. While these four studies report differences in the effects of PFAS on neurodevelopment in children, Gump et al. (193) and Vuong et al. (196) each reported significant reductions in neurodevelopment related to PFOS and PFHxS exposures. As each exposure has not been investigated in relation to other childhood developmental outcomes, there is considered to be evidence to support a negative association between PFOS and PFHxS and neurodevelopment. Further, the significant association between PFOSA and impaired inhibition responses in children is considered to be evidence for a negative association between PFOSA and neurodevelopment. We evaluated both of these studies to have a high risk of bias. Therefore, a clear conclusion between PFOS, PFHxS and PFOSA and adverse neurodevelopmental outcomes in children cannot be determined for the exposure-effect associations without further research. We considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Attention deficit hyperactivity disorder

Seven studies investigated the association between PFAS exposure and the development of ADHD during childhood. (195, 198-203) Overall, the studies present inconsistent evidence to support an increased risk of ADHD related to elevated PFAS levels, with both significant and non-significant results stated for PFOA, PFOS, PFHxS and PFNA exposures.

In 571 children aged 12 to 15-years old that participated in the 1999–2000 or 2003–2004 waves of the NHANES study, Hoffman et al. (198) reported a significant positive association between ADHD and exposure levels of PFOA, PFOS and PFHxS (OR per-1 μg/L increase in serum PFAS (CI); 1.12 (1.01, 1.23), 1.03 (1.01, 1.05) and 1.06 (1.02, 1.11), respectively). Hoffman et al. (198) further observed a positive association for PFNA, which was not statistically significant. Lien et al. (199) found a significant association between ADHD diagnostic measures and elevated PFNA exposure levels, including an inverse association for inattention (group mean difference in test scores <50th percentile vs ≥90th percentile (CI); -2.11 (-3.99, -0.23)) and a positive association for combined measure of hyperactivity and inattention (group mean difference in test scores <50th percentile vs ≥90th percentile (CI); -0.01 (-1.06, 1.09)). The study stated no significant results related to PFOA, PFOS and PFUdA exposures.

In a study of 10,546 children aged 5 to 18-years old enrolled in the C8 Health Project, Stein & Savitz (202) found a significant association between elevated PFHxS exposure and ADHD diagnosis (adjusted OR Q4-Q1 (CI); 1.53 (1.15, 2.04)), however the association was not significant when adjusting for children that had been diagnosed with ADHD and had been prescribed medication for the condition. Stein & Savitz (202) reported non-significant results related to PFOA, PFOS and PFNA exposures. In another cohort study of 320 mother-infant pairs enrolled in the C8 Study, Stein et al. (195) found a significant negative association between prenatal PFOA exposure levels and ADHD in children between the age of 6 to 12-years old (linear regression β for ADHD behavioural measurements (CI); -8.5 (-16.1, -0.8)). Liew et al. (200) found no significant association between PFAS levels and ADHD in children aged 9 to 15-years old for PFOA, PFOS, PFHxS, PFNA, PFDA and PFHpS. Ode et al. (201) also reported no significant effects related to PFOA, PFOS and PFNA and Strøm et al. (203) found no association for PFOA and PFOS. Thus, across the seven evaluated studies there was inconsistent evidence related to PFAS exposure and the development of ADHD, specifically related to increased levels of PFOA, PFOS, PFHxS and PFNA.

Autism

The relationship between prenatal exposure to PFAS and autism in children was reported in three studies. (200, 204, 205) In a cohort study of 175 mother-child pairs from the Health Outcomes and Measures of the Environment (HOME) study, Braun et al. (204) reported a negative association between PFOA exposure and autistic behaviours (regression coefficient β (CI); -2.0 (-4.4, 0.4)), and no association for PFNA and PFHxS exposure. The study further concluded sex-related differences for the association between PFOS and autistic behaviours, with a positive association found for boys only (β (CI); 3.8 (1.3, 6.3)). Social, Repetitive and Stereotypic (SRS) behaviour scores for girls were not associated with prenatal PFOS exposure. As autistic behaviour was measured using reports of SRS scores provided by the child's mother, the study was determined to have a high risk of bias. In contrast, Oulhote et al. (205) found a significant positive association between PFOA exposure levels at 5-years old and autism screening scores in children at 7-years old in a cohort of 567 children from the Faroe Islands (adjusted mean difference in behavioural test scores per 2-fold increase in PFOA (CI); 0.68 (0.25, 1.11)). The study did not report any significant results related to additional PFAS, including PFOS, PFHxS, PFNA and PFDA, and further, nor for prenatal PFAS exposure levels or PFAS at 7-years old. Liew et al. (200) found no significant association between PFAS levels and Autism in children aged 9 to 15-years old, including PFOA, PFOS, PFHxS, PFNA, PFDA and PFHpS. Therefore, the association between PFAS exposure levels and autism is inconsistent across the 3 studies for the effects of PFOA and PFOS exposures.

Behavioural problems

Three studies investigated the effect of PFAS exposure on behavioural problems in children. (194, 205, 206) Høyer et al. (194) stated a significant positive association between PFOS exposure levels and behavioural problems in children aged 5 to 9-years old (β per-1 natural log increase PFOS (CI); 1.0 (0.1, 2.0)); however, results were only significant for children from Greenland enrolled in the INUENDO cohort study. Oulhote et al. (205) found a significant positive association between a doubling of PFOA, PFNA, and PFDA concentrations and increases in total Strengths and Difficulties Questionnaire scores (point increases (CI); 1.03 (0.11, 1.95), 0.72 (0.07, 1.38), 0.78 (0.01, 1.55), respectively). The study reported inconsistent results related to PFOS and PFHxS exposure levels and behavioural problems in children, and no significant results were reported in relation to PFAS exposure levels at 7-years old or prenatal PFAS exposure levels. Fei & Olsen (206) reported no association between PFOA and PFOS exposure levels and behavioural problems in children at 7-years of age. As for other neurodevelopmental outcomes, the associations reported across the three evaluated studies are largely inconsistent; however, there is evidence to suggest a positive effect related to PFNA and PFDA exposures, as reported by Oulhote et al. (205). While there are no studies to suggest PFNA and PFDA are not associated with an increase in behavioural problems in children, the study by Oulhote et al. (205) was evaluated to have a high risk of bias, and should be interpreted with caution. As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Learning problems

Stein & Savitz (202) reported the association between childhood exposure to PFAS and learning problems between 5 to 18-years old. Through a cross-sectional study of children

enrolled in the C8 Health Project, Stein & Savitz (202) found a positive association between exposure to PFHxS and learning problems in children and adolescents (adjusted OR Q4-Q1 (95% CI); 1.59 (1.21, 2.08)). The study found no association related to exposure to PFOA, PFOS and PFNA. The study was determined to have a high risk of bias as the learning problems were reported in the study by the child's parent and were not based on a validated scale or score.

Neurophysiological outcomes

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Depressive symptoms		
Depression in children	PFOA, PFOS	Inadequate evidence
Depression in adults	PFOA, PFOS, PFHxS, PFNA, PFDA, PFOSA, PFBS, PFHpA, PFUdA, Me-PFOSA-AcOH, Et-PFOSA-AcOH, PFDoA	Inadequate evidence
Memory impairment	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Sleep effects	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpA, Me-PFOSA-AcOH, Et-PFOSA-AcOH, PFBS, PFDoA	Inadequate evidence

Depression

Berk et al. (207) and Strøm et al. (203) investigated the association between PFAS exposure and depression. The results reported in the studies are conflicting; however, the studies differed significantly in their design and outcome measurement. Berk et al. (207) reported a negative association between PFOA, PFHxS, PFNA and PFDA exposure and depressive symptoms in adults from the NHANES survey (multivariate prevalence ratios (CI); 0.63 (0.44, 0.89), 0.67 (0.49, 0.92), 0.63 (0.43, 0.92) and 0.62 (0.45, 0.86), respectively). The cross-sectional study further found no association between PFOS, PFOSA, PFBS, PFHpA, PFUdA, Me-PFOSA-AcOH, Et-PFOSA-AcOH and PFDoA exposure and depressive symptoms. In agreement with Berk et al. (207), Strøm et al. (203) reported no association between PFOS and depression, although the study was based on diagnosis in children and young adults through a prospective cohort study. Strøm et al. (203) also concluded no association between PFOA and depression, in contrast to Berk et al. (207) The risk of bias was determined to be high for the Berk et al. (207) study and moderate for the Strøm et al. (203) study.

Memory impairment

The relationship between PFAS exposure and memory impairment in adults was investigated in two studies. (208, 209) Gallo et al. (208) investigated the relationship between PFAS exposure and self-reported memory impairment in adults over the age of 50-years who were enrolled in the C8 Health Project. Gallo et al. (208) found that exposure to PFOA, PFOS, PFHxS and PFNA were negatively associated with adult's self-reported memory problems (adjusted OR Q5-Q1 (CI); 0.79 (0.71, 0.88), 0.85 (0.76, 0.94), 0.89 (0.79, 0.99), and 0.89 (0.80, 0.99), respectively). Power et al. (209) studied self-reported memory difficulties in adults aged between 60 to 85-years old using the NHANES between the years 1999–2008. Power et al. (209) found that a doubling of PFOA, PFOS, PFHxS and PFNA serum concentration was negatively associated with memory problems (OR (CI); 0.92 (0.78, 1.09), 0.90 (0.78, 1.03), 0.93 (0.82, 1.06), and 0.91 (0.79, 1.04), respectively), although these were not statistically significant. These studies were cross-sectional and drew participants from the USA. Both studies were determined to have a high risk of bias due to their self-report

measurement of the memory impairment, and therefore the evidence presented by both studies to support a negative association between PFOA, PFOS, PFHxS and PFNA exposure levels and memory impairment should be considered with caution.

Sleep effects

Shiue (210) investigated the association between elevated PFAS exposure levels and sleeping problems in 18–85-year old participants in the NHANES study between 1999 and 2000. The study found a significant positive association between urinary Me-PFOSA-AcOH and PFBS concentrations and the odds of a person feeling unrested during the day (OR (CI); 1.24 (1.02, 1.51), and 1.42 (1.02, 1.98), respectively). In addition, Shiue (210) found significant positive associations with higher urinary Et-PFOSA-AcOH and PFDoA concentrations (cut-points not stated) and the odds of a person waking at night (OR (CI); 1.50 (1.08, 2.09), and 1.72 (1.08, 2.73), respectively). Shiue (210) found no significant associations related to PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpA and PFOSA. Therefore, there is evidence to support an association between elevated serum Me-PFOSA-AcOH, Et-PFOSA-AcOH, PFBS and PFDoA levels and adverse sleep effects in adults. However, it is important to consider the high risk of bias assessment associated with this study, due to the use of self-reported sleeping patterns in the study. As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Cancers

A total of 20 papers investigating PFAS exposure and cancer were evaluated. (61, 155, 159, 185, 211-226) Eight papers examined the mortality experience or cancer incidence of three different occupational cohorts at different time points: three papers into APFO production workers at Cottage Grove, Minnesota; three papers into PFOA production workers at Parkersburg, West Virginia, and three papers into perfluoroctanesulphonyl fluoride (POSF) production workers at Decatur, Alabama. In addition, one study examined episodes of care for different cancer outcomes from health insurance providers from workers in Decatur, Alabama. Thirteen papers, including one which also examined mortality, examined the relationship between estimated or measured exposure and cancer incidence. Twelve of these were conducted in the United States, two in Denmark, and one in each of Sweden, Greenland, Greece and Australia. Four of the papers used C8 Health Project community cohort study data. The papers from Greece and Australia were small cross-sectional studies using opportunistic testing of blood and tissues from diseased and non-diseased populations. Most of the papers were conducted among populations where exposure had been estimated or modelled based on blood testing at a single point in time and many of the papers only examined exposure to a single PFAS. The papers are summarised in Appendix 12.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Bladder cancer	PFOA, PFOS	Inadequate evidence
Kidney cancer	PFOA	Limited evidence
Liver cancer	PFOA, PFOS	Inadequate evidence
Prostate cancer	PFOA, PFOS, PFNA, PFHxS, PFDA, PFDoA	Inadequate evidence
Pancreatic cancer	PFOA, PFOS	Inadequate evidence
Colorectal cancer	PFOA, PFOS	Inadequate evidence
Breast cancer	PFOA, PFOS, PFNA, PFHxS	Inadequate evidence
Testicular cancer	PFOA	Limited evidence
Thyroid cancer	PFOA	Inadequate evidence

Bladder cancer

One of the five papers evaluating mortality, and one of the seven studies evaluating incidence, found an association of bladder cancer with PFAS. Alexander et al. (211) examined mortality in a cohort of workers at a DuPont facility manufacturing POSF—a pre-cursor to PFOS. The study of 2,083 workers engaged at the plant for at least one year found that exposure to PFOS based on work history was associated with an increased standardised mortality ratios for cancer of bladder and other urinary organs (standardised mortality ratio (SMR) (95% CI); 12.77 (2.6, 37.35)) based on three cases. Olsen et al. (222) examined health claims data for this cohort and did not observe any association with bladder cancer. In a further follow-up of this occupational cohort using improved ascertainment of incident cases, Alexander et al. (212) did not observe an association between exposure and incidence of bladder cancer (standardised incidence ratio (SIR) (95% CI); 1.28 (0.64, 2.29)). Leonard et al. (221) did not

observe an association between PFOS exposure and bladder cancer mortality in an occupational cohort. Eriksen et al. (217) did not observe an association between PFOS and bladder cancer incidence in the general Danish population.

Steenland et al. (185) interviewed 73% (4391/6026) workers or their next of kin in an update of a highly-exposed occupational cohort that was inclusive of follow-up time from Steenland & Woskie (155). They observed a significant negative trend for bladder cancer across quartiles of PFOA with analysis without a lag period (p=0.04). (185) Lundin et al. (159) did not observe an association between PFOA and mortality from bladder cancer, which was consistent with an earlier analysis of this cohort. (218) In a combined analysis of the C8 Health Project cohort and a nearby DuPont occupational cohort, Barry et al. (213) did not observe an association between PFOA and bladder cancer incidence. Similarly, Vieira et al. (225) in an overlapping study conducted a geographic analysis of cancers in the C8 Health Project area of Ohio and West Virginia and did not identify an association with bladder cancer.

Kidney cancer

An association of kidney cancer with PFAS was found in one of three papers evaluating its mortality and one of the three papers evaluating its incidence. Steenland & Woskie (155) updated mortality data for an occupational cohort study of workers exposed to PFOA originally conducted by Leonard, et al. (221) and found elevated risks for kidney cancer (SMR (Q4-Q1) (95% CI); 2.82 (1.13, 5.81)). Barry et al. (213) used C8 Health Project data and found an association for a 1-unit increase in ln-transformed cumulative exposure to PFOA in relation to kidney cancer (HR (95% CI); 1.10 (0.98, 1.24)), the P-value was 0.10. In a study that overlapped in terms of study population and follow-up period. Vieira et al. (225) conducted a case control study of residents of different water supply districts in West Virginia and Ohio and identified weak positive association between PFOA and kidney cancer incidence (OR (Q4-Q1) (95% CI); 2.0 (1.0, 3.9)), in individual data, with some evidence a of dose response relationship. This association was little evident in area level data. Raleigh et al. (223) studied mortality and incidence and Leonard et al. (221) studied mortality among AFPO workers and found no association between PFOA and kidney cancer.

Liver cancer

None of the nine papers investigating the association between PFAS and liver cancer incidence and mortality reported statistically significant findings. Leonard et al. (221) and Steenland & Woskie (155) examined mortality from liver cancer in an occupational cohort, which was not associated with PFOA exposure. Barry et al. found no association between PFOA and liver cancer in the C8 Health Project. (213) In a geographic analysis as part of the C8 Health Project, Vieira et al. (225) found no association between PFOA and liver cancer incidence. Alexander et al., (211) in an occupational cohort study found no association between PFOS and liver cancer mortality. From the same cohort, Olsen et al. (222) found no association between episodes of care for liver cancer with PFOS exposure. Raleigh et al. (223) found no association between PFOA exposure and mortality or incidence of liver cancer. Eriksen et al. (217) investigated PFOS and PFOA in the Danish population and found no association with liver cancer. In a small cross-sectional study, Yeung et al. (226) tested for nine different PFAS in blood serum and liver of 79 patients undergoing liver transplant for liver cancer and found marginally higher levels in these patients than a small number of 34 control patients. However, sampling for this study was opportunistic in nature and could have been subject to selection bias.

Prostate cancer

An association between prostate cancer and PFAS exposure was identified in two of five papers evaluating mortality and none of the nine papers evaluating incidence. In an occupational cohort study of 3,993 employees, Lundin et al. (159) found an association between prostate cancer mortality and high levels of exposure to AFPO (HR (95% CI); 6.2 (1.1, 37.7)) based on job classification and duration of employment. Leonard et al. (221) observed a lower mortality rate of prostate cancer among an occupational cohort when compared to the United States general population (SMR (95% CI); 51.8% (26.8, 90.5)). However, in an update of this study, Steenland & Woskie did not observe an association between PFOA and mortality from prostate cancer from the same cohort. (155) In another occupational cohort, Raleigh et al., Lundin et al. and Gilliland & Mandel did not observe an association between prostate cancer mortality and PFOA exposure. (159, 218, 223) In the Danish birth cohort, Eriksen et al. (217) found a weak association between prostate cancer incidence and PFOS when comparing the highest quartile with the lowest (incidence rate ratio (Q4-Q1) (95% CI); 1.38 (0.99, 1.33)), although this association was not statistically significant. They did not observe an association for PFOA. In a study of 25,412 men from the C8 study, Ducatman et al. (216) examined prostate specific antigen levels among men in the C8 cohort study and found no association between PFAS and prostate specific antigen levels. Hardell et al. (219) conducted a case control study of 201 cases of prostate cancer and 186 population-based controls and found no overall association with the six PFAS chemicals measured. However, when analysis was adjusted for men who had a first degree relative had a history of prostate cancer there were positive associations with both PFOA (OR (95% CI); 2.6 (1.2, 6.0)) and PFOS (OR (95% CI); 2.7 (1.04, 6.8)).

Steenland et al. (185) did not identify an association between PFOA and incidence of prostate cancer among exposed workers in Ohio. Barry et al. (213) did not observe an association between prostate cancer and PFOA in the C8 Health Project. Similarly, in a geographic analysis of the C8 Health Project, Vieira et al. (225) did not observe an association between PFOA and prostate cancer. Grice et al. (61) found no association between self-reported prostate cancer and PFOS exposure in exposed workers. Olsen et al. (222) did not observe an association between PFOS and episodes of care for prostate cancer.

Colorectal cancer

Neither of the two papers examining mortality identified an association between colorectal cancer and exposure to PFAS. Gilliland & Mandel (218) did not identify an association between occupational exposure to PFOA and colorectal cancer. Similarly, Leonard et al. (221) did not observe an association between occupational exposures to PFOA and colorectal cancer.

Among six papers examining incidence, there were two papers that identified an association between PFAS and colorectal cancer. Innes et al. (220) conducted a large cross-sectional study among C8 Health Project study participants and found a strong inverse relationship between colorectal cancer and increasing blood concentration of PFOS (OR (Q4-Q1) (95% CI); 0.2 (0.2, 0.3)) and PFOA (OR (Q4-Q1) (95% CI); 0.6 (0.4, 0.9)) after adjusting for potential confounders. Vieira et al. (225) used a geographical approach to analysing data from cancer cases and controls (who were patients with cancers other than the cancers hypothesized to be caused by PFAS exposure) in the C8 study area using water supply areas of residence and historical measurements of PFAS in the supplied water to estimate PFAS exposure. There

was a weak positive association between colorectal cancer incidence and high exposure to PFOA (OR (95% CI); 1.3 (1.0–1.7)).

Grice et al. (61) did not identify an association between self-reported colorectal cancer and PFOS among exposed workers. In a similar occupational cohort, Olsen et al. (222) did not identify an association between occupational exposure to PFOS and episodes of care for colorectal cancer. Steenland et al. (185) did not identify an association between colorectal cancer incidence and PFOA exposure. Barry et al. (213) did not identify an association between colorectal cancer incidence and PFOA in the C8 Health Project.

Breast cancer

For breast cancer, none of the four papers evaluating mortality found an association between breast cancer and PFAS.

Two of the six papers evaluating incidence found an association with PFAS. Bonefeld-Jørgensen et al. (215) conducted a case cohort study of breast cancer in Danish women finding that increased PFHxS was negatively associated with this disease (RR (Q4-Q1) (95% CI); 0.41 (0.17, 0.96)) for women in the highest quartile versus the lowest quartile in women ≤40 years of age. The study also found that increased PFOSA was weakly positively associated with disease in these women (RR (95% CI); 2.45 (1.00, 6.00)) in the highest quartile versus lowest quartile in women ≤40 years of age. In a study of 31 breast cancer cases and 115 controls in Greenland, Bonefeld-Jørgensen et al. (214) found an association with higher blood levels of PFOS concentration modelled as a continuous variable (OR per ng/mL increase PFOS (95% CI); 1.01 (1.00, 1.02)).

Grice et al. (61) did not find an association between self-reported breast cancer and PFOS exposure at work. In another occupational cohort, Raleigh et al. (223) did not find any association between breast cancer and occupational exposure to PFOA. Barry et al. (213) did not observe an association between PFOA and breast cancer incidence in the C8 Health Project study. Similarly, in geographic analysis of the C8 Health Project, Vieira et al. (225) did not observe an association between breast cancer and PFOA exposure.

Testicular cancer

None of three papers evaluating mortality from testicular cancer and both of the two papers evaluating incidence found an association with PFAS. (155, 218, 221) Two overlapping papers investigating testicular cancer in the C8 Health Project identified associations with PFOA. Barry et al. (213) observed a comparatively strong and consistent association between PFOA exposure and testicular cancer: HR was 1.34 (95% CI 1.00, 1.79)) for log estimated exposure fitted as a continuous variable, and in quartiles of exposure it was 1.04 (95% CI 0.26, 4.22) Q2, 1.91 (95%CI 0.47, 7.75) Q3, and 3.17 (95% CI 0.75, 13.45) Q4 (P-0.94). Similarly, in a geographic analysis of testicular cancer in the C8 Health Project, the OR for testicular was higher in one of six water districts contaminated with PFOA: Little Hocking (OR 5.1 (95% CI); 1.6, 15.6). (225) However, there was no overall association with testicular cancer in this study.

Thyroid cancer

Four papers evaluated the association between PFOA and thyroid cancer incidence (three papers) and mortality (one paper). The three papers examining incidence did not find an association between PFAS and thyroid cancer. (61, 213, 225) Leonard et al. (221) conducted

an occupational cohort study of mortality in 6,027 men and women working in a DuPont ammonium perfluorooctanoate factory between 1948–2002 and found elevated risks for thyroid and other endocrine cancers (SMR (95% CI); 6.286 (1.297, 18.369)) in workers with any exposure to PFOA when compared to non-exposed workers.

Other cancers

In the cohort studies examining incidence of and mortality from cancer in people exposed to PFAS, there were many additional cancers studied that showed little or no evidence of any association with PFAS. They included: oesophageal, stomach, respiratory, larynx, lung, pancreas, central nervous system, lymphatic and haematopoietic, and bone cancers, and melanoma, Hodgkin lymphoma, and leukaemia. Vieira et al, (225) examined the relationship between PFOA and 18 different cancers. Non-Hodgkin lymphoma was associated with the highest level of exposure to PFOA (OR (95% CI); 1.8 (1.0, 3.4)). This study also found a weak association between PFOA and brain cancer at moderate levels of exposure (OR (95% CI); 1.8 (1.1, 3.2)), but not at high (OR (95% CI); 0.6 (0.2, 1.6)) or very high (OR (95% CI); unable to be estimated) levels of exposure. Steenland & Woskie (155) found an association between higher levels of exposure to PFOA among workers from the DuPont Chemical plant and mesothelioma mortality (SMR without lag analysis (Q4-Q1) (95% CI); 6.27 (2.04, 14.63)), although the authors concluded that there may have been confounding by job type and duration of employment giving rise to higher exposure to asbestos in certain occupations.

Diabetes

In total, 11 evaluated papers the association of PFAS exposure with diabetes outcomes in children and adults. The main exposure of interest was PFOA and a range of health outcomes were investigated in the papers, including type I and type II diabetes, gestational diabetes and diabetic mortality. All reviewed papers were determined to have a moderate to high risk of bias. The papers are summarised in Appendix 13.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Type I diabetes	PFOA, PFOS	Inadequate evidence
Type II diabetes	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
Gestational diabetes	PFOS, PFOA, PFNA, PFDA, PFHxS Et-PFOSA-AcOH, Me-PFOSA-AcOH	Inadequate evidence
All diabetes (unspecified)	PFOA, PFOS, PFNA, PFUdA PFHpA, PFHxS, PFOSA	Inadequate evidence

Type I diabetes

Predieri et al. (227) and Steenland & Woskie (155) reported on the effect of PFAS exposure on the onset of type 1 diabetes. Predieri et al. (227) investigated the association between PFOA and PFOS exposure and type I diabetes diagnosis in a case-control study of 44 Italian children. The study investigators concluded that children with type 1 diabetes had a significantly higher serum PFOS level than heathy controls (PFOS concentration (ng/mL) ± standard deviation (SD); cases: 1.53±1.50; controls: 0.55±0.15). Predieri et al. (227) stated that there was no difference between serum PFOA measurements in cases and controls. In agreement, Steenland & Woskie (155) reported no significant relationship between PFOA exposure and the development of type 1 diabetes in a cohort of exposed workers. This study did not evaluate the effects of exposure to PFOS.

Predieri et al. (227) suggest the potential use of serum PFOS levels as a biomarker for the development of type I diabetes; however, this conclusion should be considered with caution as the study was evaluated to have a high risk of bias. The largest concern in this study was the temporality of the exposure-disease relationship, as PFAS serum concentration was determined after type I diabetes diagnosis.

Type II diabetes

Karnes et al. (228) Lin et al. (163) and MacNeil et al. (229) all investigated PFAS exposure and type II diabetes and reported no association for PFOA. Lin et al. (163) further found no association between PFOA, PFOS and PFUdA and type II diabetes in a cohort of Taiwanese children and young adults aged 12 to 30-years old. The study investigators reported significantly higher circulating serum adiponectin—a glucose regulating compound as a marker of type II diabetes—for increasing quartiles of PFNA (p for trend, 0.01). There is evidence available for the effect of PFNA exposure. The study by Lin et al. (163) was evaluated to have a high risk of bias. As with papers into type I diabetes, future papers on type II diabetes

need to consider the temporality of the association to reduce the risk of bias. As there was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Gestational diabetes

Shapiro et al. (230) and Zhang et al. (231) investigated the association between PFAS exposure and the development of gestational diabetes in pregnant women. Both papers were cross-sectional in design and tested reconception serum for a range of PFAS, including PFOA and PFOS. Shapiro et al. (230) and Zhang et al. (231) found no association for PFOS exposure and gestational diabetes. Zhang et al. (231) reported a statistically significant association between PFOA and gestational diabetes (OR per SD increase (95% CI); 1.86 (1.14, 3.02)). Shapiro et al. (230) found no relationship between PFOA, PFNA, PFDA, PFHxS, Et-PFOSA-AcOH and Me-PFOSA-AcOH and gestational diabetes, and Zhang et al. (231) concluded no association for PFHxS. Shapiro et al. (2016) identified an association between impaired glucose tolerance for the second quartile of PFHxS (OR (95% CI); 3.5 (1.4, 8.9)). With the exception of PFOA, no other exposures were associated with the development of gestational diabetes in pregnant women. The use of self-reported measures to determine gestational diabetes by Zhang et al. (231) and the high percentage of missing data on gestational diabetes in Shapiro et al. (230) were potential areas of concern relating to study design.

Unspecified diabetes

Lind et al. (232) and Su et al. (233) examined the association between PFAS exposure and unspecified diabetes. Each study investigated exposure to several PFAS; however, PFOA, PFOS and PFNA were the only PFAS common to both authors. Lind et al. (232) determined a positive relationship between PFNA exposure and diagnosis of diabetes (OR (95% CI); 1.96 (1.19, 3.22)), and no association for PFOA, PFHpA, PFHxS, PFOS, PFOSA and PFUdA. Su et al. (233) concluded no association for PFOA, PFNA and PFUdA exposure and diabetes, and found a positive association for PFOS (OR (Q4-Q1); 3.37 (1.18, 9.65)). Overall, these papers each conclude no association for PFOA exposure and diabetes, and present conflicting results for the association for PFNA and PFOS.

The association between PFAS exposure and mortality caused by diabetes was further examined by Leonard et al. (221), Lundin et al. (159) and Steenland & Woskie (155) Each study was based on occupation cohorts of workers exposed to PFOA in the United States. None of the papers identified an association between PFOA exposure and mortality due to diabetes. However, there was significant potential for biased measurement of exposure and outcome.

Cardiovascular effects

We evaluated a total of nine papers focused on the effect of PFAS exposure on cardiovascular outcomes in children and adults. Most studies analysed the effect of PFOA on the development of cardiovascular disease and hypertension. The main health outcome was mortality caused by a specific cardiovascular disease, including heart disease and stroke. All papers were determined to have a moderate or high risk of bias. The papers are summarised in Appendix 14.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Cardiovascular disease		
All cardiovascular diseases	PFOA	Inadequate evidence
Coronary heart disease	PFOA	Inadequate evidence
Peripheral arterial disease	PFOA	Inadequate evidence
Cardiovascular disease mortality	PFOA	Inadequate evidence
Stroke mortality	PFOA	Inadequate evidence
Hypertension	PFOA, PFOS	Inadequate evidence
Carotid atherosclerotic vascular disease	PFOA, PFOS, PFNA, PFUdA	Inadequate evidence

Cardiovascular disease

We evaluated six papers investigating the association between PFAS exposure and cardiovascular disease. (128, 155, 159, 234-236) All of the studies examined the effect of PFOA on a specific cardiovascular disease diagnosis or mortality, including coronary heart disease, peripheral arterial disease and cerebrovascular disease (stroke). The studies presented conflicting results. However, it is difficult to make direct comparisons, as the health outcomes were not the same for all studies.

Shankar et al. (236) found a positive association between PFOA exposure and unspecified cardiovascular disease (OR (Q4-Q1) (95% CI); 2.01 (1.12, 3.60)) and peripheral arterial disease diagnosis (OR (Q4-Q1) (95% CI); 1.78 (1.03, 3.08)) in 1,216 subjects from NHANES. Lundin et al. (159) also reported a positive association between PFOA exposure and mortality caused by cerebrovascular disease (stroke) (HR (95% CI); 2.1 (1.0, 4.6)) in a cohort study of 3,993 DuPont workers exposed to AFPO. In contrast, Mattsson et al. (234), Sakr et al. (235) and Steenland & Woskie (155) found no association between PFOA and cardiovascular disease diagnosis and mortality. The effect of other PFAS on cardiovascular disease was only considered by Mattsson et al. (234).The case-control study concluded no association between PFOS, PFNA, PFDA, PFHpA, PFHxS, PFUdA and PFDoA and coronary heart disease diagnosis in adults.

From the above findings, the association between PFOA exposure and stroke and peripheral arterial disease is unclear. For coronary heart disease, all evaluated papers determined that there was no association between PFOA exposure and the disease diagnosis and mortality in

adults. This may indicate that PFOA exposure is not related to coronary heart disease, although all papers evaluated were considered to have a moderate to high risk of bias.

Hypertension

Geiger et al. (237), Min et al. (238) and Winquist & Steenland (128) investigated the relationship between PFAS exposure and diagnosis of hypertension. Through a cross-sectional analysis of the NHANES dataset, Geiger et al. (237) found no association between PFOA and PFOS exposure and hypertension in children aged 12 to 18-years old. Similarly, Min et al. (238) also used the NHANES dataset but reported a positive association between PFOA and hypertension diagnosis in adults (OR (Q1-Q4) (95% CI); 1.71 (1.23, 2.36)). Winquist & Steenland (128) used the C8 Health Project cohort to study the association between PFOA exposure and hypertension in adults and concluded there was no association.

Only one study investigated the association between PFAS exposure and hypertension in children, with conflicting results for the association in adults. Therefore, no clear conclusions can be made for the health outcome. Further, we considered all three studies to have potential for a high risk of bias.

Carotid intima-media thickness

Lin et al. (239) investigated the association between PFAS exposure and carotid intima-media thickness, as an indicator of carotid atherosclerotic vascular disease. Through a cohort study of individuals aged 12 to 30 years, Lin et al. (239) reported a positive association between PFOS exposure and carotid intima-media thickness (mean of carotid intima-media thickness (Q4-Q1) standard error (SE); 0.451 (0.006)), with no association for PFOA, PFNA and PFUdA. Similar to the studies on hypertension, the temporality of the association between PFAS and carotid intima-media thickness was unknown in the study resulting in the study having a high potential risk of bias.

Overweight and obesity

We evaluated nine papers examining the effect of PFAS exposure on overweight and obesity in children and adults. The studies mainly investigated exposure to PFOA and PFOS, however, the effect of additional PFAS exposure was also considered. The main health outcome evaluated in the studies was overweight in childhood, adulthood and during pregnancy, reported as measured using BMI and waist circumference. All papers were determined to have a moderate to high risk of bias. The papers are summarised in Appendix 15.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Childhood overweight and obesity		
Childhood BMI	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Waist circumference	PFOA, PFOS	Inadequate evidence
Childhood risk of overweight	PFOA, PFOS	Inadequate evidence
Height to waist ratio <0.5	PFOA, PFOS	Inadequate evidence
Adulthood overweight and obesity		
Adulthood BMI	PFOA	Inadequate evidence
Waist circumference	PFOA	Inadequate evidence
Adulthood risk of overweight	PFOA	Inadequate evidence
Gestational weight gain	PFOA, PFOS, PFHxS, PFNA, PFDA, PFOSA, PFHpS, PFHpA	Inadequate evidence

Childhood overweight and obesity

Andersen et al. (240), Braun et al. (241), de Cock et al. (242) and Høyer et al. (243) investigated the association between PFAS exposures and overweight and obesity in children. All of the studies were based on analysis of mother-child pairs enrolled in prospective birth cohorts. The research was mainly focused on the effect of prenatal exposure to PFOA and PFOS on BMI and waist measurements in young children. Andersen et al. (240) and de Cock et al. (242) concluded no association between PFOA and PFOS exposure and BMI in children aged seven years and less than one year old, respectively. Braun et al. (241) supported this conclusion of exposure to PFOS, PFNA and PFHxS in children aged 18 years or younger. Andersen et al. (240) also reported no association between waist circumference at 7-years old and prenatal exposure to PFOA and PFOS. Høyer et al. (243) further found no association between PFOA and PFOS exposure and the risk of a child being overweight between the ages of five and 9-years old.

Whilst most of the studies concluded no association between PFAS exposure and indicators of childhood overweight and obesity, Braun et al. (241) and Høyer et al. (243) reported conflicting results. In the HOME study, Braun et al. (241) reported a positive association between maternal PFOA levels and BMI gains for children from 2 to 8-years old (BMI z score (T3-T1) (95% CI); 0.44 (0.23, 0.64). Høyer et al. (243) reported a positive association between

PFOS exposure and a waist to height ratio of less than 0.5 for children aged five to nine years (RR (95% CI); 1.38 (1.05, 1.82)). Høyer et al. (243) identified similarly elevated results for PFOA, which were not statistically significant. These studies present some evidence that PFOA and PFOS exposures may increase childhood overweight and obesity in young children. However, due to the moderate risk of bias presented by each of the studies, and the conflicting results presented by Andersen et al. (240) and de Cock et al. (242), the findings should be considered with caution.

Adulthood overweight and obesity

Barry et al. (244) and Halldorsson et al. (245) investigated the association between PFOA exposure and overweight and obesity in adults. The two studies presented conflicting results and the measurement of overweight and obesity was not the same. In a Danish birth cohort, Halldorsson et al. (245) reported a positive association among female offspring at 20 years of age between maternal PFOA exposure and waist circumference (RR (Q4-Q1) (95% CI); 3.0 (1.3, 6.8)) and for overweight and obese as measured by BMI (adjusted RR (Q4-Q1) (95% CI); 3.1 (1.4, 6.9)). No association was observed for male offspring. Barry et al. (244) reported no association between PFOA exposure and BMI, stating there was no increased risk of an adult being overweight or obese as a result of the exposure. Whilst the cohort studies did not present the same results, there was inconsistent use of measures of overweight and obesity. Current health guidelines suggest that a combination of waist circumference and BMI measurements should be used to determine overweight and obesity in adults.

Gestational weight gain

Ashley-Martin et al. (246), Jaacks et al. (247) and Rylander et al. (248) studied the effects of PFAS exposure on weight gain during pregnancy. Ashley-Martin et al. (246) and Jaacks et al. (247) both reported a positive association between PFOS exposure and gestational weight gain. In a trans-Canadian study of 2001 pregnant women, Ashley-Martin et al. (246) report that maternal PFOS levels were positively associated with gestational weight gain (regression coefficient β (95% CI); 0.39 (0.02, 0.75)). Similar associations of borderline statistical significance were observed for PFOA, and no association was reported for PFHxS. Using data from the LIFE study in Michigan and Texas, Jaacks et al. (247) reported a significantly positive association with gestational weight gain in women with a BMI < 25 kg/m^2 (adjusted regression coefficient (95% CI); 280.29 (13.71, 546.86)) but not in women with BMI ≥ 25 kg/m^2. All authors reported no association between other PFAS exposures and overweight and obesity measurement during pregnancy, including PFOA, PFHxS, PFNA, PFDA, PFOSA, PFHpS and PFHpA. A clear limitation of the studies was that the weight of the child at birth was not considered as a covariate; however, Jaacks et al. (247) justified their measurement of gestational weight gain through only including female participants that had a normal BMI before their pregnancy. Though the measurement of gestational weight gain is defined to be an indicator of weight retention after pregnancy, it is difficult to interpret the effect of PFAS exposure on the gestational weight gain as most studies were evaluated to have moderate to high risk of bias.

Immunological outcomes

We evaluated 15 papers investigating the effect of PFAS exposure on the immune system in children and adults. In the papers, exposure to PFOA and PFOS was primarily studied in relation to several health outcomes, including the overall function of the immune system and the efficacy of vaccinations. All papers that were evaluated were determined to have a moderate to high risk of bias. A summary of the papers is provided in Appendix 16. All immunological health outcomes were ineligible for meta-analysis.

Vaccine response

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Diphtheria	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHPA, PFUdA, PFDoA	Limited evidence; PFOA, PFOS, PFHxS, PFDA
Tetanus	PFOA, PFOS, PFHxS, PFNA, PFDA, PFHPA, PFUdA, PFDoA	Inadequate evidence
Measles, mumps and rubella		
Measles	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Mumps	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Rubella	PFOA, PFOS, PFHxS, PFNA	Limited evidence; PFOA, PFOS Inadequate evidence; PFHxS, PFNA
Influenza	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence

Antibody responses to routine childhood vaccinations provide insights into the potential immunological effects of PFAS. Epidemiological studies have investigated the effects of PFAS on antibody responses from different vaccines. Three papers related to the same cohort of 656 Faroese children who had a diet high in marine life and were presumed to have higher exposure to PFAS. The mean PFOS blood concentration in mothers was 27.3 ng/mL (IQR 9.9 ng/mL) and for PFOA was 3.2 ng/mL (IQR 1.45 ng/mL). In 587 children, the mean PFOS was 16.7 ng/mL (IQR 17.6 ng/mL) and for PFOA was 4.06 ng/mL (IQR 1.63 ng/mL).

Diphtheria vaccine

Four papers investigated the association between exposure to PFAS and antibody response to diphtheria vaccination. (249-252) Grandjean et al. (249) and Mogensen et al. (250) in near identical analyses used the same study population from the Faroese birth cohort. Each study reported no statistically significant relationship between PFHxS and diphtheria antibody response, and Grandjean et al. (249) further concluded no association of PFDA and PFNA in children. Both Grandjean et al. (249) and Mogensen et al. (250) used structural equation models for the association between antibodies and PFAS concentrations. Both investigators conducted multiple analyses and identified that PFOS, PFOA were significantly associated

with a reduction in antibody in children aged 5-years old and at follow-up at 7-years old. For PFHxS, PFNA and PFDA only 1–2 analyses out of the eight different models identified a significant reduction in antibodies. Mogensen et al. (250) estimated that the concentration of diphtheria antibodies decreased by 51.8% (95% CI; 24.6, 68.5) for a doubling of childhood PFAS exposure.

Grandjean et al. (252) reported findings from the same Faroe cohort study with follow-up to 13-years old. At age 13-years old, using structural equation modelling among children who had not visited an emergency room, PFOS (percent change (95% CI); -32.8% (-47.9, -13.3)), PFOA (percent change (95% CI); -19.9% (-35.4, -0.5), PFHxS (percent change (95% CI); -16.2% (-29.3, -0.6), PFNA (percent change (95% CI); -23.3% (-35.3, -9.0) and PFDA (percent change (95% CI); -20.7% (-30.7, -9.2) was associated with decreased diphtheria antibody concentration for a doubling of the PFAS concentration in children at 7-years old.

Kielsen et al. (251) studied diphtheria antibody response after vaccination in 12 healthy adults from Denmark. The authors found a significant reduction in diphtheria antibodies 4–10 days post vaccination and exposure to PFOS (percent change (95% CI); -11.90% (-21.92, -0.33)), PFNA (percent change (95% CI); -17.90% (-27.99, -6.39)), PFDA (percent change (95% CI); -18.18% (-29.52, -5.00)), PFUnDA (percent change (95% CI); -12.11% (-22.06, -0.90)) and PFDoA (percent change (95% CI); -15.64% (-0.98, 28.14)). There was no significant association of PFOA, PFHxS and PFHpA exposure to reduced antibody concentrations in the adults, although the sample size was very small.

It is important to note that three of the four papers were on the same cohort in the Faroe Islands, making assessment of the consistency of evidence difficult. For this reason, we considered this to be limited evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Tetanus vaccine

Five papers reported tetanus antibody response, with conflicting results. Grandjean et al. (249) found a negative association with PFOA (percent change (95% CI); 28.2% (−42.7, -10.1)), and PFHxS (percent change (95% CI); 14.0% (-24.0, -2.6)). For PFNA and PFDA only one to two analyses out of the eight different models identified a significant reduction in antibodies. Mogensen et al. (250) using structural equation modelling for the same cohort of 5 and 7 year old children found the strongest negative association with PFHxS (percent change (95% CI); 22.3% (-36.3, -5.2)) followed by PFOA (percent change (95% CI); -20.5% (−38.2, 2.1)) then PFOS (percent change (95% CI) -9.1% (−32.8, -23.0)). In the follow-up study at age 13 years, Grandjean et al. (252) found no significant relationship between anti-tetanus and PFOS, PFOA, PFHxS, PFNA, and PFDA.

Granum et al. (253) investigated the antibody response to the tetanus vaccine in 99 children aged 3-years old based on maternal blood concentration in a prospective birth cohort in Norway. They found no associations with PFOA, PFOS, PFHxS and PFNA. Kielsen et al. (251) found a significant decline in tetanus antibody concentration 4–10 days post vaccination for PFUdA (percent change (95% CI); 7.88% (-14.79, -0.42) and PFDoA (percent change (95% CI); 10.78% (-19.90, -0.64), but no association with PFOA, PFOS, PFNA, PFDA, PFHxS or PFHpA.

It is important to note that three of the five papers were on the same cohort in the Faroe Islands, making assessment of the consistency of evidence difficult. We considered this to be

inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias.

Measles, mumps and rubella (MMR) vaccine

Granum et al. (253) and Stein et al. (254) examined the relationship between PFAS and antibody response to measles, mumps and rubella. Granum et al. (253) found a significant negative association with rubella antibodies and exposure levels of PFOA, PFOS, PFHxS and PFNA (estimated change (95% CI); -0.40 (-0.64, -0.17), -0.08 (-0.14, -0.02), -0.38 (-0.66, -0.11) and -1.38 (-2.35, -0.40), respectively) in children aged 3-years old. While Stein et al. (254) using NHANES weighted survey results found the same association for antibody response to mumps only in seropositive 12 to 19-year old children for PFOS (percent change (95% CI); -13.3% (-19.9, -6.2)), PFOA (percent change (95% CI); -8.9% (-14.6, -2.9)) and PFHxS (percent change (CI); -6.0% (-9.6, -2.2)). Stein et al. (254) did not find an association with mumps antibody response of PFNA. Stein et al. (254) found a significant reduction in rubella antibody response in seropositive 12–19-year old children for PFOS (percent change (95% CI); -5.9% (-9.9, -1.6)) and PFOA (percent change (95% CI); -6.6% (-11.7, -1.5)), but no association with PFHxS or PFNA. Both Granum et al. (253) and Stein et al. (254) looked at the association between the measles antibody response and PFAS. No associations were found with the response and PFOA, PFOS, PFHxS and PFNA. Granum et al. (253) also found no association between the Hib antibody response and PFOA, PFOS, PFNA and PFHxS. Thus, there is inadequate evidence for an association between PFAS and decreased antibody response to measles and mumps vaccination. We considered the evidence for a reduced antibody response to rubella vaccination to be limited after taking into account the study design, strength of effect and associated risk of bias.

Influenza vaccine

Looker et al. (255) and Stein et al. (256) investigated the effect of exposure to PFAS on antibody response to influenza vaccinations. Looker et al. (255) investigated a cohort of 403 adults who had pre- and post-influenza vaccination titres tested. Looker et al. (255) found that there was evidence of a reduced antibody response to A/H3N2 influenza vaccine by higher PFOA concentration (Logistic regression coefficient (Q2-Q1) (95% CI); -0.28 (-0.51, -0.06)), although rates of seroconversion were not significantly different. In this study, elevated PFOS did not affect antibody response. Stein et al. (256) in a study of 78 adults who were vaccinated with FluMist™—an influenza vaccine administered directly into the nose—were more likely to seroconvert with higher concentrations of PFOS, PFOA, or PFNA. However, there were few seroconversions to this intranasal vaccine. Confidence intervals around estimates were highly uncertain. Higher levels of PFHxS was not associated with seroconversion, but was negatively associated with other markers of immunity.

Infectious disease

We evaluated four papers investigating the effect of PFAS exposure on the incidence of infectious diseases in children and adults. In the papers, exposure to PFOA and PFOS were primarily studied in relation to several health outcomes, gastroenteritis and respiratory infections. All papers that were evaluated were determined to have a moderate to high risk of bias. All infectious disease outcomes were ineligible for meta-analysis.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Hospitalisations due to infection	PFOA, PFOS	Inadequate evidence
Middle ear infection	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Gastroenteritis	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Colds and influenza	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence

Hospitalisations due to infection

Fei et al. (257) reported on the association between prenatal exposure to PFAS and risk of hospitalisations due to infection during early childhood. The authors found no significant relationship between the concentration of PFOA and PFOS in maternal serum and hospitalisations due to infection in children.

Middle ear infection

Okada et al. (258) and Granum et al. (253) investigated the association between prenatal exposure to PFAS and the development of *Otitis media*, or middle ear infection, during early infancy. Okada et al. (258) reported no significant association between maternal concentrations of PFOA and PFOS during pregnancy and middle ear infections in infants during their first 18 months of life. Granum et al. (253) further found no significant relationship between PFOA, PFOS, PFHxS and PFNA in maternal serum and middle ear infections in children in their first 3 years of life.

Gastroenteritis

Granum et al. (253) reported on the effect of prenatal exposure to PFAS on the development of gastroenteritis in infants enrolled in the BraMat prospective birth cohort (a sub-cohort of the MoBa study). The study investigators reported a significant positive association between the number of self-reported gastroenteritis episodes (with vomiting or diarrhoea) during infancy (the first 3-years of life) and maternal serum concentrations of PFOA and PFHxS (multivariate β (CI); 0.31 (0.00, 0.61) and 0.35 (0.10, 0.61), respectively), and no significant association with PFOS and PFNA exposure levels. However, when Granum et al. (253) conducted an analysis on the odds of having had an episode of gastroenteritis at 3 years old (versus not having had gastroenteritis at 3 years old), the study found no significant associations between PFAS exposure and the health outcome. Therefore, Granum et al. (253) suggest that increased exposure to specific PFAS may lead to increased number of episodes, despite no apparent differences between infants who have never had the infectious disease and infants

that have. As the health outcome has not been investigated in other studies to date, it is difficult to comment on the consistency of evidence. The study was determined to have a high risk of bias due to the self-reported nature of gastroenteritis–a common childhood illness.

Colds and influenza

Granum et al. (253) and Looker et al. (255) investigated the association between PFAS exposure levels and episodes of cold and flu. After adjusting for potential confounders, Granum et al. (253) reported a significant positive relationship between episodes of the common cold in children during the first 3-years of life and maternal concentrations of PFOA and PFNA (multivariate β (CI); 0.42 (0.21, 0.62) and 0.74 (0.05, 1.43), respectively), and no association with PFOS and PFHxS exposure. In contrast, Granum et al. (253) reported no significant association between PFAS exposure and the odds of infants developing the common cold at 3 years old versus not developing the common cold at 3 years old. This finding was similar to the relationship Granum et al. (253) found for gastroenteritis. In another study, Looker et al. (255) found no significant association between PFOA and PFOS exposure and self-reported cases of cold and flu, however the research was conducted on a cohort of adults from a highly exposed community in the USA. Given the limited number of studies that are at high risk of bias, these results should be viewed with caution.

Asthma and allergic diseases

We evaluated fifteen papers investigating the effect of PFAS exposure on the immune system in children and adults. In the papers, exposure to PFOA and PFOS was primarily studied in relation to several health outcomes, including the overall function of the immune system and allergies. All papers that were evaluated were determined to have a moderate to high risk of bias. All immunological health outcomes were ineligible for meta-analysis.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Asthma	PFOA, PFOS, PFHxS, PFNA, PFDA, PFTEDA, PFDoA, PFHxA, PFHpA, PFBS	Inadequate evidence
Allergies		
Total allergies	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTrDA, PFUdA	Inadequate evidence
Total food allergies	PFOA, PFOS	Inadequate evidence
Shrimp allergy	PFOA, PFOS, PFNA	Inadequate evidence
Plant sensitivity	PFOA, PFOS, PFNA	Inadequate evidence
Cockroach sensitivity	PFOA, PFOS, PFNA	Inadequate evidence
Mould sensitivity	PFOA, PFOS, PFNA	Inadequate evidence
Allergic Rhinoconjunctivitis	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTrDA, PFUdA	Inadequate evidence
Wheezing	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTrDA, PFUdA	Inadequate evidence
Eczema	PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTrDA, PFUdA	Inadequate evidence

Asthma

Six studies investigated the association between exposure to PFAS and asthma diagnosis. (185, 253, 254, 259-261) While six studies were evaluated, the reported findings relate to four different cohorts, with two instances where results were presented of the same study population. Overall, the findings report conflicting results of the effect of PFAS exposure on asthma in children and adults.

Dong et al. (259) and Zhu et al. (261) each reported on the results from a case-control study of Taiwanese children enrolled in the Genetic and Biomarkers study of Childhood Asthma (GBCA) study. Dong et al. (259) reported a significant positive association between serum concentrations of PFOA (OR (Q4-Q1) (95% CI); 4.05 (2.21, 7.42)), PFOS (OR (Q4-Q1) (95% CI); 2.63 (1.48, 4.69)), PFHxS (OR (Q4-Q1) (95% CI); 3.83 (2.11, 6.93)), PFDA (OR (Q4-Q1) (95% CI); 3.22 (1.75, 5.94)),PFNA (OR (Q4-Q1) (95% CI); 2.56 (1.41, 4.65)), PFDoA (OR (Q4-Q1) (95% CI); 1.81 (1.02, 3.23)) and PFBS (OR (Q4-Q1) (95% CI); 1.90 (1.08, 3.37)) and asthma diagnosis in the past 12 months of children aged 10–15 years old, and no association of PFHxA and PFTrDA. Zhu et al. (261) further reported a significant positive association between PFOA, PFHxS and PFDA exposure levels and asthma diagnosis in the past 24 months of children aged 10-15 years old. The study also concluded a significant positive

relationship between PFOS, PFNA and PFBS and asthma diagnosis of boys of the same age (results available in graphical format only).

Similarly, Zhu et al. (261) reported no significant association between PFHxA and PFTEDA exposure and asthma diagnosis in children, and further concluded no significant results of PFHpA and PFDoA of children aged 10-15 years old. Zhu et al. (261) reported significant associations among males for PFOS (OR (Q4-Q1) (95% CI); 4.38 (2.02, 9.50)) for PFOA (OR (Q4-Q1) (95% CI); 4.24 (1.91, 9.42)) for PFBS (OR (Q4-Q1) (95% CI); 2.59 (1.14, 5.87)) for PFDA (OR (Q4-Q1) (95% CI); 3.45 (1.51, 7.88) for PFHxS (OR (Q4-Q1) (95% CI); 2.97 (1.33, 6.64)) and for PFNA (OR (Q4-Q1) (95% CI); 3.33 (1.46, 7.58). Similar results were seen for females, but were only significant for PFOA, PFDA, and PFHxS.

Humblet et al. (260) and Stein et al. (254) also reported on asthma outcomes in children from the same study population–the NHANES study. Humblet et al. (260) using data from the 1999-2008 waves, and Stein et al. (254) using over-lapping results from the 2005-2006 waves. In agreement, the studies concluded no significant association between PFOA, PFOS, PFHxS and PFNA serum levels and asthma diagnosis in children aged 12–19 years of age. Using the BraMat prospective birth cohort, Granum et al. (253) reported no significant association between maternal concentrations of PFOA, PFOS, PFHxS and PFNA during pregnancy and asthma in children during their first 3-years of life.

Steenland et al. (185) investigated the association between occupational exposure to PFAS and asthma in adults through a retrospective analysis of DuPont employees. The study reported a significant negative trend between estimated exposure to PFOA and medicated asthma in the employees, with the non-lag analysis (p trend, 0.05).

The five studies conducted on the association between exposure to PFAS and asthma in children reported conflicting findings. Despite presenting results on the same case-control study of Taiwanese children, Dong et al. (259) and Zhu et al. (261) reported differing results, with Zhu et al. (261) concluding that the positive association between PFOS and PFBS was significant for male adolescents only. Furthermore, the non-significant associations between PFOA, PFOS, PFHxS and PFNA, from the NHANES study reported by Humblet et al. (260) and Stein et al. (254), contrast many of the associations found by Dong et al. (259) and Zhu et al.(261). All findings related to PFAS exposure and asthma in adolescents were inconsistent, with the exception of positive association reported of PFDA and PFBS (in boys only), which were only studied by Dong et al. (259) and Zhu et al. (261).

Allergies

Four studies reported on the association between exposure to PFAS and the development of allergies in children. (254, 258, 262, 263) Goudarzi et al. (262) investigated total number of allergic diseases in 4-year old children in Japan through the Hokkaido Study. The study stated no significant association between maternal levels of PFOA, PFOS, PFHxS, PFNA, PFDA and PFUdA during pregnancy and allergic disease, and a significant negative association of PFDoA and PFTrDA in boys only (OR (Q4-Q1) (95% CI); 0.492 (0.314, 0.766)) and (OR (Q4-Q1) (95% CI); 0.647 (0.416, 1.00)) respectively. Two studies by Okada et al. (258, 263) investigated total allergic diseases during early infancy in relations to PFAS exposure using data from the Hokkaido Study. Okada et al. (263) reported no significant association between PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA and PFTrDA and total allergic diseases in the first 24-months of age. Okada et al. (258) reported on food allergies in the first 18-

months of age, including milk, egg, shrimp or other foods and found no significant associations.

Stein et al. (254) reported on the effect of PFAS exposure and IgE sensitisation during adolescents using data from the NHANES study. The study reported a significant negative association between PFOS and sensitisation to plants (geometric mean (95% CI); 13.6 (12.4, 15.0)), cockroaches and shrimp (geometric mean (95% CI); 12.5 (11.0, 14.2)), and a significant negative association for PFHxS and allergies to cockroaches and shrimp in children aged 12-19 years old (geometric mean (95% CI); 1.44 (0.924, 2.25)). Stein et al. (254) also reported a significant positive association between PFOS exposure levels and sensitivity to mould (geometric mean (95% CI); 17.0 (15.4, 18.8)). Stein et al. (254) did not identify any associations between PFOA and PFNA and IgE sensitisation in children.

Of the four studies that investigated the association between PFAS exposure and allergic diseases in children, three related to the same cohort of Japanese children from the Hokkaido Study. Though this limits the body of evidence presented on the health outcome, the studies by Goudarzi et al. (262) and Okada et al. (263) demonstrated associations between maternal exposure levels of PFDoA and PFTrDA and total allergic diseases is significant only in children 4 years-old, and not at 2 years-old. This indicates that the effect of PFAS on allergic diseases in children may not develop until later during infancy, Further, the findings presented by Stein et al. (254) regarding IgE sensitization should be the subject of future research.

Allergic rhinoconjunctivitis

Goudarzi et al. (262) reported the association between prenatal exposure to PFAS and allergic Rhinoconjunctivitis in Japanese children aged 4 years old from the Hokkaido Study. The investigators used the prospective birth cohort study to identify a significant negative association between rhinoconjunctivitis and maternal concentrations of PFNA (OR (Q4-Q1) (95% CI); 0.409 (0.192, 0.825)). A negative association was also reported for PFDoA (OR (Q4-Q1) (95% CI); 0.430 (0.176, 0.985)) and PFUdA (OR (Q4-Q1) (95% CI); 0.285 (0.099, 0.741)), though the finding was reported of rhinoconjunctivitis in boys only. Goudarzi et al. (262) found no significant association between PFOA, PFOS, PFHxS, PFDA and PFTrDA exposure and rhinoconjunctivitis in children at 4-years old. Although the findings presented by Goudarzi et al. (262) show a negative association between PFNA, PFDoA and PFUdA and allergic rhinoconjunctivitis, the findings have not be replicated.

Wheezing

Four studies investigated the association between PFAS exposure and wheezing in children. (253, 258, 260, 262) All studies reported no association between the exposure and health outcome. Granum et al. (253) and Okada et al. (258) concluded no association between prenatal exposure to PFOA and PFOS and wheezing in children during early infancy defined as the first 3-years of life and the first 18-months of life. Granum et al. (253) did not identify an association of PFHxS and PFNA. Goudarzi et al. (262) investigated wheezing in children at 4-years of age, and did not identify an association between maternal concentrations of PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTrDA and PFUdA during pregnancy and wheezing. Humblet et al. (260) did not identify an association between PFOA, PFOS, PFHxS and PFNA exposure and wheezing in children aged 12–19 years old, using data from the NHANES study.

Eczema

Four studies investigated the association between prenatal exposure to PFAS and eczema in children. (253, 258, 262, 263) Similarly to the investigations into allergies, 3 out of 4 of the studies completed on eczema were conducted from the Hokkaido Study and presented similar results. Goudarzi et al. (262) reported a significant negative association between maternal concentrations of PFDoA (OR (Q4-Q1) (95% CI); 0.566 (0.383, 0.831)) and PFTrDA (OR (Q4-Q1) (95% CI); 0.672 (0.465, 0.968)) and eczema in children aged 4-years old, and further a significant negative association of PFOA and eczema in boys (OR (Q4-Q1) (95% CI); 0.592 (0.319, 1.08)). The study reported no significant associations of PFOS, PFHxS, PFNA, PFDA and PFUdA in male and female infants. Okada et al. (263) reported a negative association between prenatal exposure to PFTrDA and eczema, though the association was only significant of girls at 24-months old (OR (Q4-Q1) (95% CI); 0.39 (0.23, 0.64)). The study further reported no significant association between PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA and PFDoA in children. In agreement, Okada et al. (258) reported no significant association between PFOA and PFOS and eczema in children in the first 18-months of life.

Granum et al. (253) investigated the association between prenatal exposure to PFAS and eczema in children aged 3-years old from the BraMat prospective birth cohort. The study also reported no significant relationship PFOA, PFOS, PFHxS and PFNA and eczema during early infancy. Findings from the four studies largely show no significant association between PFAS exposure levels and the development of eczema in children. However, the negative associations found by Goudarzi et al. (262) and Okada et al. (263) of PFTrDA and eczema in children during early infancy require further investigation. It is important to note that studies were conducted on the same cohort, making interpretation of the overall consistency of findings difficult.

Autoimmune diseases

We evaluated two papers investigating the effect of PFAS exposure on autoimmune diseases in adults. In the papers, exposure to PFOA was primarily studied in relation to health outcomes, including Crohn's disease, ulcerative colitis, rheumatoid arthritis, and multiple sclerosis. One paper that was evaluated had a moderate risk of bias, while the other had a high risk of bias. All autoimmune health outcomes were ineligible for meta-analysis.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Crohn's disease	PFOA	Inadequate evidence
Multiple sclerosis	PFOA	Inadequate evidence
Lupus	PFOA	Inadequate evidence
Rheumatoid arthritis	PFOA	Inadequate evidence
Ulcerative colitis	PFOA	Inadequate evidence

Two studies by Steenland et al. (185, 264) investigated the relationship between exposure to PFAS and autoimmune diseases in adults. Steenland et al. (185) reported positive associations between cumulative exposure to PFOA and cases of ulcerative colitis in 4,391 workers from the DuPont plant in West Virginia (RR unlagged analysis of log cumulative exposure (Q4-Q1) (95% CI); 2.86 (1.65, 4.96) and 2.74 (0.78, 9.95), respectively). Results from an analysis incorporating a 10-year lag showed a stronger association (RR 10-year lag analysis of log cumulative exposure (Q4-Q1) (95% CI); 3.05 (1.56, 5.96) and 6.57 (1.47, 29.40), respectively). Steenland et al. (185) reported a significant positive association between exposure to PFOA and self-reported cases of rheumatoid arthritis (RR unlagged analysis of PFOA categories (Q4-Q1) (95% CI); 4.45 (0.99–19.9)). In contrast, Steenland et al. (264) reported no association between PFOA levels and rheumatoid arthritis and further reported no significant association between the exposure and cases of Crohn's disease, lupus and multiple sclerosis. Thus, these studies provide inadequate evidence for an association between increased PFOA exposure levels and an increased risk of rheumatoid arthritis, Crohn's disease, lupus and multiple sclerosis. As Steenland et al. (185) was only a single study reporting this statistically significant association we considered this to be inadequate evidence of a health effect after taking into account the study design, strength of effect and associated risk of bias. Future studies should consider the use of validated cases of autoimmune diseases, as the use of self-reported measures by Steenland et al. (264) contributed to a high risk of bias assessment for the study.

Skeletal outcomes

We included five papers on the association of PFAS exposure with the development of skeletal conditions in adults. All of the five studies they reported on were based on populations of adults living in the United States. Four were cross-sectional studies and 1 was a cohort study; 3 were non-overlapping analyses of data from the NHANES study. The studies investigated a wide range of skeletal outcomes in men and women, with PFOA and PFOS the main exposures of interest. All studies were evaluated to have a high risk of bias. Summaries of the papers are available in Appendix 17. All skeletal health outcomes were ineligible for meta-analysis.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Osteoarthritis	PFOA, PFOS	Inadequate evidence
Osteoporosis	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Bone mineral density		
Lumbar spine	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Total femur	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Femur neck	PFOA, PFOS, PFHxS, PFNA	Inadequate evidence
Hip	PFOA, PFOS	Inadequate evidence
Bone fractures		
All	PFOA, PFOS	Inadequate evidence
Hip, wrist and spine	PFOA, PFOS	Inadequate evidence
Hip	PFOA, PFOS	Inadequate evidence
Wrist	PFOA, PFOS	Inadequate evidence
Spine	PFOA, PFOS	Inadequate evidence

Osteoarthritis

Three studies investigated the association between exposure to PFAS and osteoarthritis in adults. (185, 265, 266) Innes et al. (265) reported a significant positive association between PFOA exposure levels and the development of osteoarthritis (OR (Q4-Q1) (95% CI); 1.3 (1.2, 1.5)). Uhl et al. (266) reported an elevated OR of borderline significance, which was significant for females when stratified by sex (OR (Q4-Q1) (95% CI); 1.98 (1.24, 3.19)). Steenland et al. (185) found no significant association between occupational exposure to PFOA and osteoarthritis in a cohort of DuPont workers. For PFOS, Innes et al. (265) reported a significant negative association with osteoarthritis (OR (Q4-Q1) (95% CI); 0.8 (0.7, 0.9)), while Uhl et al. (266) reported a significant positive association (OR (Q4-Q1) (95% CI); 1.77 (1.05, 2.96)). In contrast to the reported effect of PFOA, Uhl et al. (266) found no significant relationship between PFOS and osteoarthritis when results were stratified by sex.

The findings of both PFOA and PFOS were inconclusive and all studies were determined to have a high risk of bias. Although the results were inconsistent, there was a clear difference in the level of exposure between the three studies, with Innes et al. (265) and Steenland et al. (185) studying highly exposed communities and Uhl et al. (266) using NHANES data that is broadly of the United States population.

Osteoporosis

Khalil et al. (267) determined the association between PFAS and the development of osteoporosis in adults from the NHANES study. The study found differing associations by sex, with a significant positive association for women for PFOA (OR (Q4-Q1) (95% CI); 2.59 (1.01, 6.67)), PFHxS (OR (Q4-Q1) (95% CI); 13.20 (2.72, 64.15) and PFNA (OR (Q4-Q1) (95% CI); 3.23 (1.44, 7.21)). There were no significant associations identified among men. There was no association identified for PFOS and osteoporosis among men or women. As the results stated by Khalil et al. (267) were associated with a high risk of bias and have not been replicated by another study to date, the findings should be interpreted with caution.

Bone mineral density

Khalil et al. (267) and Lin et al. (268) investigated the association between PFAS exposure and bone mineral density in adults using data from the NHANES study (participants from the 2009–2010 waves and 2005–2006 waves, respectively). Each study measured the density of the lumbar spine and also investigated other bones in the human body, including the hip and femur. Overall, the results were conflicting of the association between PFAS and bone mineral density, with inconsistencies within each study and between the two studies.

Lin et al. (268) found a significant negative association between PFOS and bone mineral density of the lumbar spine (change in total bone mineral density (95% CI); -0.022 (-0.038, -0.007)) of women not in menopause, while Khalil et al. (267) did not identify an association. Khalil et al. (267) reported a significant negative association between PFNA and bone mineral density of the lumbar spine (regression coefficient β (continuous) (95% CI); -0.043 (-0.073, -0.013)) of post-menopausal women and no significant association for PFHxS. Both Khalil et al. (267) and Lin et al. (268) found no association between PFOA exposure and bone mineral density of the lumbar spine.

Khalil et al. (267) further investigated the association between PFAS exposure and bone mineral density of the femur. The study reported a significant negative association between PFOA and bone mineral density of the femur neck (regression coefficient β (continuous) (95% CI); -0.017 (-0.033, -0.001)) in women only, and no association between PFOA and bone mineral density of the total femur. For PFOS exposure, Khalil et al. (267) reported a significant negative relationship with bone mineral density of the total femur (regression coefficient β Q1-Q4 (CI); -0.044 (-0.074, -0.014)) and the femur neck (regression coefficient β (Q4-Q1) (95% CI); -0.034 (-0.059, -0.009)) in women, and a significant negative association of the bone mineral density of the femur neck (regression coefficient β (Q4-Q1) (95% CI); -0.046 (-0.078, -0.015)) in men. The study found a significant negative association between PFHxS and PFNA and total femur bone mineral density (regression coefficient β Q1-Q4 (95% CI); -0.044 (-0.074, -0.014) and β (Q4-Q1) (95% CI); -0.040 (-0.077, -0.003), respectively) in women, and a significant negative relationship between PFNA and bone mineral density of the femur neck (regression coefficient β (continuous) (95% CI); -0.025 (-0.049, -0.001)). Lin et al. (268) further determined the association between PFOA and PFOS and bone mineral density of the hip and found no significant relationship between the exposures and health outcome in adults.

The findings reported by Khalil et al. (267) and Lin et al. (268) suggest an overall negative association between PFAS exposure and bone mineral density, however the results are conflicting and further, the significant associations found relate to small changes in total bone mineral density. In the study by Khalil et al. (267), several negative associations were reported

for PFOA, PFOS, PFHxS and PFNA bone mineral density in women, and one association between PFOS and bone mineral density of the femur neck was found in men. Lin et al. (268) reported a significant negative finding of the association between PFOS and bone mineral density of the lumbar spine in women, though this contrasted the results of men and the results reported by Khalil et al. (267).

Bone fractures

Lin et al. (268) examined the effect of PFAS exposure on bone fractures in adults. Using data from the NHANES, the study found no significant association between PFOA and PFOS exposure and instances of bone fractures of the hip, wrist and spine.

Respiratory outcomes

Three of the evaluated papers reported on the association of PFAS exposure with respiratory outcomes in adults. Two papers reported on the association between occupational exposure to PFOA and respiratory disease mortality in one study. The third reported on the association between PFAS exposure and lung disease in pregnant women living in a region of the USA with drinking water contaminated with PFOA (C8 Study). Both studies were determined to have a moderate risk of bias. The papers are summarised in Appendix 18. All respiratory health outcomes were ineligible for meta-analysis.

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
COPD	PFOA	Inadequate evidence
Bronchitis	PFOA	Inadequate evidence
Emphysema	PFOA	Inadequate evidence
Lung disease	PFOA	Inadequate evidence

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is an umbrella term used for three respiratory conditions; emphysema, chronic bronchitis and chronic asthma. Leonard et al. (221) and Steenland & Woskie (155) each reported on the association between occupational exposure to PFOA and COPD mortality in a 50-year retrospective cohort study of DuPont workers from West Virginia. The analysis conducted by Leonard et al. (221) began with exposure in 1948, 4 years prior to the period of exposure in the analysis by Steenland & Woskie. (155) Neither analysis reported a statistically significant association between PFOA exposure and COPD related death in the cohort. Leonard et al. (221) also reported on bronchitis mortality and emphysema mortality separately and found no significant associations between them and estimated PFOA exposure.

Lung disease

Nolan et al. (70) investigated the association between modelled PFOA exposure and diagnosis of lung disease in pregnant women. The study concluded that there was no statistically significant association between the two.

Discussion

Overview of results

Using standard systematic review methods, we identified 221 published papers reporting original results of research into the association in humans between exposure to PFAS chemicals and health outcomes. We classified the outcomes in 12 broad categories: neonatal, infant and maternal outcomes, reproductive outcomes, metabolic outcomes, thyroid outcomes, neurodevelopmental outcomes, cancer, diabetes, cardiovascular outcomes, overweight and obesity, immunological outcomes, skeletal outcomes and respiratory outcomes. The majority of studies examined health outcomes of exposure to PFOS or PFOA; some also examined other PFAS chemicals. There were many health outcomes for which one or more studies found a statistically significant association with exposure to PFAS. Most of the evidence for individual health outcomes across multiple studies, however, suggested there was no consistent evidence of an association. Where we did find an association between PFAS exposure and a health outcome, the body of evidence for it was generally insufficient to be confident of a real effect of the exposure or was inconsistent across multiple studies and thus possibly a result of uncontrolled bias or confounding in the studies.

Of the 148 health outcomes investigated, we found *sufficient evidence* for an association of exposure to PFOA and PFOS with high blood total cholesterol concentration (hypercholesterolaemia). In general terms, hypercholesterolaemia is associated with an increased risk of cardiovascular disease due to the build-up of cholesterol in arteries, particularly those that supply blood to the heart muscle. We found evidence of a positive association between exposure to PFOA and hypercholesterolaemia levels in 12 of 19 relevant papers. Further, eight of 18 papers that reported on PFOS exposure and total cholesterol levels also found a positive association. These associations were observed in both children and adults. We considered seven papers on PFOA and total cholesterol and five on PFOS and total cholesterol for meta-analysis, but the variety of different ways in which the findings were reported made meta-analysis impractical.

All the relevant studies of hypercholesterolaemia were judged to be at moderate or high risk of bias and the distribution across these two categories was similar in studies showing an association and those not showing an association. Since the studies were mostly cross-sectional studies, temporality was a frequent reason for a high risk of bias classification. We saw no grounds for a “reverse causation hypothesis”, that is that hypercholesterolaemia caused an increase in blood concentrations of PFAS. We also found no plausible hypothesis for confounding that might explain the observed associations and evidence that PFAS chemicals accumulate in the liver adds biological plausibility to a positive association of PFAS chemicals and total blood cholesterol concentration. The studies that did not show an association were generally quite a lot smaller than those that did show an association. We consider, therefore, there to be sufficient evidence that elevated PFOA and PFOS concentrations in blood increase total blood cholesterol concentration. The observed increases in concentration were quite small, and thus likely to have limited effects on health.

It is uncertain to which type of cholesterol these findings relate, HDL cholesterol (sometimes called ‘good’ cholesterol) or LDL cholesterol (sometimes called ‘bad’ cholesterol). Across the studies in which HDL and LDL cholesterol were measured separately, the evidence for positive

associations of PFOA and PFOS with each was insufficiently consistent for a confident conclusion. This finding with respect to LDL cholesterol is inconsistent with the findings of Rappazzo et al. (47) who conducted a broad-ranging systematic review on health effects of PFAS in children and adolescents. They found consistent evidence of a positive association between PFAS and LDL cholesterol, but not HDL cholesterol, in five relevant papers. We included 13 more papers in children and adolescents than Rappazzo et al. (47) referred to, which may explain the difference between our findings and theirs.

We found *limited evidence* for an association between PFOA and PFOS chemicals and seven health effects; one of which, high blood uric acid (hyperuricaemia) concentration, like high blood cholesterol concentration, was a health-related metabolic outcomes. Two other health outcomes were related to renal function: impaired glomerular filtration rate and chronic kidney disease. The body of evidence relevant to hyperuricaemia (and hypercholesterolaemia) was much greater than that for the other renal outcomes.

We found *limited evidence* for an association between exposure to PFOA and PFOS and higher blood uric acid levels. As with hypercholesterolemia, elevated blood uric acid is a predictor of risk for a number of chronic diseases, including cardiovascular disease. Six of seven papers reported that PFOA exposure was positively associated with uric acid levels. Similarly, four of six papers reported that elevated PFOS levels were associated with hyperuricemia. Results for both were significant in adults and in children and adolescents.

We found *limited evidence of a health effect* for an association between PFOA exposure and kidney cancer and testicular cancer. While based on relatively weak evidence, this finding is concordant with the IARC evaluation of PFOA, which was made in 2014 and published in 2017. (269) There was inadequate evidence of an association between PFAS and other cancers studied.

We found *limited evidence of a health effect* for an association between PFAS exposure and reduced vaccine antibodies. There was evidence of a negative association between PFAS and antibody levels of diphtheria after vaccination of children or adults. Reduced diphtheria antibody concentrations were reported for PFOA, PFOS, PFHxS and PFDA exposures. There was *limited evidence* of the effect of PFOA and PFOS on reduced rubella antibody levels. For antibody levels for other vaccines there was *inadequate evidence* of an effect of PFAS. However, there were only one to three papers reporting on each of these exposure-effect associations.

We found *inadequate evidence of a health effect* for most individual health outcomes. Previous systematic reviews, have concluded that PFAS exposure was associated with some of these health outcomes. Lam et al. (42) and Johnson et al. (21) concluded that elevated maternal PFOS levels reduced infants' birth weights. However, we found the results of these studies to be inconsistent across the 28 papers evaluated for the health outcomes in this systematic review. While our conclusions conflict with those of Lam et al. (42) and Johnson et al. (21), a more recent systematic review by Bach et al. (41) found, like this review, the results to be inadequate evidence of an association. Similarly, we found inadequate evidence for a positive association of PFAS exposure with asthma in children and late menarche. Rappazzo et al. (47) reported a consistent positive association with asthma but not with age at menarche.

Overall, we found *inadequate evidence of an association* of PFAS exposure with the majority of health outcomes covered by this systematic review. In many instances, there was a large

body of evidence in support of lack of any association; for these outcomes, it may be reasonable to consider that there is evidence of lack of association between PFAS and the health outcomes in question. However, the quality of studies was highly variable for most health outcomes, the study populations very different and the evidence for lack of association, therefore, generally inconclusive.

Study quality

The quality of studies covered by this systematic review was assessed using a multi-domain risk of bias tool for each study design. Studies were generally considered to be at moderate or high risk of bias. Only 3.6% (8/221) of papers evaluated were considered to be at low risk of bias.

Cross-sectional studies included in this systematic review were all evaluated to have a high risk of bias, predominantly due to participation rates, the uncertain temporality of exposure and self-reported measures of disease outcomes. When PFAS exposure levels were measured at the same time as the health outcome was measured, reverse causality is a possibility (health outcome causes high PFAS levels). Cross-sectional studies also commonly included the administration of a health outcome questionnaire around the time of PFAS measurement, which raises the possibility that knowledge of one influences recall or measurement of the other.

Many studies attempted to control for confounding, particularly in cohort and case control studies in which investigators measured many exposures and other variables. However, it can be difficult to correctly control for confounding, particularly when the possible confounders are largely unknown and potentially important confounders are not measured. Studies of the association between elevated PFAS and low birth weight provide an example. It has been suggested that GFR may confound the association between elevated blood levels of PFAS and low birth weight, due to the fact that poor kidney function is associated both with reduced excretion of chemicals from the body and low birth weight infants. (270) Few earlier studies measured and adjusted for GFR.

Limitations

As with any systematic review, this study has limitations in its design and conduct. The broad search strategy we used enabled us to investigate the effects of many PFAS exposures. However, the method restricted our ability to examine specific health outcomes in detail. We did not include disease- or health condition-specific terms in our search strategy, as we did not want to bias our search towards any particular health outcomes. However, this reduced our chance of finding studies that did not mention the general terms used in our strategy syntax—exposure, health, outcome or disease. Further, our search strategy included a specific cut-off date and did not encompass studies published after February 7th 2017. Given the public and scientific interest in PFAS, there are many recently published papers that are relevant to this review. While possible consequences of exposure to PFAS for human health remain a public issue, updating this review will be a useful and cost-effective way of seeking to resolve it. Publication bias, which we did not seek to evaluate given the relatively few reports addressing each exposure-disease association studied, is a potential limitation of this, as it is of any, systematic review. Most studies evaluated in this review, however, showed little or no evidence of an association of PFAS exposure with the studied health outcomes. Publication bias tends to favour publication of ‘positive’ studies.

We were only able to conduct meta-analyses for a few key outcomes and the results were potentially conflicting with our overall assessment of the exposure-outcome association, particularly when comparatively few of the relevant studies could be included in the meta-analysis. We were not able to include many studies because of the way results were presented in published papers. We did not try to combine continuous and categorical measurements of exposure in the one meta-analysis, and we also separated log-transformed and untransformed PFAS measures. As a result, the four meta-analyses we did were based on only some of the papers evaluated for each subject health outcome and, therefore, the overall measure of association may not have reflected all the reported measures of association. Due to the large number of papers evaluated in this review, we did not attempt to access raw data from published papers, which could have allowed us to produce results in a more consistent form. Finally, in some instances we observed considerable heterogeneity in the meta-analysis. For these reasons, the results of the meta-analyses should be interpreted with caution, particularly where they contradict the overall assessment.

We included in this review research papers from study populations with very different exposures to PFAS, particularly in relation to the concentration and duration of exposure. There are three distinct types of study populations: (1) people who were heavily exposed to PFAS through their work, (2) people who were exposed to more moderate levels of PFAS through specific instances of contamination of the environment, and (3) people who were exposed to PFAS at community background levels. We reviewed studies in each of these exposure-defined population sub-groups. However, the exposure and consequent blood concentrations of chemicals were orders of magnitude higher in occupational cohorts (e.g. DuPont workers), than in people exposed to background levels (e.g. mothers in the Danish Birth Cohort). It is possible that some health effects would have been newly observed or more evident if more highly-exposed people had been studied.

We attempted to access all grey literature, but it was difficult to cover all possible grey literature sources as each site had to be searched individually. It is hoped, though, that this problem was substantially ameliorated by the use of Google for ‘catch all’ searches. It was difficult to identify sometimes whether the work was subsequently published in the scientific literature; though we think duplication is unlikely. We also found that many government reports were inaccessible to the public.

Finally, due to the scale of this systematic review, we had to use a team of people to review papers. This may have led to some inconsistency in how reviews were conducted. We attempted to minimise this by training our reviewers, and using standardised tools and data abstraction forms.

Conclusions

The health effects of PFAS have been extensively studied. However, there is still a need for high-quality research to increase the consistency of the findings for outcomes for which there is suspicion but limited or no consistency in the findings to date. In our review of 221 papers, we found *sufficient evidence* of an association of PFOA and PFOS exposure with hypercholesterolemia. We found *limited evidence* of an association for PFOA and PFOS with hyperuricemia. We also found *limited evidence* of an association between PFAS and decreased GFR, increased chronic kidney disease, kidney cancer and testicular cancer, and

impaired vaccine antibody response to diphtheria and rubella, although there were fewer studies of these outcomes. Hypercholesterolemia and hyperuricemia are associated with increased risks of chronic diseases, including cardiovascular conditions. Although there is limited evidence for the association between PFOA and PFOS exposure and high cholesterol and uric acid levels in the blood, the public health implications of these findings are mitigated somewhat by the treatability of these metabolic states and that the effects are likely to be small.

For most health outcomes, the body of evidence provides inadequate evidence of health effects of PFAS. In addition, the evidence relating to health effects that we judged to provide *limited* or *sufficient evidence for a health effect* may change with the conduct of further studies, particularly when there were relatively few studies contributing evidence to these evaluations. It may be important to prioritise for further research health outcomes for which we found inadequate evidence of an association with PFAS exposure and where there were only a few studies of these outcomes. Where there are exposure-effect associations in the results of these studies, they are findings that have not been disproven to date. We were unable to conduct meta-analyses for many outcomes due to the nature of data analyses in the published papers. There may well be value in fostering international collaborations to pool data for harmonised analysis of results for some key health outcomes.

Our systematic review examines epidemiological studies of the association of PFAS exposure with a large number of health outcomes in humans. The papers evaluated included the results of studies conducted on highly exposed populations, including the effect of community-level exposure to PFAS and the effect of prolonged occupational exposure to the substances. Overall, this systematic review finds that the literature does not currently provide evidence of health effects from PFAS exposures and many health outcomes. There is, though, limited evidence for associations of PFOA and PFOS exposure with adverse effects on metabolism and renal function, and some cancers and vaccine derived immunity. Further research is needed on a number of exposure-effect associations that have been less-well studied.

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Appendices

Appendix 1: Scientific database search strategy and results

PRISMA 2009 Flow Diagram – Amended of PFAS Systematic Review Protocol

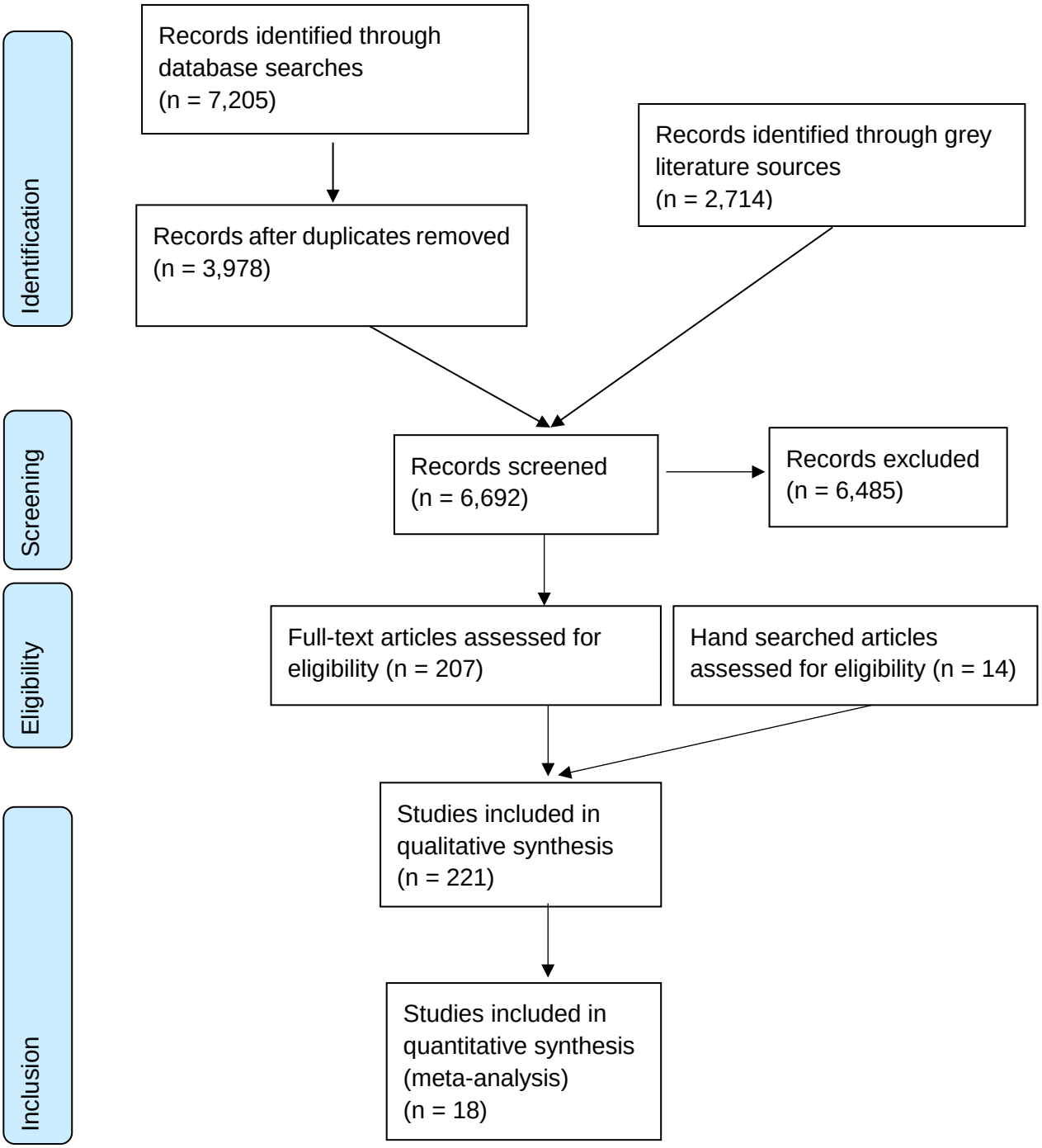

Figure 10: PRISMA 2009 Flow Diagram showing details of the PFAS study.

Source: http://prisma-statement.org/documents/PRISMA%202009%20flow%20diagram.pdf

Appendix 2: Quality assessment criteria

Cross-sectional study design

Cross-sectional studies were evaluated based on the following criteria:

- Study location
- Study population
- Participation (response) rate
- Nature, measurement and temporality of the exposure
- Missing exposure data
- Nature and measurement of the disease
- Missing disease data
- Control of confounding
- Conflict of interest.

Cohort study design

Cohort studies were evaluated based on the following criteria:

- Study location
- Exposed population and selection of exposed cohort
- Comparison population and selection of comparison cohort
- Participation (response) rates
- Nature and measurement of the exposure
- Nature and measurement of the disease
- Follow-up methods
- Completeness of follow-up
- Missing exposure data
- Control of confounding
- Conflict of interest.

Case-control study design

Case-control studies were evaluated based on the following criteria:

- Study location
- Study population
- Definition and selection of case and controls
- Participation (response) rates
- Nature, measurement and temporality of the exposure
- Missing exposure data

- Control of confounding
- Conflict of interest.

Nested case-control study design

Nested case-control studies were evaluated based on the following criteria:

- Study location
- Study population
- Exposed population and selection of exposed cohort
- Definition and selection of case and controls
- Participation (response) rates
- Nature, measurement and temporality of the exposure
- Follow-up methods
- Control of confounding
- Conflict of interest.

Case-cohort study design

Case-cohort studies were evaluated based on the following criteria:

- Study location
- Study population
- Study cohorts
- Definition and selection of case and cohort sample
- Participation (response) rates
- Nature, measurement and temporality of the exposure
- Missing exposure data
- Follow-up methods
- Counting person time
- Control of confounding
- Conflict of interest.

Appendix 3: Risk of bias—cross-sectional studies

(Adapted from the Newcastle-Ottawa tool of QA of clinical case-control studies of use in the Prostate Cancer Foundation of Australia and Cancer Council Australia auspiced – *Development of Clinical Practice Guidelines of PSA Testing and Early Management of Test-Detected Prostate Cancer*)

Population sampled of participants

1. A general population bounded only by area and time (low risk)
2. A general population bounded by area and time with one or more additional defining characteristics that are probably unrelated to exposure or outcome (moderate risk)
3. A general population bounded by area and time with one or more additional defining characteristics that are probably related to exposure or outcome OR some population other than in (1) or (2) OR insufficient information to tell (high risk)

Participation (response) rate

1. ≥70% participation rate (≥80% response rate) (low risk)
2. ≥50 to <70% participation rate (≥60 to <80% response rate) (moderate risk)
3. <50% participation rate (<60% response rate) OR insufficient information to tell (high risk)
4. New data not being collected from participants (not applicable)

Measurement of exposure

1. Objective measurements from pre-existing records or biological assessment are blind to diseased or non-diseased status (low risk)
2. Objective measurements from pre-existing records or biological assessment are not blind or not known to be blind to diseased or non-diseased status OR structured interview is blind to diseased or non-diseased status (moderate risk)
3. Structured interview is not blind to diseased or non-diseased status OR self-administered questionnaire OR insufficient information to tell (high risk)

Temporality of exposure

1. Exposure precedes onset of disease in diseased people and is from a corresponding calendar time in non-diseased people (low risk)
2. Exposure precedes onset of disease in diseased people but exposure in non-diseased people is not from a calendar time corresponding to exposure in diseased people (moderate risk)
3. Exposure does not precede onset of disease in diseased people OR insufficient information to tell (high risk)

Missing exposure data

1. Exposure data missing in < 10 percent of participants (low risk)
2. Exposure data missing in ≥10 to <20 percent of participants (moderate risk)
3. Exposure data missing in ≥20 percent of participants OR insufficient information to tell (high risk)

Measurement of disease

1. Objective measurements from pre-existing records or clinical or pathological assessment are blind to exposed or non-exposed status (low risk)
2. Objective measurements from pre-existing records or clinical or pathological assessment are not blind to exposed or non-exposed status OR structured interview is blind to exposed or non-exposed status (moderate risk)
3. Structured interview is not blind to exposed or non-exposed status OR self-administered questionnaire OR insufficient information to tell (high risk)

Missing disease data

1. Disease data missing in < 10 percent of participants (low risk)
2. Disease data missing in ≥10 to <20 percent of participants (moderate risk)
3. Disease data missing in ≥20 percent of participants OR insufficient information to tell (high risk)

Control of confounding

Comparability of diseased and non-diseased people with respect to potentially important confounding variables (Requires prior specification of potentially important confounders)
1. Age and other potentially important confounders measured and controlled by design or in analysis (low risk)
2. Age and some but not all other potentially important confounders controlled by design or in analysis (moderate risk)
3. No potentially important confounders or only age controlled by design or in analysis OR insufficient information to tell (high risk)

Matching variables are appropriately included in the analysis
1. When non-diseased people are frequency matched to diseased people, matching variables are controlled in the analysis OR when diseased people are individually matched to non-diseased people, a conditional analysis is used or matching variables are controlled in the analysis (low risk)
2. It is not a matched design (not applicable)
3. None of the above OR insufficient information to tell (high risk)

Other covariates are appropriately included in the analysis
1. NO variable measuring the same underlying concept or lying in the same causal pathway as the exposure variable under study IS included as a covariate in the statistical analysis models (low risk)
2. ONE OR MORE variables measuring the same underlying concept or lying in the same causal pathway as the exposure variable under study IS included as a covariate in statistical analysis models OR insufficient information to tell (high risk)

Conflict of interest

1. No conflict of interest declared (low risk)
2. One or more conflicts of interest OR no conflict of interest declaration (moderate risk)

Overall rating

High risk of bias – high risk of bias in any domain
Moderate risk of bias – moderate or low risk of bias in all domains, no high risk domains
Low risk of bias – all domains low risk of bias, no moderate or high risk domains

Appendix 4: Risk of bias—cohort studies

(Adapted from the Newcastle-Ottawa tool of QA of clinical cohort studies of use in the Prostate Cancer Foundation of Australia and Cancer Council Australia auspiced - *Development of Clinical Practice Guidelines of PSA Testing and Early Management of Test-Detected Prostate Cancer*)

Exposed and comparison (unexposed) populations and selection of cohort(s)

1. Drawn from the same population (low risk)
2. Drawn from different populations but unlikely to introduce bias (moderate risk)
3. Drawn from different populations and likely to introduce bias OR insufficient information to tell (high risk)

Participation rates

1. Participation rate in exposed cohort is ≤10 percentage points different from unexposed cohort OR exposed and unexposed are from the same cohort (low risk)
2. Participation rate in exposed cohort is >10 percentage points but <20 percentage points different from unexposed cohort (moderate risk)
3. Participation rate in exposed cohort ≥20 percentage points different from unexposed cohort OR insufficient information to tell (high risk)

Nature and measurement of exposure

1. Objective measurements from pre-existing records or baseline physical or biological assessment blind to outcome status (low risk)
2. Objective measurements from pre-existing records or baseline physical or biological assessment not blind or not known to be blind to outcome status OR structured interview (moderate risk)
3. Self-administered questionnaire OR insufficient information to tell (high risk)

Was outcome of interest present at the time to which the exposure measurement refers?
1. No (low risk)
2. Yes but outcome unlikely to affect exposure measurement (moderate risk)
3. Yes and outcome likely to affect exposure measurement OR insufficient information to tell (high risk)

Nature and measurement of outcome

1. Objective outcome measurement unlikely to be influenced by exposure (low risk)
2. Objective outcome measurement possibly influenced by exposure (moderate risk)
3. Objective outcome measurement probably influenced by exposure OR self-reported outcome OR insufficient information to tell (high risk)

Follow-up methods

1. Active or passive follow-up of participants with methods of ascertainment of outcome and death clearly described AND with methods of ascertainment of emigration from population-at-risk clearly described or censoring at date of last follow-up OR there is a plausible estimate of >90% follow-up (low risk)
2. Active or passive follow-up with methods of ascertainment of outcome, death and emigration from population-at-risk not clearly described OR there is a plausible estimate of 70 – 90% follow-up (moderate risk)

3. Active or passive follow-up with methods of ascertainment of one or more of outcome, death or emigration not described OR there was probably <70% follow-up OR insufficient information to tell (high risk)

Sufficiency of follow-up time and completeness of follow-up

Was follow-up long enough of outcome to occur as a consequence of measured exposure?
(Requires prior specification of a sufficient follow-up period)
1. Yes (low risk)
2. No OR insufficient information to tell (high risk)

Completeness of follow-up
1. Follow-up methods are the same and likely to achieve the same completeness of follow-up in exposed and unexposed participants (low risk)
2. Completeness of follow-up in exposed and unexposed participants is unlikely to be the same but difference between the two is, or would be likely to be, small (<10%) (moderate risk)
3. Completeness of follow-up in exposed and unexposed participants is very unlikely to be the same and difference between the two is, or is likely to be, large (>10%) OR insufficient information to tell (high risk)

Missing data on exposure

Difference in missing data of exposure between those with or without the outcome
1. Difference in missing data of exposure <10 percentage points (low risk)
2. Difference in missing data of exposure ≥10 to <20 percentage points (moderate risk)
3. Difference in missing data of exposure ≥20 percentage points (high risk) OR insufficient information to tell (high risk)

Control of confounding

Comparability of exposed and non-exposed cohorts with respect to potentially important confounding variables
(Requires prior specification of potentially important confounders)
1. Age and other potentially important confounders measured and controlled by design or in analysis (low risk)
2. Age and some but not all other potentially important confounders controlled by design or in analysis (moderate risk)
3. No potentially important confounders or only age controlled by design or in analysis OR insufficient information to tell (high risk)

Covariates are appropriately included in the analysis
1. NO variable measuring the same underlying concept or lying in the same causal pathway as the exposure variable under study IS included as a covariate in the statistical analysis models (low risk)
2. ONE OR MORE variables measuring the same underlying concept or lying in the same causal pathway as the exposure variable under study IS included as a covariate in statistical analysis models OR insufficient information to tell (high risk)

Conflict of interest

1. No conflict of interest declared (low risk)
2. One or more conflicts of interest OR no conflict of interest declaration (moderate risk)

Overall rating

High risk of bias – high risk of bias in any domain
Moderate risk of bias – moderate or low risk of bias in all domains, no high risk domains
Low risk of bias – all domains low risk of bias, no moderate or high risk domains

Appendix 5: Risk of bias—case-control studies

(Adapted from the Newcastle-Ottawa tool of QA of clinical case-control studies of use in the Prostate Cancer Foundation of Australia and Cancer Council Australia auspiced – *Development of Clinical Practice Guidelines of PSA Testing and Early Management of Test-Detected Prostate Cancer*)

Definition and selection of cases and controls

Definition of cases

1. Outcome precisely specified and with pathological or other objective confirmation (low risk)
2. Outcome precisely specified but without known pathological or other objective confirmation OR outcome precisely specified, self-reported and cases blind to hypotheses related to outcome (moderate risk)
3. Outcome imprecisely specified OR outcome self-reported and cases not blind to hypotheses related to outcome OR insufficient information to tell (high risk)

Definition of controls

1. Objective evidence of no past history of outcome of interest (low risk)
2. Self-report of no past history of outcome of interest OR insufficient information to tell (moderate risk)

Selection of cases and controls

1. Drawn from the same population (low risk)
2. Drawn from different populations but unlikely to introduce bias (moderate risk)
3. Drawn from different populations and likely to introduce bias OR insufficient information to tell (high risk)

Participation (response) rates

Participation (response) rate of cases

1. ≥70% participation rate (≥80% response rate) (low risk)
2. ≥50 to <70% participation rate (≥60 to <80% response rate) (moderate risk)
3. <50% participation rate (<60% response rate) OR insufficient information to tell (high risk)
4. Not applicable – new data not being collected from participants

Participation (response) rate of controls

1. ≥60% participation rate (≥70% response rate) (low risk)
2. ≥40 to <60% participation rate (≥50 to <70% response rate) (moderate risk)
3. <40% participation rate (<50% response rate) OR insufficient information to tell (high risk)
4. New data not being collected from participants (not applicable)

Difference in participation rate (response rate) between cases and controls

1. Participation (response) rate in cases ≤10 percentage points different from controls (low risk)
2. Participation (response) rate in cases is >10 to ≤15 percentage points different from controls (moderate risk)
3. Participation (response) rate in cases is >15 percentage points different from controls OR insufficient information to tell (high risk)

Measurement of exposure

1. Objective measurements from pre-existing records or biological assessment blind to case or control status (low risk)
2. Objective measurements from pre-existing records or biological assessment not blind to case or control status OR structured interview blind to case or control status (moderate risk)
3. Structured interview not blind to case or control status OR self-administered questionnaire OR insufficient information to tell (high risk)

Was the same method used to measure exposure in cases and controls?
1. Yes (low risk)
2. No OR insufficient information to tell (high risk)

Temporality of exposure

1. Exposure precedes onset of disease in cases and is from a corresponding calendar time in controls (low risk)
2. Exposure precedes onset of disease in cases but exposure in controls is not from a calendar time corresponding to exposure in cases (moderate risk)
3. Exposure does not precede onset of disease in cases OR insufficient information to tell (high risk)

Missing exposure data

Difference in missing data of exposure between cases and controls
1. Difference in missing data of exposure < 10 percentage points (low risk)
2. Difference in missing data of exposure ≥10 to <20 percentage points (moderate risk)
3. Difference in missing data of exposure ≥20 percentage points OR insufficient information to tell (high risk)

Control of confounding

Comparability of cases and controls with respect to potentially important confounding variables (Requires prior specification of potentially important confounders)
1. Age and other potentially important confounders measured and controlled by design or in analysis (low risk)
2. Age and some but not all other potentially important confounders controlled by design or in analysis (moderate risk)
3. No potentially important confounders or only age controlled by design or in analysis OR insufficient information to tell (high risk)

Matching variables are appropriately included in the analysis
1. When controls are frequency matched to cases, matching variables are controlled in the analysis OR when controls are individually matched to cases, a conditional analysis is used or matching variables are controlled in the analysis (low risk)
2. It is not a matched design (not applicable)
3. None of the above OR insufficient information to tell (high risk)

Other covariates are appropriately included in the analysis
1. NO variable measuring the same underlying concept or lying in the same causal pathway as the exposure variable under study IS included as a covariate in the statistical analysis models (low risk)

2. ONE OR MORE variables measuring the same underlying concept or lying in the same causal pathway as the exposure variable under study IS included as a covariate in statistical analysis models OR insufficient information to tell (high risk)

Conflict of interest

1. No conflict of interest declared (low risk)
2. One or more conflicts of interest OR no conflict of interest declaration (moderate risk)

Overall rating

High risk of bias – high risk of bias in any domain
Moderate risk of bias – moderate or low risk of bias in all domains, no high risk domains
Low risk of bias – all domains low risk of bias, no moderate or high risk domains

Appendix 6: Risk of bias—nested case-control studies

(Adapted from the Newcastle-Ottawa tool of QA of clinical cohort studies of use in the Prostate Cancer Foundation of Australia and Cancer Council Australia auspiced - *Development of Clinical Practice Guidelines of PSA Testing and Early Management of Test-Detected Prostate Cancer*)

Cohorts

Selection of exposed and unexposed cohorts of the cohort study
1. Drawn from the same population (low risk)
2. Drawn from different populations but unlikely to introduce bias (moderate risk)
3. Drawn from different populations and likely to introduce bias OR insufficient information to tell (high risk)

Participation (response) rates of exposed and unexposed cohorts in the cohort study
1. Participation rate in exposed cohort is ≤10 percentage points different from unexposed cohort OR exposed and unexposed are from the same cohort (low risk)
2. Participation rate in exposed cohort is >10 percentage points but <20 percentage points different from unexposed cohort (moderate risk)
3. Participation rate in exposed cohort ≥20 percentage points different from unexposed cohort OR insufficient information to tell (high risk)

Definition and selection of cases and controls

Definition of cases
1. Outcome precisely specified and with pathological or other objective confirmation (low risk)
2. Outcome precisely specified but without known pathological or other objective confirmation OR outcome precisely specified, self-reported and cases blind to hypotheses related to outcome (moderate risk)
3. Outcome imprecisely specified OR outcome self-reported and cases not blind to hypotheses related to outcome OR insufficient information to tell (high risk)

Definition of controls
1. Objective evidence of no past history of outcome of interest (low risk)
2. Self-report of no past history of outcome of interest OR insufficient information to tell (moderate risk)

Selection of cases and controls
1. Cases and controls are randomly selected from all available cases and controls; controls matched to cases by risk set* (either at selection or during analysis) (low risk)

2. Only one of the two criteria in 1 is met (moderate risk)

3. Neither criterion in 1 is met OR insufficient information to tell (high risk)

*Risk set defined by sex, age group, date of entry into cohort and date of case-defining event

Participation (response) rates in the nested case-control study

Participation (response) rate of cases
1. ≥70% participation rate (≥80% response rate) (low risk)
2. ≥50 to <70% participation rate (≥60 to <80% response rate) (moderate risk)

3. <50% participation rate (<60% response rate) OR insufficient information to tell (high risk)
4. New data not being collected from cases (not applicable)

Participation (response) rate of controls
1. ≥60% participation rate (≥70% response rate) (low risk)
2. ≥40 to <60% participation rate (≥50 to <70% response rate) (moderate risk)
3. <40% participation rate (<50% response rate) OR insufficient information to tell (high risk)
4. New data not being collected from controls (not applicable)

Difference in participation rate (response rate) between cases and controls
1. Participation (response) rate in cases ≤10 percentage points different from controls (low risk)
2. Participation (response) rate in cases is >10 to ≤15 percentage points different from controls (moderate risk)
3. Participation (response) rate in cases is >15 percentage points different from controls OR insufficient information to tell (high risk)
4. New data not being collected from either or both of cases and controls (not applicable)

Nature, measurement and temporality of exposure

Measurement of exposure
1. Objective measurements from pre-existing records or baseline physical or biological assessment blind to outcome status (low risk)
2. Objective measurements from pre-existing records or baseline physical or biological assessment not blind or not known to be blind to outcome status OR structured interview (moderate risk)
3. Self-administered questionnaire OR insufficient information to tell (high risk)

Was the outcome that defined cases present at the time of exposure measurement?
1. No (low risk)
2. Yes but outcome unlikely to affect exposure measurement (moderate risk)
3. Yes and outcome likely to affect exposure measurement OR insufficient information to tell (high risk)

Was the same method used to measure exposure in cases and controls?
1. Yes (low risk)
2. No OR insufficient information to tell (high risk)

Temporality of exposure
1. Exposure precedes onset of disease in cases and is from a corresponding calendar time in controls (low risk)
2. Exposure precedes onset of disease in cases but exposure in controls is not from a calendar time corresponding to exposure in cases (moderate risk)
3. Exposure does not precede onset of disease in cases OR insufficient information to tell (high risk)

Missing exposure data

Difference in missing data of exposure between cases and controls
1. Difference in missing data of exposure <10 percentage points (low risk)
2. Difference in missing data of exposure ≥10 to <20 percentage points (moderate risk)
3. Difference in missing data of exposure ≥20 percentage points (high risk) OR insufficient information to tell (high risk)

Follow-up

Follow-up methods

1. Active or passive follow-up of participants with methods of ascertainment of outcome and death clearly described AND with methods of ascertainment of emigration from population-at-risk clearly described or censoring at date of last follow-up OR there is a plausible estimate of >90% follow-up (low risk)
2. Active or passive follow-up with methods of ascertainment of outcome, death and emigration from population-at-risk not clearly described OR there is a plausible estimate of 70 – 90% follow-up (moderate risk)
3. Active or passive follow-up with methods of ascertainment of one or more of outcome, death or emigration not described OR there was probably <70% follow-up OR insufficient information to tell (high risk)

Was follow-up long enough of outcome to occur as a consequence of measured exposure?
(Requires prior specification of a sufficient follow-up period)

1. Yes (low risk)
2. No OR insufficient information to tell (high risk)

Completeness of follow-up

1. Follow-up methods are the same and likely to achieve the same completeness of follow-up in exposed and unexposed participants (low risk)
2. Completeness of follow-up in exposed and unexposed participants is unlikely to be the same but difference between the two is, or would be likely to be, small (<10%) (moderate risk)
3. Completeness of follow-up in exposed and unexposed participants is very unlikely to be the same and difference between the two is, or is likely to be, large (>10%) OR insufficient information to tell (high risk)

Control of confounding

Comparability of cases and controls with respect to potentially important confounding variables
(Requires prior specification of potentially important confounders)

1. Age and other potentially important confounders measured and controlled by design or in analysis (low risk)
2. Age and some but not all other potentially important confounders controlled by design or in analysis (moderate risk)
3. No potentially important confounders or only age controlled by design or in analysis OR insufficient information to tell (high risk)

Matching variables are appropriately included in the analysis

1. When controls are frequency matched to cases, matching variables are controlled in the analysis OR when controls are individually matched to cases, a conditional analysis is used or matching variables are controlled in the analysis (low risk)
2. It is not a matched design (not applicable)
3. None of the above OR insufficient information to tell (high risk)

Covariates are appropriately included in the analysis

1. NO variable measuring the same underlying concept or lying in the same causal pathway as the exposure variable under study IS included as a covariate in the statistical analysis models (low risk)
2. ONE OR MORE variables measuring the same underlying concept or lying in the same causal pathway as the exposure variable under study IS included as a covariate in statistical analysis models OR insufficient information to tell (high risk)

Conflict of interest

1. No conflict of interest declared (low risk)
2. One or more conflicts of interest OR no conflict of interest declaration (moderate risk)

Overall rating

High risk of bias – high risk of bias in any domain
Moderate risk of bias – moderate or low risk of bias in all domains, no high risk domains
Low risk of bias – all domains low risk of bias, no moderate or high risk domains

Appendix 7: Neonatal, infant and maternal

Summary table of neonatal, infant and maternal health outcomes

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Andersen et al., 2010 (52)	Aarhus, Denmark	Cohort Study	1,400 randomly sampled mother-infant pairs enrolled in the Aarhus Birth Cohort. Mothers gave birth to a singleton from 1996–2002.	PFOA, PFOS; maternal serum.	Birth weight, height at 5 and 12 months, weight at 5 and 12 months and BMI at 5 and 12 months.	High	Significant negative association between PFOA and birth weight. Significant negative association between PFOS and weight at 12 months and BMI at 12 months.
Apelberg et al., 2007 (53)	Baltimore, Maryland, USA	Cross-sectional study	293 infants who participated in the Baltimore THREE study and were born from 26 November – 16 March 2005 at the Johns Hopkins Hospital. All live birth, singleton deliveries occurring in the labour and delivery suite at the hospital were eligible of participation in the study. Newborns with major congenital anomalies likely to affect foetal growth were excluded.	PFOA, PFOS; umbilical cord serum.	Birth weight, birth length, gestational age, head circumference and PI.	High	Significant negative association between PFOA and PFOS and head circumference and PI. No significant associations between PFOA and PFOS and gestational age, birth weight and length.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Arbuckle et al., 2013 (54)	Ottawa, Ontario, Canada	Cross-sectional study	126 women who had their obstetrical care provided by physicians and midwives at the Ottawa Hospital from 2005–2008.	PFOA, PFOS, PFHxS, PFNA; umbilical cord serum.	Gravidity, gestational age, mode of delivery and birth weight.	High	Significant negative association between PFOS and PFHxS and gravidity. Significant positive associations between PFNA and gravidity. Significant positive association between PFOS and gestational age. Significant positive association between PFOA, PFOS and PFNA and vaginal delivery compared to caesarean. No significant association between PFOA and birth weight.
Bach et al., 2016 (55)	Aarhus, Denmark	Cohort Study	1,533 randomly sampled mother-infant pairs enrolled in the Aarhus Birth Cohort between. Women were nulliparous and gave birth to a singleton from 2008–2013.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnDA, PFHpS; maternal serum.	Birth weight, length and head circumference.	Moderate	No significant associations between PFAS and birth weight, length and head circumference.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Bae et al., 2015 (86)	Michigan and Texas, USA	Cohort study	223 women with singleton pregnancies enrolled in the LIFE study. Women were recruited preconception from 2005–2009 from 16 counties in Michigan and Texas.	PFOA, PFOS, PFNA, PFDA, PFOSA, Et-PFOSA-AcOH, Me-PFOSA-AcOH; maternal serum.	Secondary sex ratio and odds of having a male infant.	High	Significant dose-response relationship between paternal Me-PFOSA-AcOH and odds of having a male infant only. No significant association between PFAS and secondary sex ratio.
Buck Louis et al., 2016 (84)	Michigan and Texas, USA	Cohort Study	344 women with singleton pregnancies enrolled in the LIFE study. Women were recruited preconception from 2005–2009 from 16 counties in Michigan and Texas.	PFOA, PFOS, PFNA, PFOSA, PFDA, Me-PFOSA-AcOH, Et-PFOSA-AcOH; maternal serum.	Pregnancy loss (undefined).	High	No significant associations between PFAS and pregnancy loss.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Chen et al., 2012 (56)	New Taipei, Taiwan	Cohort Study	429 mother-infant pairs enrolled in the Taiwan Birth Panel Study. Mothers were recruited from one medical centre in Taipei and one local hospital and two clinics in New Taipei from April 2004–January 2005.	PFOA, PFOS, PFNA, PFUdA; umbilical cord serum.	Preterm birth, low birth weight, birth length, head circumference, PI and birth weight <10th percentile of gestational age.	Moderate	Significant positive association between PFOS and preterm birth and small birth weight of gestational age. Significant negative association between PFOS and head circumference. Significant positive association between PFNA and birth length. Significant negative association between PFNA and PI. No significant association between PFOS and low birth weight, birth length and PI. No significant association between PFOA, PFNA and PFUdA and preterm birth, low birth weight, head circumference and small weight of gestational age. No significant association between PFOA and PFUdA and birth length and PI.
Darrow et al., 2013 (57)	Mid-Ohio River Valley, USA	Cohort study	1,630 mothers enrolled in the C8 Health Project between 2005 and 2006, who reported at least one live birth from 2005–2010.	PFOA, PFOS; maternal serum.	Preterm birth, low birth weight and pregnancy induced hypertension.	High	No significant association between PFOA and PFOS and preterm birth, low birth weight and pregnancy induced hypertension.
Darrow et al., 2014 (82)	Mid-Ohio River Valley, USA	Cohort study	1,129 mother-infant pairs enrolled in the C8 Health Project from 2005–2006.	PFOA, PFOS; maternal serum	Miscarriage.	High	No significant association between PFOA and PFOS and miscarriage.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
De Cock et al., 2016 (58)	Zwolle, Netherlands	Cohort study	91 mother-infant pairs enrolled in the OBELIX cohort and recruited from January 2011–January 2013.	PFOA, PFOS; umbilical cord serum.	Birth weight.	Moderate	Significant positive association between PFOS and birth weight. No significant association between PFOA and birth weight.
Fei et al., 2007 (59)	Denmark	Cohort study	1,399 mother-infant pairs randomly selected from the DNBC. Mothers were recruited nationwide by their general practitioner (GP) early in pregnancy between March 1996 and November 2002.	PFOA, PFOS; maternal serum.	Preterm birth, low birth weight and birth weight <10th percentile of gestational age.	Moderate	No significant associations between PFOA and PFOS and preterm birth, low birth weight and small weight of gestational age.
Fei et al., 2008 (81)	Denmark	Cohort study	1,399 mother-infant pairs randomly selected from the DNBC. Mothers were recruited nationwide by their GP early in pregnancy from March 1996–November 2002.	PFOA, PFOS; maternal serum.	Placental weight, birth length, head circumference and abdominal circumference.	Moderate	No significant associations between PFOA and PFOS and placental weight, birth length, head circumference and abdominal circumference.
Govarts et al., 2016 (60)	Flanders, Belgium	Cohort study	248 newborn-mother couples enrolled in the FLEHS II cohort and recruited from the general population of the five provinces of Flanders from August 2008–July 2009 using a multistage sampling procedure.	PFOA, PFOS; umbilical cord serum.	Birth weight.	High	No significant association between PFOA and PFOS and birth weight.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Grice et al., 2007 (61)	Decatur, Alabama, USA	Cohort study	439 pregnant women who gave birth to live singletons and were current, retired, and former employees with a cumulative employment of ≥1 year at a facility that manufactures POSF-based chemicals and specialty films.	PFOS; maternal serum.	Birth weight.	High	No significant association between PFOS and birth weight. This study was ineligible of quantitative analysis as PFOS exposure was estimated not measured.
Hamm et al., 2010 (62)	Alberta, Canada	Cohort study	252 pregnant women who gave birth to live singletons without evidence of malformations, and who delivered at ≥22 weeks of gestation.	PFOA, PFOS PFHxS; maternal serum.	Preterm birth, low birth weight, length of gestation (weeks) and birth weight <10th percentile of gestational age.	Moderate	Significant negative associations between PFHxS and preterm birth. Significant positive associations between PFHxS and length of gestation. No significant associations between PFOA and PFOS and preterm birth and length of gestation. No significant associations between PFAS and birth weight and birth weight <10th percentile of gestational age.
Jensen et al., 2015 (83)	Odense, Denmark	Case-cohort study (with additional case-control analyses)	392 mothers enrolled in the Odense Child Cohort. The cohort included pregnant women residing in the Municipality of Odense, Denmark from January 1, 2010–December 31, 2012 were recruited at 8–16 weeks gestation. 51 of the women suffering a miscarriage were matched on parity and gestational day of serum sampling with 204 delivering women.	PFOA, PFOS, PFHxS, PFNA, PFDA; maternal serum.	Miscarriage.	High	Significant positive association between PFNA and PFDA and miscarriage. No significant association between PFOA, PFOS and PFHxS and miscarriage.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Kim et al., 2011 (63)	Seoul, South Korea	Cross-sectional study	17 mothers aged >25 years old and their neonates from the general population of Seoul in 2007.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnDA; maternal and umbilical cord serum.	Birth weight.	High	No significant association between PFAS and birth weight. This study was ineligible of quantitative analysis as the results of the association between PFAS and birth weight were not published – Only raw birth weights of each participant were provided by the authors.
Kishi et al., 2015 (64)	Hokkaido, Japan	Cohort study	306 mother–child pairs enrolled in the Hokkaido Study conducted from July 2002–October 2005.	PFOS; maternal serum.	Birth weight.	High	Significant negative association between PFOS and birth weight in female infants only.
Kwon et al., 2016 (65)	Seoul, South Korea	Cohort Study	268 mother-infant pairs enrolled in the Ewha Birth and Growth Retrospective Cohort from 2006 –2010. Pregnant women receiving prenatal care between 24 and 28 weeks of gestation were included in the study.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFTrDA; umbilical cord serum.	Birth weight.	Moderate	Significant negative association between PFOA, PFOS, PFNA, PFDA and PFUdA and birth weight. No significant association between PFHxS, PFDoA and PFTrDA and birth weight.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Lee et al., 2013 (66)	Gyeongbuk County, South Korea	Cross-sectional study	70 pregnant women who visited the regional hospitals of delivery in Gyeongbuk county, South Korea, from March–August 2011. All the deliveries were singleton births without congenital malformations.	PFOA, PFOS, PFHxS; maternal and umbilical cord serum.	Parity, birth weight, birth length, head circumference and PI.	High	Significant negative association between PFHxS and birth weight of cord blood concentrations only. Significant negative association between PFOS and PI of umbilical cord concentrations only. No significant associations between PFAS and parity, birth length and head circumference.
Lee et al., 2016 (67)	Seoul, South Korea	Cross-sectional study	118 newborns at the Cheil Woman's Hospital in Seoul, South Korea from June–November 2008.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnDA, PFDoA; umbilical cord serum.	Birth weight.	High	No significant association between PFAS and birth weight.
Lenters et al., 2016 (68)	Greenland, Kharkiv, Ukraine and Warsaw, Poland	Cohort study	1,710 pregnant women were enrolled in the INUENDO cohort from June 2002–May 2004 during routine antenatal care visits at a) local hospitals or clinics in 19 municipalities and settlements throughout Greenland, b) three hospitals and eight antenatal clinics in Kharkiv, Ukraine, and c) a large central hospital in Warsaw, Poland.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnDA, PFDoA, PFHpA; maternal serum.	Birth weight.	High	Significant negative association between PFOA and birth weight only.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Liew et al., 2014 (89)	Denmark	Case-cohort study	156 offspring who were diagnosed with congenital cerebral palsy enrolled in the DNBC from 1996–2002. 550 randomly sampled infants (controls) of the cohort without cerebral palsy.	PFOA, PFOS, PFHxS, PFNA, PFHpS, PFDA; maternal serum.	Cerebral Palsy.	Moderate	Significant positive association between PFOA, PFOS and PFHpS and Cerebral Palsy in male infants only. No significant association between PFAS and Cerebral Palsy in female infants.
Maisonet et al., 2012 (69)	Avon, Great Britain	Cohort study	447 mother-infant pairs enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC) from April 1991–December 1992.	PFOA, PFOS, PFHxS; maternal serum.	Birth weight, birth length, gestational age, PI and weight at 20 months old.	High	Significant negative association between PFOA, PFOS and PFHxS and birth weight. Significant negative association between PFOS and PFHxS and birth length. No significant association between PFOA, PFOS and PFHxS and gestational age and PI. Significant positive association between PFOS and weight at 20 months old.
Monroy et al., 2008 (80)	Hamilton, Ontario, Canada	Cohort study	89 mother-infant pairs enrolled in the Family Study from January 2004–June 2005.	PFOA, PFOS, PFHxS, PFNA; maternal and umbilical cord serum.	Birth weight.	High	No significant association between PFAS and birth weight. The association between PFHxS and PFNA and birth weight was ineligible of quantitative analysis as the results were not published.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Nolan et al., 2009 (70)	Washington County, Ohio, USA	Cross-sectional study	1,555 live born neonates born to mothers residing in Washington County, Ohio from January 1, 2003–September 1, 2005.	PFOA; estimated maternal exposure.	Birth weight, preterm birth and gestational age.	High	No significant association between PFOA and birth weight, preterm birth and gestational age. This study was ineligible of quantitative analysis as PFOS exposure was estimated not measured.
Nolan et al., 2010 (85)	Washington County, Ohio, USA	Cross-sectional study	1,548 live born neonates born to mothers residing in Washington County, Ohio from January 1, 2003–September 1, 2005.	PFOA; estimated maternal exposure.	Multiple congenital abnormalities and birth complications.	High	No significant association between PFOA and congenital abnormalities and birth complications. This study was ineligible of quantitative analysis as PFOA exposure was estimated not measured.
Robledo et al., 2015 (71)	Michigan and Texas, USA	Cohort Study	324 parent-child groups with singleton pregnancies enrolled in the LIFE study. Women were recruited preconception from 2005–2009 from 16 counties in Michigan and Texas.	PFOA, PFOS, PFNA, PFOSA, PFDA, Me-PFOSA-AcOH, Et-PFOSA-AcOH; maternal and paternal serum.	Birth weight, birth length, head circumference and PI.	High	No significant associations between PFAS and birth weight, birth length, head circumference and PI.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Savitz et al , 2012 (72)	Mid-Ohio Valley, USA	Cohort study	11,737 pregnancies that occurred from–2006 in women enrolled in the C8 Health Project.	PFOA; estimated maternal exposure.	Miscarriage, stillbirth, preeclampsia, preterm birth, birth weight and birth defects.	High	Significant positive association between PFOA and preeclampsia. No significant associations between PFOA and miscarriage, stillbirth, preterm birth, birth weight and birth defects. This study was ineligible of quantitative analysis as PFOA exposure was estimated not measured.
Savitz et al., 2012 (73)	Mid-Ohio Valley, USA	Case-cohort study (with additional case-control analyses)	106 cases of stillbirth, 224 cases of preeclampsia, 3,613 cases of preterm birth, 918 cases of low birth weight and 353 cases of small-of-gestational age from mother-infant pairs enrolled in the C8 Health Project from 1990–2004. 4,547 births linked to a survey with residential history data of the C8 Health Project (additional case-control analyses).	PFOA; estimated maternal exposure.	Birth weight, birth weight <10th percentile of gestational age, preeclampsia, stillbirth, preterm birth (<32 weeks and <37 weeks).	High	No significant association between PFOA and birth weight, birth weight <10th percentile of gestational age, preeclampsia, stillbirth, preterm birth (<32 weeks and <37 weeks). This study was ineligible of quantitative analysis as PFOA exposure was estimated not measured.
Shi et al., 2016 (74)	Beijing, China	Cross-sectional study	170 mother-infant pairs recruited from the Haidian Maternal and Child Health Hospital from February 2012–June 2012. Women were native Chinese and infants were singleton live-born without congenital anomalies.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA; umbilical cord serum.	Birth weight, birth length and PI.	High	No significant association between PFAS and birth weight, birth length and PI

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Starling et al., 2014 (87)	Norway	Case-cohort study	500 cases of validated preeclampsia from pregnant women enrolled in the Norwegian Mother and Child Cohort Study from 2003–2007. 567 control pregnancies without pre-eclampsia sampled randomly from cohort.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnDA, PFHpS; maternal serum.	Preeclampsia.	Moderate	No significant association between PFAS and preeclampsia.
Stein et al., 2009 (75)	Mid-Ohio Valley, USA	Cohort study	5,663 mother-infant pairs enrolled in the C8 Health Project from August 2005–July 2006.	PFOA, PFOS; maternal serum.	Miscarriage, preeclampsia, preterm birth, birth weight and birth defects examined through retrospective analysis.	High	Significant positive association between PFOS and low birth weight. No significant association between PFOA and PFOS and miscarriage, preterm birth, preeclampsia and birth defects.
Stein et al., 2014 (88)	Mid-Ohio Valley, USA	Cohort study	10,105 mother-infant pairs enrolled in the C8 Health Project from August 2005–July 2006.	PFOA, PFOS; estimated maternal exposure.	Gastrointestinal, kidney, brain, craniofacial, eye, limb, genitourinary and heart birth defects.	High	Significant positive association between PFOA and brain birth defects. No significant association between PFOA and gastrointestinal, kidney, craniofacial, eye, limb, genitourinary and heart birth defects. This study was ineligible of quantitative analysis as PFAS exposure was estimated not measured.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Wang et al., 2016 (76)	Central Taiwan, Taiwan	Cohort study	223 mother-infant pairs enrolled in the Taiwan Maternal and Infant Cohort Study from December 2000–November 2001. All pregnant women who visited the local clinics in central Taiwan were invited to participate in the study.	PFOA, PFNA, PFDA, PFUnDA, PFDoA;	Birth weight, birth length, head circumference and birth weight <10th percentile of gestational age.	High	Significant negative association between PFNA, PFDA, PFUnDA and PFDoA and birth weight in female infants only. Significant negative association between PFDoA and head circumference in female infants only. Significant negative association between PFDA and PFUnDA and birth weight <10th percentile of gestational age.
Washino et al., 2009 (77)	Sapporo, Hokkaido, Japan	Cohort study	514 mother-infant pairs enrolled in the Hokkaido Study from July 2002–October 2005. Mothers were enrolled at 23–35 weeks gestation. All subjects were native Japanese and residents of Sapporo or surrounding areas.	PFOA, PFOS;	Birth weight, birth length, chest circumference and head circumference.	Moderate	Significant negative association between PFOS and birth weight. No significant association between PFOA, PFOS and birth length, chest circumference and head circumference.
Whitworth et al., 2012 (78)	Norway	Cohort study	950 mother-infant pairs enrolled in the Norwegian Mother and Child Cohort Study. Mothers were enrolled between 2003 and 2004 at approximately 17 weeks gestation.	PFOA, PFOS;	Preterm birth, birth weight, birth weight <10th percentile of gestational age and birth weight >90th percentile of gestational age.	Moderate	Significant negative association between PFOA and PFOS and preterm birth. No significant associations between PFOA and PFOS and birth weight, birth weight <10th percentile of gestational age and birth weight >90th percentile of gestational age.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Wu et al., 2012 (79)	Shantou City, China	Cohort study	108 women from Guiyu and 59 women from Chaonan, Shantou City, China who were healthy pregnant women, were recruited from two hospitals in Guiyu and Chaonan from May–July 2007. Participants from Chaonan were used as the comparison cohort in the study.	PFOA;	Gestational age, birth weight, birth length, PI and Apgar score at 5-minutes after birth.	High	Significant negative association between PFOA and gestational age, birth weight, birth length and Apgar score. No significant association between PFOA and PI.

Appendix 8: Reproductive

Summary table of reproductive health outcomes

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Bach et al., 2015 (102)	Department of Obstetrics and Gynaecology, Aarhus University Hospital, Denmark	Cohort study	*random sample *n=1372 from Aarhus Birth Cohort, 2008–13 *nulliparous *pregnancy fully or partly planned *blood sample between 9 and 20 weeks' gestation *live birth, singleton neonate *complete information on exposures, outcomes, and covariates	PFHxS PFHpS PFOS PFOA PFNA PFDA PFUdA	Fecundity (time to pregnancy) Infertility	Moderate	* no association between exposure to PFAS and longer time to pregnancy or infertility in Danish nulliparous women
Barrett et al., 2015 (100)	Tromsø, Norway	Cohort study	*eligible n=178, from Energy Balance and Breast Cancer Aspects (EBBA-I) study, 2000-02 * Age range 25–35 y.o. * Self-described regular menstrual cycles 22–38 days * No use of hormonal contraceptives within past 6 months * excluded: pregnant or had breast fed within previous 6 months; women with known infertility history, gynaecological disorders, or chronic illness (e.g. type 2 diabetes or hypothyroidism).	PFOS PFOA PFNA PFDA PFUdA PFHxS PFOSA	E2 and P were measured in saliva samples collected daily of a single menstrual cycle.	Low	Results demonstrate that PFOS and PFOSA may be associated with decreased production of E2 and P in reproductive age women.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Buck Louis et al., 2012 (114)	USA	Cohort study	* Operative sample * n=495 * aged 18–44 y.o., scheduled of laparoscopy/-otomy at one of 14 participating clinical sites in the Salt Lake City or San Francisco area, 2007–09 * excluded: history of surgically confirmed endometriosis (prevalent disease); aged <18 or >44 years; history of cancer other than nonmelanoma skin cancer; had used injectable hormones within past 2 years; currently breastfeeding of ≥ 6 months	PFOS PFOA PFNA PFDA PFHxS	Endometriosis	Moderate	* Select PFAS were associated with an endometriosis diagnosis

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Buck Louis et al., 2013 (103)	16 counties in two U.S. states (Michigan and Texas)	Cohort study	* The Longitudinal Investigation of Fertility and the Environment (LIFE) study 2005-09 * 501 committed couples, planning pregnancy in next 6 months * Male partner ≥ 18 y.o. * Female partner 18–40 y.o. * Menstrual cycles 21–42 days * No history of injectable hormonal contraception in past year, discontinuing contraception or off < 2 months * Able to communicate in English or Spanish * excluded: either/both partners reported clinically diagnosed infertility	PFOSA	Fecundity (time to pregnancy)	High	* No association between PFOSA and TTP

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Buck Louis et al., 2015 (111)	Michigan and Texas, USA	Cohort Study	* LIFE Study cohort * 501 couples discontinuing contraception from 2005-09	PFDA PFNA PFOA PFOS PFOSA	Semen quality	Low	* select PFAS associated with certain semen end points *most significant associations observed of PFOSA but with results in varying directions * PFOSA associated with smaller sperm head area and perimeter, a lower percentage of DNA stainability, and a higher percentage of bicephalic and immature sperm * PFDA, PFNA, PFOA, and PFOS were associated with lower percentage of sperm with coiled tails
Campbell et al., 2016 (115)	USA	Cohort study	* data from 2003–04 and 2005–06 National Health and Nutrition Examination Survey (NHANES) cycles * n=20,470, restricted to women 20 – 50 y.o. with self-reported data on doctor-diagnosed endometriosis (n=2,493) * further restricted to those with serum measurements of perfluoroalkyl substances (n=753).	PFOA PFOS PFHxS PFNA	Endometriosis	High	* geometric mean levels of PFNA, PFOA and PFOS were significantly higher among women reporting endometriosis * endometriosis associated with select quartiles of PFOA, PFNA, and PFOS

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Christensen et al., 2011 (118)	United Kingdom	Cohort study	* Avon Longitudinal Study of Parents and Children (ALSPAC) * prospective cohort study * approx 14,000 pregnant women residing in Avon (UK) * expected delivery date between April 1, 1991 and December 31, 1992	PFOSA PFOS PFHxS PFOA PFNA Et-PFOSA-AcOH Me-PFOSA-AcOH PFDA	Age at menarche	High	* participants had nearly ubiquitous exposure to most PFAS examined * PFAS exposure did not appear to be associated with altered age at menarche of their offspring
Dhingra et al., 2016 (116)	Mid-Ohio Valley, USA	Cohort study	1. Retrospective Cohort * n=8,759 women exposed to PFOA-contaminated water from one of 6 affected public water districts, or from contaminated private well of at least 1 year * or participant worked at the DuPont plant * inclusion: complete exposure and menopause history, and women ≥ 40 y.o., who did not report menopause beofe age 40 years 2. Prospective Cohort, n=3,334 * premenopausal women at 2005-06 C8HP survey * serum PFOA measurements at that time	PFOA	Onset of menopause	High	* earlier age at menopause is not associated with PFOA exposure

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Fei et al., 2009 (104)	Denmark	Cohort study	* random selection, n=1,400 * DNBC * recruited 1996 – 02 who provided the first maternal blood sample, gave birth to a single live born child without congenital malformation, and completed all 4 telephone interviews * of these 160 women with unplanned pregnancies or unknown TTP (time to pregnancy) were excluded * final analysis n=1,240 women	PFOS PFOA	Fecundity (time to pregnancy)	High	* suggest that PFOA and PFOS exposure at plasma levels seen in the general population may reduce fecundity
Itoh et al., 2016 (90)	Sapporo, Hokkaido, Japan	Cohort study	* prospective birth cohort (Sapporo cohort of the Hokkaido study), 189 mother–infant pairs recruited in 2002–05 * both prenatal maternal and cord blood samples * excluded: Women who delivered twins, who experienced miscarriage, stillbirth, relocation, or voluntarily withdrew	PFOS PFOA	Measurement of: oestradiol (E2), total testosterone (T), progesterone (P4), inhibin B, insulin-like factor 3, steroid hormone binding globulin, FSH, and LH, and prolactin (PRL)	Low	* foetal synthesis and secretion of reproductive hormones may be affected by in-utero exposure to measurable levels of PFOS and PFOA

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Jensen et al., 2014 (121)	Denmark and Finland	Cohort study	* cases and controls sampled from ongoing joint prospective birth cohort study, University Hospital of Copenhagen, Denmark, and Turku University Hospital, Finland * pregnant women recruited from 1997 – 01 in Denmark and from 1997 – 99 in Finland * children examined at birth and 3 months of age using standardized procedures * Finland: additional cases with cryptorchidism recruited at birth from total hospital cohort during 1997–02 * total n=215 participants: in Denmark n=59 participants (29 cases and 30 controls) and in Finland n=156 (78 cases, 78 controls).	PFOS PFOA	Congenital cryptorchidism	High	* no statistically significant association between cord blood PFOA and PFOS levels and congenital cryptorchidism was found; however, the study was small and larger studies are warranted
Joensen et al., 2009 (34)	Denmark	Cohort study	* 105 Danish men from the general population * median age, 19 years * examined in 2003	PFOA PFOS	Sperm quality Measurement of testosterone, oestradiol, SHBG, LH, FSH, and inhibin B in semen samples	High	* high PFAS (PFOS and PFOA) levels associated with fewer normal sperm * high levels of PFAS may contribute to the otherwise unexplained low semen quality often seen in young men

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Joensen et al., 2013 (99)	Denmark	Cohort study	* 2008–09, 247 healthy young Danish men from general population of Denmark randomly selected among men participating in an on-going study surveying semen quality	PFHxS PFHpS PFOS PFOA PFNA PFDA	Measurement of testosterone, oestradiol, sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and inhibin B in semen samples	Low	* PFOS levels negatively associated with testosterone (T), calculated free testosterone (FT), free androgen index (FAI) and ratios of T/LH, FAI/LH and FT/LH * other PFAS were found at lower levels than PFOS and did not exhibit the same associations * PFAS levels were not significantly associated with semen quality
Jørgensen et al., 2014 (105)	Greenland, Poland, Ukraine	Cohort study	* 938 pregnant women from Greenland (448), Poland (203) and Ukraine (287) included in INUENDO cohort	PFOS PFOA PFHxS PFNA	Fecundity (time to pregnancy)	High	* no consistent evidence that environmental exposure to PFAS is impairing female fecundity
Knox et al., 2011 (101)	West Virginia, USA	Cohort study	* 25,957 women 18 – 65 y.o. from the C8 Health Project including subjects from 6 public water districts contaminated by PFOA from the DuPont Plant near Parkesburg, WV, between August 2005 and August 2006	PFOA PFOS	Onset of menopause	High	* these data suggest that PFAS are associated with endocrine disruption in women and further research on mechanisms is warranted

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Kristensen et al., 2013 (119)	Denmark	Cohort study	* daughters of women enrolled in a Danish population-based cohort established 1988–89 that were followed up at ~ 20 y.o. The final study population consisted of 343 daughters of which 254 had attended the clinical examinations and 89 only had answered the questionnaire	PFOA PFOS	Age at menarche	High	* in adjusted regression analyses, daughters exposed to higher levels of PFOA in utero had a 5.3 (95% confidence interval: 1.3; 9.3) months later age of menarche compared with the reference group of lower PFOA. Crude (p =0.05) and adjusted (p = 0.01) trend tests also indicated a relationship between higher prenatal PFOA exposure and delay of menarche
Lopez-Espinosa et al. , 2011 (120)	Mid-Ohio Valley, USA	Cohort study	* Participants were 3,076 boys and 2931 girls 8-18 y.o. who participated in C8 Health Project between August 2005 and July 2006 (living in contaminated water districts)	PFOA PFOS	Age at puberty	High	* study suggests later age of pubertal maturation is correlated with PFOS in boys and later age of menarche is correlated with PFOA and PFOS concentrations in girls * our results suggest later age at having reached puberty of 3 to 6 months across the range of concentrations found in this population of both boys and girls

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Lopez-Espinosa et al., 2016 (91)	Mid-Ohio Valley, USA	Cohort study	* 2,292 children (6–9 y.o.) from the C8 Health Project 2005–06 who lived near a chemical plant in the Mid-Ohio Valley (USA) with local contamination from PFOA	PFHxS PFOA PFOS PFNA	Oestradiol and total testosterone levels were measured in serum samples	High	* to our knowledge, this is the first study suggesting PFAS associated with lower levels of IGF-1 and sex hormones in young children * boys: PFOA concentrations were significantly associated with testosterone levels; PFOS with oestradiol, testosterone, and IGF-1; and PFNA with IGF-1 * girls: significant associations found between PFOS and testosterone and IGF-1; and PFNA and IGF-1 * both sexes: association magnitudes decreased monotonically across quartiles of both testosterone and IGF-1 in relation to PFOS, and of IGF-1 and PFNA in girls
Lum et al., 2017 (106)	Michigan and Texas, USA	Cohort study	* 501 couples from Michigan and Texas recruited in 2005–09 upon discontinuation contraception	PFOSA Et-PFOSA-AcOH Me-PFOSA-AcOH PFDA PFNA PFOS PFOA	Fecundity (time to pregnancy) Menstrual cycle length	High	* associations observed between two perfluoroalkyl substances and menstrual cycle length changes, and between select perfluoroalkyl substances and diminished fecundity at some (but not all) concentrations

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Lyngsø et al., 2014 (113)	Greenland, Poland and Ukraine	Cohort study	* 1,623 pregnant women * from INUENDO cohort * enrolled during antenatal care visits between June 2002 and May 2004	PFOS PFOA	Menstrual cycle length	High	* higher exposure levels of PFOA associated with longer menstrual cycles in pooled estimates of all three countries
Maisonet et al., 2015 (92)	Avon, United Kingdom	Cohort study	* 72 female daughters born between April 1991 and December 1992 of mothers enrolled in the ALSPAC cohort who had prenatal concentrations of PFAS and serum testosterone and SHBG concentrations at age 15 years available of the analysis	PFOS PFOA PFHxS PFNA	Total testosterone and SHBG concentrations measured in serum from daughters at 15 y.o.	Moderate	* findings were based on a small study sample and should be interpreted with caution * however, they suggest that prenatal exposure to some PFAS may alter testosterone concentrations in females
Olsen et al., 1998 (93)	USA	Cohort study	* two cross-sectional studies of 111 and 80 production workers in 1993 and 1995 respectively, in the same plant	PFOA	Hormone levels in serum: cortisol, dehydroepiandrosterone sulfate (DHEAS), oestradiol, FSH, 17[alpha]-hydroxyprogesterone (17-HP), free testosterone, total testosterone, LH, prolactin, TSH and SHBG	High	* reasonable assurance that, in this production setting, there were no significant hormonal changes associated with PFOA at the serum levels measured

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Raymer et al., 2012 (94)	Durham, NC, USA	Cohort study	* Duke University Medical Center's IVF Clinic recruited a total of 256 men to participate in this study between 2002-05	PFOS PFOA	Semen quality and hormone concentrations	High	* no indication that PFOA or PFOS was significantly associated with volume, sperm concentration, percent motility, swim-up motility and concentration, and directional motility * FSH not associated with either PFOA or PFOS * LH positively correlated with plasma PFOA and PFOS, but not semen PFOS
Taylor et al., 2014 (117)	USA	Cohort study	* 501 natural menopausal women and 431 women with hysterectomy 20–65 y.o. in NHANES (National Health and Nutrition Examination Survey)	PFOS PFOA PFNA PFHxS	Onset of menopause	High	* findings suggest a positive association between PFAS and menopause; however, at least part of the association may be due to reverse causation * regardless of underlying cause, women appear to have higher PFAS concentrations after menopause
Toft et al., 2012 (112)	Greenland, Poland, Ukraine	Cohort study	* n=588 (97%) of partners of pregnant women from Greenland, Poland and Ukraine included in INUENDO cohort	PFOS PFOA PFHxS PFNA	Semen quality	Moderate	* the most robust finding in the present study was the negative associations between PFOS exposure and sperm morphology suggesting adverse effects of PFOS on semen quality, possibly due to interference with the endocrine activity or sperm membrane function.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Toft et al., 2016 (95)	Denmark	Cohort study	* Danish National Patient Registry * 270 cryptorchidism cases, 75 hypospadias cases, and 300 controls with stored maternal amniotic fluid samples available in a Danish pregnancy-screening biobank 1980–96) were selected	PFOS	Amniotic fluid measurements, hormone levels and Cryptorchidism and Hypospadias cases	Low	* environmental PFOS exposure was associated with steroid hormone and INSL3 concentrations in amniotic fluid, but * not associated with cryptorchidism or hypospadias in our study population
Tsai et al., 2015 (96)	Taipei, Taiwan	Cohort study	* 540 subjects, 12–30 y.o. from a 1992– 00 mass urine screening population were recruited to an established cohort from 2006–08 via mail or/and telephone invitations	PFOA PFOS PFNA PFUdA	Serum reproductive hormone levels including testosterone, SHBG, and FSH were measured	Moderate	* serum concentrations of PFOA, PFOS, and PFUdA were negatively associated with the serum levels of SHBG, FSH, and testosterone in the young Taiwanese population * these effects were the strongest in females aged 12–17 years
Velez et al., 2015 (107)	Canada	Cohort study	* Maternal-Infant Research on Environmental Chemicals (MIREC) cohort study *n=2,001 women recruited before 14 weeks of gestation between 2008-11	PFOA PFOS PFHxS	Fecundity (time to pregnancy) Infertility	Low	* results add to the evidence that exposure to PFOA and PFHxS, even at lower levels than previously reported, may reduce fecundity
Vested et al., 2013 (97)	Aarhus, Denmark	Cohort study	* 169 male offspring (19–21 y.o.) from a pregnancy cohort established in Aarhus, Denmark, 1988–89	PFOS PFOA	Semen quality Hormone levels	Low	* in-utero exposure to PFOA may affect adult human male semen quality and reproductive hormone levels

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Vestergaard et al., 2012. (108)	Denmark	Cohort study	* 222 Danish first-time pregnancy planners during the years 1992–95 of whom blood sample was available of analysis	PFOS PFOA PFHxS PFNA PFDA Me-PFOSA-AcOH Et-PFOSA-AcOH PFOSA	Self-reported fecundity (time to pregnancy)	High	* these findings suggest that exposure to PFAS affects TTP (time to pregnancy) only to a small extent, if at all
Whitworth et al. , 2012 (109)	Norway	Cohort study	* 910 women enrolled in the Norwegian Mother and Child Cohort Study	PFOS PFOA	Self-reported fecundity	High	* no associations observed between PFAS and subfecundity among nulliparous women
Whitworth et al., 2016 (110)	Norway	Cohort study	* analysis based on a Whitworth et al, 2012 case-based study of PFAS and subfecundity among MoBa (Norwegian Mother and Child Cohort Study) women with a live birth, enrolled in 2003–04 * retrospective study, n=451 primiparous women	PFOSA PFBA PFHpA PFOA PFNA PFDA PFUdA PFDoA PFTrDA PFTeDA PFHxS PFHpS PFOS	Self-reported fecundity (time to pregnancy)	High	* weakly decreased fecundability OR showed limited support of an association between plasma PFOSA concentrations and TTP among primiparous women

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Zhou et al., 2016 (98)	Taipei, Taiwan	Cohort study	* Cohort of the Genetics and Biomarkers study of Childhood Asthma (GBCA) - sample of 225 healthy adolescents (102 boys and 124 girls aged 13–15 years during 2009–10), was selected from 7 public schools, who had no personal or family history of asthma	PFOS PFOA PFBS PFDA PFDoA PFHxA PFHxS PFNA PFTEDA	Serum reproductive hormone levels including testosterone and oestradiol	Moderate	* higher levels of PFAS coincide with lower testosterone and higher oestradiol levels * more significant associations of PFAS with reproductive hormone were found in males than in females

Appendix 9: Metabolic

Summary table of metabolic outcomes

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Château-Degat et al., 2010 (145)	Nunavik, Northern Quebec, Canada	Cross-sectional study	*n=716 randomly sampled Nunavik Inuit adults aged 18-74 years during 2004	PFOS	Total cholesterol, HDL-C, cholesterol to HDL-C ratio, LDL-C, non HDL-C and TG	Moderate	*significant positive association between PFOS and cholesterol to HDL-C ratio, HDL-C and TG (women only) *no significant association between PFOS and total cholesterol, non-HDL-C and LDL-C
Costa et al., 2009 (122)	Italy	Cohort study	*n=53 males from 20 – 63 years old from a PFOA production department in 2007 (37 current workers and 16 past workers) *n=107 males from the same PFOA department in 2007 that were not exposed to PFOA (executive clerks and blue collar workers) in the control group	PFOA	Total cholesterol, HDL-C and TG, uric acid and creatine concentration, Biochemical liver parameters (albumin, α1-α2-ß-γ globulins, bilirubin, AST, ALT, ALP, GGT)	High	*significant positive association between PFOA and total cholesterol *no significant association between PFOA and HDL-C and TG * positive association between serum PFOA and uric acid levels * no association between serum PFOA and creatinine *Significant negative association between total bilirubin and PFOA *Significant positive association between PFOA and ALT, GGT, ALP and α2-globulin

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Darrow et al., 2016 (157)	Parkersburg, West Virginia, USA	Cross sectional study	Participants of the C8 Health project	Modelled PFOA	Liver biomarkers: alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), bilirubin Liver disease	High	Positive association between PFOA and ALT, no association with GGT, negative association with direct bilirubin. Did not observe evidence that PFOA increases the risk of clinically diagnosed liver disease.
Dhingra et al., 2016 (153)	Mid-Ohio Valley, USA	Cohort study	* Those in C8 Health project with Kidney Disease and those without as control	Serum PFOA, but modelled	Chronic Kidney Disease (self-reported and confirmed through medical records)	High	* no association between Chronic Kidney Disease and exposure to PFOA

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Emmett et al., 2006 (18)	Little Hocking, USA	Cross-sectional	* Stratified random sample of persons from households who had resided in Little Hocking Water Association district of at least 2 years	Serum PFOA	Blood urea nitrogen, creatinine, Albumin, bilirubin, Liver disease	High	*no significant positive association between serum PFOA and markers of several potential health effects from PFOA in a sample of residents from a community with markedly elevated serum PFOA compared with general population levels *no data was extracted, poorly written and it was unclear which of the many endpoints would relate to kidney disease. * No significant positive relationship between serum PFOA and liver function tests.
Eriksen et al., 2013 (123)	Denmark	Cross-sectional study	*n=753 women and men 50–65 years of age, born in Denmark and with no previous cancer diagnosis, were enrolled in the prospective Danish Diet, Cancer and Health (DCH) cohort between 1993 and 1997	PFOA PFOS	Total cholesterol	High	*significant positive association between PFOA and PFOS and total cholesterol
Fan et al., 2014 (160)	Ohio/West Virginia, USA	Cross sectional study	C8 Health Project population	PFOA, PFOS, PFHxS, PFPeA, PFHxA, PFHpA, PFNA, PFDA, PFUnA, PFDoA	Bilirubin, ALT, AST, LDH as a measure of Gilbert syndrome, only GS assessed as disease outcome	High	PFHxA seen at significantly higher concentrations in GS men and women

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Fisher et al., 2013 (134)	Canada	Cross-sectional study	*n=2700 women (non-pregnant) and men aged 18-74 that participated in the Canadian Health Measures Survey (Cycle 1 2007–2009)	PFOA PFOS PFHxS	Total cholesterol, HDL, cholesterol to HDL ratio, LDL, non HDL and TG,	Moderate	*significant positive association between PFHxS and total cholesterol, cholesterol to HDL ratio, LDL, and non HDL *no significant association between PFAS and HDL and TG * no association between PFAS and metabolic syndrome or glucose homeostasis
Fitz-Simon et al., 2013 (135)	Ohio and West Virginia, USA	Cohort study	*n=560 adults enrolled in the C8 Health Project between 2005-2006, with follow-up in 2010	PFOA PFOS	Change in total cholesterol, HDL, LDL and TG over 4 years of follow-up	High	*positive association between PFOS and PFOSA and LDL-C

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Fleisch et al., 2016 (164)	Boston, Massachusetts	Cohort	* Pregnant women recruited to Project Viva, * prospective cohort study of prenatal exposures and offspring health 1999 – 02 during first prenatal visit and their children	PFOA, PFOS, PFNA, PFHxS, PFDA in maternal plasma and in child plasma	Leptin, adiponectin and homeostatic model assessment of insulin resistance in mid-childhood	High	* no evidence of adverse effect of early-life PFAS exposure on metabolic function in mid-childhood * children with higher PFAS concentrations had lower insulin resistance * prenatal PFAS plasma concentrations not associated with leptin, adiponectin, or HOMA-IR in mid-childhood in unadjusted (data not shown) or covariate-adjusted analyses * mid-childhood PFAS concentrations not associated with leptin or adiponectin measured at the same time in unadjusted (data not shown) or adjusted analyses except of consistently lower leptin in children in higher quartiles (Q2–4) of PFOA plasma concentrations * children with higher PFAS concentrations had lower HOMA-IR with weaker associations in covariate-adjusted versus unadjusted models * child PFAS plasma concentrations not associated with leptin or adiponectin * prenatal PFAS plasma concentrations not associated with leptin, adiponectin, or HOMA-IR in offspring

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Frisbee et al., 2010 (129)	Mid-Ohio Valley, USA	Cross-sectional study	*n=12,476 children aged <18 years old enrolled in the C8 Health Project from 2005-2006	PFOA PFOS	Total cholesterol, HDL-C, LDL-C and TG	High	*significant positive association between PFOA and PFOS and total cholesterol and LDL *significant negative association between PFOS and HDL *no significant association between PFOA and PFOS and TG
Fu et al., 2014 (132)	Henan, China	Cross-sectional study	*n=133 randomly selected males and females aged 0 – 88 years old who visited the Yuanyang Red Cross Hospital for a health check-up from October – November 2011	PFOA PFOS PFNA PFDA PFUdA	Total cholesterol, HDL-C, LDL-C and TG	High	*no significant association between PFAS and total cholesterol, HDL-C, LDL-C and TG
Gallo et al., 2012 (158)	USA, Mid-Ohio Valley	Cross sectional study	C8 Health Project participants	PFOS, PFOA	ALT, GGT, bilirubin	High	A small but clear linear association between PFOA and PFOS serum concentrations and ALT, a marker of hepatocellular injury, was observed in this large population-based sample of individuals with exposure to PFAA. Some suggestion of an association of PFOA and GGT, although not clear. PFOA concentration has a positive association with direct bilirubin up to 40 ng/mL, followed by a decrease of bilirubin levels after this peak.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Geiger et al., 2013 (152)	USA	Cross-sectional	* NHANES Children 12-18 y.o.	Serum PFOS and PFOA	Hyperuricemia	High	* serum perfluoroalkyl chemical levels are significantly associated with hyperuricemia in children even at the lower “background” exposure levels of the US general population * positive associations between exposure to PFOS and PFOA and elevated serum uric acid levels
Geiger et al., 2014 (130)	USA	Cross-sectional study	*n= 815 participants ≤18 years of age from the National Health and Nutrition Examination Survey 1999–2008	PFOA PFOS	Total cholesterol, HDL-C, LDL-C and TG	High	*significant positive association between PFOA and PFOS and total cholesterol *no significant association between PFOA and PFOS and HDL-C, LDL-C and TG
Kataria et al., 2015 (149)	USA	Cross-sectional	NHANES Children 12-19 y.o.	PFOS, PFOA, PFNA, PFHxS	Uric acid, estimate of glomerular filtration rate	High	* higher concentrations of PFOA and PFOS, but not PFHxS or PFNA in serum are associated with decrements in eGRF and increase in Uric Acid, independent of numerous confounders including demographic features, other environmental exposures and lifestyle variables

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Lin et al., 2009 (161)	USA	Cross-sectional	* NHANES participants >12 y.o.	PFOS, PFOA, PFHxS, PFNA	Metabolic syndrome, blood glucose, HOMA-IR	High	* PFNA correlated with a lower prevalence of metabolic syndrome and hyperglycaemia in adolescents inversely correlated with the prevalence of the metabolic syndrome *PFOS associated with increased blood insulin, HOMA-IR
Lin et al., 2011 (163)	Taipei, Taiwan	Cross-sectional	* Children year 1-12 taking part in a urine screening	PFOS, PFOA, PFNA, PFUdA	Adiponectin, metabolic syndrome, insulin, glucose	High	* higher levels of serum PFNA associated with higher levels of serum adiponectin and lower levels of insulin * high levels of PFOS associated with higher insulin levels in adults * no relationship of PFOA, PFOS, PFUdA, and the sum of all four PFAS found to glucose homeostasis, adiponectin level, lipid profile, and inflammatory markers * PFNA not associated with glucose levels
Lundin et al., 2009 (159)	3M Company, Cottage Grove, Minnesota	Cohort	3M employees from an APFO production facility	APFOA/PFOA	Mortality (liver cancer, cirrhosis of the liver)	High	No association between APFO levels and liver cancer, cirrhosis of the liver

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Maisonet et al., 2015 (136)	Avon, UK	Cross-sectional study	*n=88 females aged 15 years old and enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC) during their mother's pregnancy. All females were born between April, 1991 and December, 1992	PFOA PFOS	Total cholesterol, HDL-C, LDL-C and TG at 7 and 15 years old	High	*no significant association between PFAS and total cholesterol, HDL-C, LDL-C and TG at 7 and 15 years old
Mundt et al., 2007 (146)	USA	Cohort study	*n=630 employees of a polymer production facility between 1 January 1989 and 1 July 2003	PFNA	Total cholesterol, HDL-C, LDL-C, VLDL and TG	High	*no significant association between PFNA and total cholesterol, HDL-C, LDL-C, VLDL and TG *study was ineligible for quantitative analysis as PFNA exposure was estimated not measured
Nelson et al., 2010 (133)	USA	Cross-sectional study	*n=2,094 partipcants aged 12-80 years old that completed the 2003-2004 NHANES	PFOS PFOA PFHxS PFNA	Total cholesterol, , HDL-C, non-HDL and LDL-C	High	*significant positive association between PFOS and total cholesterol and non-HDL *significant negative association between PFHxS and total cholesterol, non-HDL and LDL-C *no significant association between PFAS and HDL
Olsen et al., 1999 (144)	Antwerp, Belgium and Decatur, Alabama, USA	Cross-sectional study	*n=178 male employees (1995) and 148 male employees (1997) from 3M plants in Antwerp and Decatur	PFOS	Total cholesterol, HDL and TG	High	*no significant association between PFOS and total cholesterol, HDL and TG *study was ineligible for quantitative analysis as Olsen et al, 2007 presented results on the same cohort of 3M employees

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Olsen et al., 2000 (137)	Cottage Grove, Minnesota, USA	Cross-sectional study	111 male employees (1993), 80 males employees (1995) and 74 male employees (1997) from a 3M plant	PFOA	Total cholesterol, HDL, LDL and TG	Moderate	*No significant association between PFOA and total cholesterol, HDL, LDL and TG *study was ineligible for quantitative analysis as Olsen et al, 2007 presented results on the same cohort of 3M employees
Olsen et al., 2003 (138)	Antwerp, Belgium and Decatur, Alabama, USA	Cross-sectional study	255 and 263 male and female employees from 3M plants in Antwerp and Decatur, respectively	PFOA PFOS	Total cholesterol, HDL and TG	High	*no significant association between PFOA and PFOS and total cholesterol and TG *results for HDL were ineligible for quantitative analysis as mean HDL was the only outcome stated, rather than regression coefficients indicating mean change in HDL or risk estimates *PFOA results in the study was ineligible for quantitative analysis as Olsen et al, 2007 presented results on the same cohort of 3M employees
Olsen et al., 2007 (139)	Antwerp, Belgium, Decatur, Alabama, USA and Cottage Grove, Minnesota, USA	Cross-sectional study	*n=552 male employees from 3M plants (n=206 for Antwerp, n=131 for Cottage Grove and n=215 for Decatur)	PFOA	Total cholesterol, HDL-C, LDL-C and TG	High	*significant positive association between PFOA and TG for Antwerp employees only *no significant association between PFOA and total cholesterol, HDL-C and LDL-C * no association between PFOA and total cholesterol or LDL * negative association with HDL * inconsistent triglyceride associations

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Olsen et al., 2012 (140)	Cottage Grove, Minnesota, USA	Cohort study	*n=179 male and female employees for a 3M plant who did not take lipid lowering medications and participated in a follow-up study from baseline	PFOA PFOS	Total cholesterol, non-HDL-C, HDL-C and total cholesterol to HDL-C ratio	High	*significant negative association between PFOA and total cholesterol to HDL-C ratio *no significant association between PFOA and PFOS and total cholesterol, non-HDL-C and HDL-C *study was ineligible for quantitative analysis as Olsen et al, 2007 presented results on the same cohort of 3M employees and did not quantify the association between PFOA and cholesterol measurements
Qin et al., 2016 (147)	Taipei, Taiwan	Cross-sectional	* Control sample of the Genetics and Biomarkers study of Childhood Asthma (GBCA) in Taiwan. 225 healthy children 12 – 15 y.o.	PFBS, PFHxS, PFOS, PFNA, PFOA, PFDA	Uric acid (hyperuricemia) in children	High	* only PFOA showed a significant effect on hyperuricemia
Rantakokko et al., 2015 (156)	Kuopio, Finland	Cohort study	161 subjects from an ongoing Kuopio Obesity Surgery Study	PFOA, PFOS, PFHxS, PFHxA, PFNA, PFDA, PFUnA	ALT, Liver histology	Moderate	The maximally adjusted model showed a significant negative association at baseline between PFOA, PFNA, PFDA, PFHxS and sum of PFCAs and lobular inflammation (2-4 foci per 200x field). No association were found with other liver histology parameter at baseline. PFHxA had significant positive association ($p = 0.011$) with ALT at 12 months in the model adjusted for age, fasting insulin and weight change (results not shown).

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Rotander et al., 2015 (141)	Australia	Cross-sectional study	*n=149 firefighter employees of AFFF training facilities in Australia	PFOA PFOS PFHxS	Total cholesterol, HDL-C, LDL-C and TG, uric acid concentration	High	*no significant association between PFOA, PFOS and PFHxS and total cholesterol, HDL-C, LDL-C and TG * no association of PFAS serum levels and uric acid
Sakr et al., 2007 (125)	Washington, West Virginia USA	Cohort study	*n=454 workers at the Washington Works site who were involved in the use of Ammonium Perfluorooctanoate (APFO)	PFOA	Total cholesterol, HDL-C, LDL-C and TG	High	*significant positive association between PFOA and total cholesterol *no significant association between PFOA and HDL-C, LDL-C and TG *study was ineligible for quantitative analysis as Sakr et al, 2007 (b) presented results on the same cohort of APFO employees
Sakr et al., 2007 (124)	Washington, West Virginia USA	Cross-sectional study	*n=1025 active workers at the Washington Works site who were involved in the use of Ammonium Perfluorooctanoate (APFO)	PFOA	Total cholesterol, HDL-C, LDL-C and VLDL-C	High	*significant positive association between PFOA and total cholesterol, LDL-C and VLDL-C *no significant association between PFOA and HDL-C
Shankar et al., 2011 (151)	USA	Cross-sectional	* NHANES ≥20 y.o.	PFOS and PFOA	Serum uric acid	High	* PFOS and PFOA positively associated with elevated uric acid/hyperuricemia, independent of confounding factors
Shankar et al. 2011 (154)	USA	Cross-sectional	* NHANES ≥20 y.o.	PFOS and PFOA	eGFR	High	* PFOS and PFOA positively associated with chronic kidney disease

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Skuladottir et al., 2015 (126)	Aarhus, Denmark	Cross-sectional study	*n=854 Danish women who gave birth between April 1988 and January 1989 and were enrolled in the Aarhus Birth Cohort	PFOA PFOS	Total cholesterol	Moderate	*significant positive association between PFOA and PFOS and total cholesterol
Starling et al., 2014 (142)	Norway	Cross-sectional study	*n=891 pregnant women enrolled in the Norwegian Mother and Child (MoBa) Cohort Study between 2003 and 2004	PFOA PFOS PFHxS PFNA PFDA PFUnDA PFHpS	Total cholesterol, HDL-C, LDL-C and TG	High	*significant positive association between PFOS and total cholesterol *positive association between PFAS and HDL-C, with no significance stated *no significant association between PFAS and LDL-C and TG
Steenland et al., 2009 (127)	Ohio and West Virginia, USA	Cross-sectional study	*n=46,294 adults who were community residents in Ohio or West Virginia aged over 18 years old and who were enrolled in the C8 Health Project between 2005 and 2006	PFOA PFOS	Total cholesterol, HDL-C, cholesterol to HDL-C ratio, LDL-C, non HDL-C and TG	Moderate	*significant positive association between PFOA and PFOS and total cholesterol, cholesterol to HDL-C ratio, LDL-C, non HDL-C and TG *no significant association between PFOA and PFOS and HDL-C
Steenland et al., 2010 (150)	Ohio and West Virginia, USA	Cross-sectional	* Participants in the C8 Health Project 2005-2006 ≥ 20 y.o.	PFOS, PFOA	Uric acid/hyperuricemia	High	* elevated risk of hyperuricemia in highest quartile. * PFOS showed a similar relationship with uric acid as did PFOA, but with less pronounced trends

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Steenland & Woskie 2012 (155)	DuPont plant West Virginia, USA	Cohort	* PFOA production plant workers	PFOA	Mortality from chronic renal disease	Moderate	* elevated mortality of chronic renal disease compared to other DuPont workers
Timmermann et al., 2014 (162)	Odense, Denmark	Cross-sectional	8-10 y.o. children from Odense	PFOS, PFOA	Leptin, adiponectin, insulin, glucose and triglyceride concentrations were assessed in 8-12 y.o. children	High	* no significant associations between PFOS and PFOA exposure and BMI, skinfold thickness, waist circumference, adiponectin, and leptin after adjustment * among overweight children, increased PFAS concentrations associated with higher insulin and triglyceride concentrations * PFOS and PFOA concentrations did not affect the glucose levels among overweight children, and the increased insulin concentrations thereof led to increased HOMAbeta and HOMA-IR levels. In contrast, * no associations between the PFAS and markers of glycaemic control among normal-weight children; and * no association between PFAS concentrations and indicators of adiposity among any children in this study

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Wang et al., 2012 (143)	Changshu City, Jiangsu Province, China	Cross-sectional study	*n=132 residents of Changshu City and 55 male employees of fluorochemical plants in Changshu City between May 2010 and October 2011	PFOA	Total cholesterol, HDL-C, HDL-C to LDL-C ratio, LDL-C and TG	High	*significant negative association between PFOA and HDL-C and HDL-C to LDL-C ratio in employees only *no significant association between PFOA and total cholesterol, LDL-C and TG
Watkins et al., 2013 (148)	Ohio and West Virginia, USA	Cross-sectional	* Children ≥1 <18 y.o. taking part in the C8 Health project	PFOS, PFOA, PFHxS, PFNA	eGFR	High	*positive association between serum PFOA and reduced kidney function indicated by eGFR. * PFOS, PFHxS and PFNA also associated with eGFR in the same way
Winquist & Steenland 2014 (128)	Mid-Ohio Valley, USA	Cohort study	*n=28,541 community members from the C8 Health Project and n=3,713 workers from a Mid-Ohio Valley chemical plant enrolled in the cohorts between 2008 and 2011	PFOA	Hypercholesterolemia	High	*significant positive association between PFOA and medicated hypercholesterolemia *study was ineligible for quantitative analysis as PFOA exposure was estimated not measured
Zeng et al., 2015 (131)	Taipei, Taiwan	Cross-sectional study	*n=225 children aged 12 – 15 from the control group of the Genetic and Biomarkers study for Childhood Asthma (GBCA) selected from 7 schools in Taipei from 2009 to 2010	PFOA PFOS PFHxS PFNA PFDA PFDoA PFTEDA PFBS PFHxA	Total cholesterol, HDL-C, LDL-C and TG	Moderate	*significant positive association between PFOA, PFOS, PFNA and PFBS and total cholesterol *significant positive association between PFOA, PFOS and PFNA and LDL-C and TG *no significant association between PFAS and HDL-C

Appendix 10: Thyroid

Summary table of thyroid effects

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Berg et al., 2015 (173)	Northern Norway	Cohort study	*n=391 pregnant women from The Northern Norway Mother-and-Child Contaminant Cohort Study (MISA) from June 2007 to October 2009	PFOS PFDA PFUdA	TSH, T3 and FT3 levels at second trimester, 3 days and 6 weeks after delivery	Moderate	*significant positive association between PFOS and TSH *significant negative association between PFDA and T3 *significant negative association between PFUdA and FT3 *study was ineligible of quantitative analysis as Berg et al, 2015 presented results on the same cohort of infants
Berg et al., 2017 (165)	Northern Norway	Cohort study	*n=391 pregnant women from The Northern Norway Mother-and-Child Contaminant Cohort Study (MISA) from June 2007 to October 2009	PFOS PFNA PFHxS PFUdA PFDA PFHpS (during the second trimester)	Infant TSH levels at 3 days after birth and maternal TSH, T3, T4, FT3, FT4 collected during the second trimester	Moderate	*significant negative association between maternal PFDA and maternal T3 only *significant negative association between maternal PFUdA and maternal FT3 only *no significant association between maternal PFAS and infant TSH *the results of infant TSH in the study were ineligible of quantitative analysis as only the mean TSH level was stated, not the relationship between TSH and PFAS levels

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Bloom et al., 2010 (175)	New York, USA	Cross-sectional study	*n=31 New York State adults who were sportfish anglers and completed a Dioxin Exposure sub-study component between 1995 and 1997	PFOA PFOS PFDA PFNA PFUdA PFHxS	Adult TSH and FT4	High	*no significant association between PFAS and TSH and FT4 in adults
Chan et al, 2011 (187)	Alberta, Canada	Case-control study	*n=974 pregnant women who underwent a triple screen blood test at 15-20 weeks gestation	PFOA PFOS PFHxS (maternal)	Hypothyroxinemia in pregnant women (FT4 levels in the lowest 10th percentile, with normal TSH)	High	*no significant association between PFAS and hypothyroxinemia
de Cock et al., 2014 (181)	Zwolle, Netherlands	Cohort study	*n=148 mother-child pairs from the LINC study recruited between January 2011 and January 2013	PFOS PFOA (cord)	Infant T4	High	*significant positive association between PFOA and T4 in female infants only *no significant association between PFOS and T4 in infants
Emmett et al., 2006 (18)	Ohio, USA	Cross-sectional study	*n=371 residents aged 2.5 – 80 years old in a community supplied by a single water district who are known to have elevated PFOA levels compared to the general population	PFOA	TSH	High	*no significant association between PFOA and TSH *study was ineligible of quantitative analysis as the age range of participants spanned through the age categories of the meta-analysis

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Jain et al., 2013 (176)	USA	Cross-sectional study	*n=1540 adults (≥18y) from the 2007-2008 wave of the National Health and Nutrition Examination Survey (NHANES) study	PFOA PFOS PFHxS PFNA PFDA	TSH, FT3, FT4, T3 and T4 in adults	High	*significant positive association between PFOA and T3 only *no significant association between PFAS and TSH, FT3, FT4 and T4 *results of PFDA were only extracted from the study of qualitative analysis, as Webster et al, 2016 presented results on the same cohort of adults of PFOA, PFOS, PFHxS and PFNA
Kato et al., 2016 (166)	Sapporo, Japan	Cohort study	*n=392 mother-infant pairs in the Hokkaido Study on the Environment and Children's Health recruited between 2002 and 2005	PFOA PFOS (maternal)	TSH and FT4 in infants and their mothers	High	*significant positive association between maternal PFOS and infant TSH *significant negative association between maternal PFOS and maternal TSH *no significant association between maternal PFAS and maternal and infant FT4
Kim et al., 2011 (63)	Seoul, South Korea	Cohort study	*n=44 pregnant women recruited from three hospitals between August 2008 and March 2009	PFOS PFOA PFHxS PFTrDA (maternal and foetal)	TSH, T4 and T3 in infants	High	*significant negative association between maternal PFOS and PFTrDA and infant T3 *significant negative association between maternal PFTrDA and infant T4 *significant positive association between maternal PFOA and infant TSH *no significant association between foetal PFAS and No association between foetal PFAS and TSH, T4 and T3 in infants

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Kim et al., 2016 (186)	Seoul, South Korea	Case-control study	*n=40 newborn infants that visited one hospital between July 2009 and February 2010	PFOA PFOS PFHxS PFNA PFUdA PFHpS PFDA PFDoA PFTrDA PFBS PFHpA PFHxA PFPeA PFBA	Cases of congenital hypothyroidism in infants	High	*significant positive association between PFOA, PFNA, PFDA and PFUdA and congenital hypothyroidism in infants
Knox et al., 2011 (177)	Ohio, USA	Cross-sectional study	*n=50,113 adult residents in six water districts contaminated by PFOA between August 2005 and August 2006	PFOA PFOS	TSH, T4 and T3	High	*significant positive association between PFOA and PFOS and T4 *significant negative association between PFOA and PFOS and T3 *no significant association between PFAS and TSH
Lin et al., 2013 (178)	Taipei, Taiwan	Cross-sectional study	*n=545 young adults in 2006-2008 that went through a health screening programme in childhood	PFOA PFOS PFNA PFUdA	FT4 and TSH (elevated TSH; Hypothyroidism)	High	*significant positive association between PFNA and FT4 only *no significant association between PFAS and TSH

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Lopez-Espinosa et al., 2012 (167)	Ohio, USA	Cross-sectional study	*n=10,725 children aged 1-17 years living near a Teflon manufacturing facility during 2005-2006 and enrolled in the C8 Health Project	PFOA PFOS PFNA	TSH, T4 and thyroid disease	High	*significant negative association between PFOA and TSH in infants ≤ 5 years old only *significant positive association between PFOS and PFNA and T4 of children aged > 5 years old only *significant positive association between PFOA and T4 of children aged 6 – 10 only *thyroid disease was ineligible of quantitative analysis as the age range of participants spanned through the age categories of the meta-analysis
Melzer et al., 2010 (183)	USA	Cross-sectional study	*n=3,974 adults in the National Health and Nutrition Examination Survey (NHANES) of 1999–2000, 2003–2004, and 2005–2006	PFOA PFOS	Thyroid disease	High	*significant positive association between PFOA and thyroid disease
Shah-Kulharni et al., 2016 (168)	Seoul, Korea	Cross-section study	*n=279 mother-infant pairs enrolled in the EwHa Birth & Growth Retrospective Cohort (EBGRC) study	PFOS PFOA PFNA PFDA PFDoA PFTrDA PFPeA PFUdA PFHxS (cord serum)	T3, T4 and TSH in cord blood samples at birth	High	*significant negative association between PFPeA and T3 levels in boys only *significant positive association between PFPeA and T4 levels in girls only *significant negative association between PFNA and TSH in girls only *significant negative association between PFHxS and T3 in girls only

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Shrestha et al., 2015 (179)	NY, USA	Cross-sectional study	*n=87 adults aged 55 to 74 years, who lived in three demographically similar communities adjacent to the Hudson River in New York State (NYS) of 25 years or more	PFOA PFOS	TSH, FT4, T4 and T3	High	*significant positive association between PFOS and FT4 and T4 only *no significant association between PFAS and TSH and T3
Steenland et al., 2015 (185)	West Virginia, USA	Cohort study	*n=4391 workers with at least 1 day of work between 1948 and 2002 at the DuPont at their West Virginia plant	PFOA	Thyroid disease in males and females with and without a 10-year lag	Moderate	*no association between exposure to PFOA and thyroid disease in males and females *study was ineligible of quantitative analysis as PFOA exposure was estimated not measured
Tsai et al., 2017 (169)	Taipei, Taiwan	Cross-sectional study	*n=118 mother-infant pairs enrolled in the Taiwan Birth Panel Study (TBPS) between April 2004 and January 2005	PFOS PFOA PFDA PFUdA (cord blood)	TSH, T4 and T3	High	*significant negative association between PFOS and T4 in boys only *significant positive association between PFOS and TSH in boys only *no significant association between PFAS and T3
Wang et al., 2013 (172)	Norway	Cross-sectional study	*n=903 pregnant women from the Norwegian Mother and Child Cohort Study between 2003 and 2004	PFOS PFNA PFOA PFHxS PFDA PFHpS PFUdA	TSH during pregnancy (mean gestational age; 18 weeks)	High	*significant positive association between PFOS and TSH in pregnant women

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Wang et al., 2014 (171)	Taiwan	Cohort study	*n=285 pregnant women and 116 neonates enrolled in the Taiwan Maternal and Infant Cohort Study from December 2000 to November 2001	PFHxS PFOA PFOS PFNA PFDA PFUdA PFDoA PFHpA PFHxA (maternal)	Infant and maternal FT4, T4, T3 and TSH	High	*significant negative association between PFOS PFUdA and PFDoA and maternal FT4 and T4 *significant positive association between PFDA and maternal T3 *significant positive association between PFHxS and maternal TSH *significant negative association between PFNA, PFUdA and PFDoA and infant T4 *significant negative association between PFNA, PFDA, PFUdA and PFDoA and infant T3
Webster et al., 2014 (174)	Vancouver, Canada	Cohort study	*n=152 euthyroid pregnant women enrolled in the Chemicals, Health and Pregnancy Study (CHirP) from December 2006-June 2008	PFOS PFNA PFOA PFHxS	FT4, T4 and TSH at 15 and 18 weeks gestation	High	*no significant association between PFAS and fT4, TT4 or TSH of women with normal thyroid peroxidase antibody (TPOAb) levels *significant positive association between PFAS and TSH in women with high TPOAb levels only *significant negative association between PFAS and FT4 in women with high TPOAb levels only

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Webster et al., 2016 (180)	USA	Cross-sectional study	*n=1525 adults (≥18y) from the 2007-2008 wave of the National Health and Nutrition Examination Survey (NHANES)	PFOA PFOS PFHxS PFNA	TSH, T4, T3, FT4, FT3	High	*significant positive association between PFOA and FT3 in adults with normal TPOAb and iodine levels only *significant positive association between PFAS and FT3 in men with high TPOAb and low iodine only *significant negative association between PFAS and FT3 in women with high TPOAb and low iodine only *significant negative association between PFAS and FT4 in adults with high TPOAb and low iodine only *significant positive association between PFAS and TSH in adults with high TPOAb and low iodine only *significant positive association between PFHxS and PFNA and T3 in women with high TPOAb and low iodine only *significant negative association between PFAS and T4 in men high TPOAb and low iodine only
Wen et al., 2013 (182)	USA	Cross-sectional study	*n=1,181 adults who participated in the 2007-2008 or 2009-2010 waves of the NHANES study	PFOA PFOS PFNA PFHxS	T4, T3, FT4, FT3, TSH and Thyroglobulin (Tg) in adults	High	*significant positive association between PFOA and total T_3 *significant positive association between PFHxS and total T_3 and T_4 (women only)

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Winquist et al., 2014 (184)	Cottage Grove, Minnesota, USA	Cohort study	*n=32,254 adults enrolled in either the C8 Health Project or the a cohort of employees from a chemical plant in the same region	PFOA	Thyroid disease	High	*significant positive association between PFOA and thyroid disease (women only) *study was ineligible of quantitative analysis as PFOA exposure was estimated not measured
Yang et al., 2016 (170)	Beijing, China	Cross-sectional study	*n=157 mother-infant pairs recruited from volunteer pregnant women in a single hospital between January and March 2013	PFOA PFOS PFHxS PFNA PFDA PFUdA PFDoA (maternal and cord)	Maternal and infant FT3, FT4, T3, T4 and TSH	High	*significant negative association between maternal PFOA and infant FT3 *significant positive association between infant PFOS and infant FT3, T3 and T4 *significant positive association between infant PFDA and infant T3 and T4 *significant positive association between infant PFUdA and infant T4 *significant negative association between maternal PFDA, PFUdA, PFDoA and PFOS and maternal TSH *significant negative association between maternal PFDoA and maternal FT3, FT4 and T3

Appendix 11: Neurodevelopmental

Summary table of neurodevelopmental and neurophysiological outcomes

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Berk et al., 2014 (207)	USA	Cross-sectional study	*n=4, 928 adults from the 2005–2006, 2007–2008 and 2009–2010 waves of the National Health and Nutrition Examination Survey (NHANES)	PFOA PFHxS Et-PFOSA-AcOH Me-PFOSA-AcOH PFDA PFBS PFHpA PFNA PFOSA PFUA PFDoA PFOS	Depressive symptoms in adults	High	*significant negative association between PFOA, PFHxS, PFNA and PFDA and depressive symptoms
Braun et al., 2014 (204)	Greater Cincinnati area, Ohio, USA	Cohort study	*n=222 mother–child pairs enrolled in the Health Outcomes and Measures of the Environment (HOME) Study	PFOA PFOS PFHxS PFNA	Autistic behaviours in children	High	*significant negative association between PFOA and autistic behaviours *significant positive association between PFOS and SRS scores in boys only
Chen et al., 2013 (188)	Taipei, Taiwan	Cohort study	*n=239 mother-child pairs enrolled in the Taiwan Birth Panel Study	PFOA PFOS	Neurodevelopment at age 2	High	*significant negative association between PFOS and neurodevelopment
Donauer et al., 2015 (189)	Greater Cincinnati area, Ohio, USA	Cohort study	*n=389 mother–child pairs enrolled in the Health Outcomes and Measures of the Environment (HOME) Study	PFOA PFOS	Neurodevelopment at age 5 weeks	High	*significant positive association between PFOA and hypotonic behaviour *no association for 12 other neurodevelopment outcomes

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Fei et al., 2008 (190)	Denmark	Cohort study	*n=1,400 mother-child pairs enrolled in the DNBC	PFOA PFOS	Neurodevelopment at age 1 year 6 months	High	*no association between PFOA and PFOS and neurodevelopment
Fei & Olsen 2011 (206)	Denmark	Cohort study	*n=787 mother-child pairs enrolled in the DNBC	PFOA PFOS	Behavioural problems at age 7	High	*no association between PFOA and PFOS and behavioural problems
Forns et al., 2015 (191)	Norway	Cohort study	*n=989 mother-child pairs enrolled in the Norwegian Human Milk Study	PFOA PFOS	Neurodevelopment at age 6 months and 2 years	High	*no association between PFOA and PFOS and neurodevelopment
Gallo et al. , 2013 (208)	Mid-Ohio Valley, West Virginia, USA	Cross-sectional study	*n=4, 462 adults (aged over 50) enrolled in the C8 Health Project	PFOA PFOS PFHxS PFNA	Memory impairment at age 50 and over	High	*significant negative association between PFOA, PFOS, PFHxS and PFNA and memory impairment
Goudarzi et al., 2016 (192)	Hokkaido, Japan	Cohort study	*n=514 mother-child pairs enrolled in the Hokkaido Study on Environment and Children's Health	PFOA PFOS	Neurodevelopment at age 6 months and 18 months	High	*significant negative association between PFOA and neurodevelopment at age 6 months in girls only *no association between PFOA at age 18 months, and PFOS at both 6 and 18 months
Gump et al., 2011 (193)	Oswego County, New York, USA	Cross-sectional study	*n=79 children aged 9 – 11 years old who lived in Oswego County	PFOA PFOS PFHxS PFNA PFDA PFOSA	Neurodevelopment at age 9 to 11 years	High	*significant negative association between PFOS, PFNA, PFDA, PFHxS, and PFOSA and neurodevelopment

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Hoffman et al., 2010 (198)	USA	Cross-sectional study	*n= 571 children aged 12 –15 years old who participated in one of the 1999 –2000 and 2003 -2004 waves of the National Health and Nutrition Examination Survey (NHANES)	PFOA PFOS PFHxS PFNA	ADHD at ages 12 to 15 years	High	*significant positive association between PFOA, PFOS, PFHxS and ADHD
Høyer et al., 2015 (194)	Greenland, Warsaw, Poland and Kharkiv, Ukraine	Cohort study	*n=1,113 mother-child pairs enrolled in the INUENDO birth cohort	PFOA PFOS	Neurodevelopment and behavioural problems at age 5 to 9 years	High	*significant positive association between PFOA and neurodevelopment *significant positive association between PFOS and behavioural problems in children from Greenland only
Lien et al., 2016 (199)	Taiwan	Cohort study	*n=282 mother-child pairs enrolled in the Taiwan Birth Panel Study	PFOA PFOS PFNA PFUdA	ADHD at age 7 years	High	*significant positive association between PFNA and ADHD *no association for PFOA, PFOS and PFUdA
Liew et al., 2015 (200)	Denmark	Case-cohort study	*n=220 children diagnosed with ADHD and 220 children diagnosed with autism between birth (1996 – 2002) and 2011 *controls were children without ADHD or autism who visited a GP across Denmark (50% total GPs in Denmark participated in the study)	PFOA PFOS PFHxS PFNA PFDA PFHpS	ADHD and autism at age 9 – 15 years	Moderate	*no association between PFAS and ADHD or autism during childhood
Ode et al., 2014 (201)	Malmo, Sweden	Case-control study	*n=206 children diagnosed with ADHD	PFOA PFOS PFNA	ADHD	Moderate	* no association between PFOA, PFOS and PFNA and ADHD.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Oulhote et al., 2016 (205)	Faroe Islands	Cohort study	*n=567 children	PFOA PFOS PFHxS PFNA PFDA	Behavioural problems at age 7	High	* no association between prenatal PFAS exposure and exposure at 7 years old with behavioural problems at 7 years *positive association between PFOA, PFNA and PFDA exposure at 5 years with behavioural problems at 7 years
Power et al., 2013 (209)	USA	Cross-sectional study	*n=1,766 adults (60 - 85 y.o.) from the 1999–00 and 2003–04, 2005–06, and 2007–08 waves of the National Health and Nutrition Examination Surveys (NHANES)	PFOA PFOS PFHxS PFNA	Difficulty remembering	High	*significant negative association between PFOA, PFOS, PFHxS and PFNA and memory impairment
Shiue 2016 (210)	USA	Cross-sectional study	*n=5,563 adults aged between 18 and 85 years old, who participated in the 1999-2000 waves of the National Health and Nutrition Examination Survey (NHANES)	PFOA PFOS PFHxS PFNA PFDA PFUdA PFDoA PFHpA PFBS PFOSA Me-PFOSA-AcOH Et-PFOSA-AcOH	Self-reported sleeping patterns and disorders	High	*significant positive association between Me-PFOSA-AcOH and PFBS and feeling unrested during the day *significant positive association between Et-PFOSA-AcOH and PFDoA and waking at night

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Stein & Savitz, 2011 (202)	Mid-Ohio Valley, West Virginia, USA	Cross-sectional study	*n=10,546 children (5 – 18 y.o.) enrolled in the C8 Health Project	PFOA PFOS PFHxS PFNA	ADHD and learning problems at age 5 – 18 y.o.	High	*significant positive association between PFHxS and ADHD *significant positive association between PFHxS and learning problems
Stein et al., 2013 (195)	Mid-Ohio Valley, West Virginia, USA	Cohort study	*n=320 mother-child pairs (6 – 12 y.o.) enrolled in the C8 Health Project	PFOA	Neurodevelopment and ADHD (based on development profile not diagnosis) at age 6 – 12 y.o.	High	* no association between PFOA and neurodevelopment * negative association between PFOA and ADHD
Stein et al., 2014 (197)	Mid-Ohio Valley, West Virginia, USA	Cohort study	*n=321 mother-child pairs (6 – 12 y.o.) enrolled in the C8 Health Project	PFOA	Behavioural problems aged 6 – 12 y.o.	High	* no association between PFOA and behavioural problems
Strøm et al., 2014 (203)	Aarhus, Denmark	Cohort study	*n=876 mother-child pairs from the Danish Foetal Origins 1988 Cohort	PFOA PFOS	ADHD diagnosis and depression diagnosis	Moderate	* no association between PFOA and PFOS and ADHD or depression diagnosis
Vuong et al., 2016 (196)	Greater Cincinnati area, Ohio, USA	Cohort study	*n=256 mother–child pairs enrolled in the Health Outcomes and Measures of the Environment (HOME) Study	PFOA PFOS PFHxS PFNA PFDA	Neurodevelopment at 5 and 8 years old	High	*negative association between PFOS and PFHxS and neurodevelopmental outcomes * no association of PFOA, PFNA and PFDA

Appendix 12: Cancer

Summary table of cancer outcomes

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Alexander et al., 2003 (211)	Decatur, Alabama, USA	Cohort study	*n=2,083 employees (83% male) from a plant manufacturing Perfluoroctanesulphonyl fluoride (POSF)	PFOS	Malignant neoplasm mortality, digestive system cancer mortality, oesophagus cancer mortality, large intestine cancer mortality, liver cancer mortality, respiratory system cancer mortality, lung cancer mortality, breast cancer mortality, urinary organ cancer mortality, bladder cancer mortality, melanoma mortality, and haemopoietic and lymphatic system cancer mortality	High	*significant association between PFOS and bladder cancer mortality. Small number of cases (n=3) *study was ineligible for quantitative analysis as PFOS exposure was estimated not measured
Alexander & Olsen 2007 (212)	Decatur, Alabama, USA	Cohort study	*n=2,083 employees (83% male) from a plant manufacturing Perfluoroctanesulphonyl fluoride (POSF)	PFOS	Bladder cancer incidence	High	*no significant association between PFOS and bladder cancer incidence *study was ineligible for quantitative analysis as PFOS exposure was estimated not measured

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Barry et al., 2013 (213)	Mid-Ohio Valley, Ohio, USA	Cohort study	*n=32,254 adults who resided in area where water was contaminated or worked in chemical plant manufacturing PFOA	PFOA	Bladder cancer incidence, brain cancer incidence, breast cancer incidence, cervical cancer incidence, colorectal cancer incidence, oesophagus cancer incidence, kidney cancer incidence, leukaemia incidence, liver cancer incidence, lung cancer, incidence, lymphoma cancer incidence, melanoma incidence, oral cancer incidence, ovarian cancer incidence, pancreatic cancer incidence, prostate cancer incidence, soft tissue cancer incidence, stomach cancer incidence, testicular cancer incidence, thyroid cancer incidence, uterine cancer incidence	High	*significant association between PFOA and bladder and testicular cancer incidence *study was ineligible of quantitative analysis as PFOS exposure was estimated not measured
Bonefeld-Jørgensen et al., 2011 (214)	Greenland	Case control study	*n=146: 31 breast cancer cases and 115 controls	PFOS PFOA	Breast cancer, incidence	High	* higher level of poly-fluorinated chemicals and legacy persistent organic pollutants in breast cancer cases indicating that the level of serum POPs in particularly PFCs might be risk factors in the development of breast cancer in Greenlandic Inuit.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Bonefeld-Jørgensen et al., 2014 (215)	Denmark	Nested case control study	*n=483 women: 250 diagnosed with breast cancer and 233 controls	PFOS PFOA PFNA PFHxS PFOSA	Breast cancer incidence	Low	* study does not document PFAS as overall causes of breast cancer in Danish premenopausal women but does not rule out that such and association may exist and more studies are needed.
Ducatman et al., 2015 (216)	Ohio, USA	Cross sectional study	*n=25,412 men	PFOS PFOA PFOS PFNA	Prostate specific antigen level	High	* findings do not provide evidence of an association between perfluoroalkyl acids and prostate-specific antigen.
Eriksen et al., 2009 (217)	Denmark	Case cohort study	*n=2012 persons; 1240 cancer patients and 772 sub-cohort comparison group	PFOA PFOS	Prostate cancer incidence, bladder cancer incidence, pancreatic cancer incidence, liver cancer incidence	Moderate	* there was no clear difference in risk of these cancers in relation to PFOS or PFOA level.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Gilliland & Mandel 1993 (218)	Not stated; authors from Minneapolis, Minnesota	Cohort study	*n=3537 men and women working for at least 6 months in a PFOA plant in Chemical Division.	PFOA	Causes of mortality	High	* Among men the cardiovascular standardized mortality rate was .68 (95% CI, .58 to .80) and the all-gastrointestinal diseases was .57 (95%CI, .29 to .99). There was no significantly increased cause-specific standardized mortality ratio for men or women. Ten years of employment was associated with a 3.3 fold increase (95% CI, 1.02 to 10.6) in prostate cancer mortality, based on a small number of deaths.
Grice et al., 2007 (61)	Decatur, Alabama United States	Cross sectional survey	1400 of 2083 employees of PSOF manufacturing plant	PFOS	Colon cancer incidence, Melanoma incidence, Prostate cancer incidence	High	* Investigations did not find an association between working in a PFOS-exposed job and cancers.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Hardell et al., 2014 (219)	Örebro, Sweden	Case control study	*n=387; 201 newly diagnosed cases of prostate cancer and 186 population-based controls	PFHxS PFOS PFOA PFNA PFDA PFUnDA	Prostate cancer incidence	High	* In this study a higher risk was found in the case group with hereditary prostate cancer. Clearly our results show an interaction between gene and environment. The possible mechanism is unknown. We found no association with clinical stage of the disease.
Innes et al., 2014 (220)	West Virginia, USA	Cross sectional study	*47,359 adults, with 208 cases of primary colorectal cancer	PFOS PFOA	Colorectal cancer incidence	High	* strong inverse linear association between PFOS and likelihood of CRC diagnosis and a significant, although more modest inverse association between PFOA and diagnosis of CRC.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Leonard et al., 2008 (221)	Parkersburg, West Virginia, USA	Cohort study	n= 5454 employees on the DuPont employee registry	PFOA	Buccal cavity and pharynx cancer mortality, Digestive system mortality, Respiratory system cancer mortality, Breast cancer mortality, Prostate mortality, Testes and other male genital organ cancer mortality, Kidney cancer mortality, Bladder cancer mortality, Melanoma, Central nervous system cancer mortality, Thyroid and other endocrine gland cancer mortality, Bone cancer mortality, Lymphatic and hematopoietic tissue cancer mortality,	Moderate	* Estimates of most standardized mortality ratios were around 1, with some elevated but not significant. * Thyroid cancer was elevated and significant, but with small numbers of observed cases.
Lundin et al., 2009 (159)	Cottage Grove, Minnesota, USA	Cohort study	*n=807 employees (80% male) from the Cottage Grove 3M Chemical Plant	PFOA	Prostate cancer mortality, pancreatic cancer mortality and bladder cancer mortality	High	*no significant association between PFOA and prostate cancer mortality, pancreatic cancer mortality and bladder cancer mortality *study was ineligible of quantitative analysis as PFOA exposure was estimated not measured

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Olsen et al., 2004 (222)	Decatur, Alabama United States	Cohort study	652 employees of PFOS manufacturing plant compared to 659 film plant employees	PFOS	Colon cancer incidence, Liver cancer incidence, Rectum cancer incidence, Lower respiratory tract cancer incidence, Melanoma incidence, Bladder cancer incidence, Prostate cancer incidence, Thyroid cancer incidence	High	* Investigations did not find an association between working in a PFOS-exposed job and cancers.
Raleigh et al., 2014 (223)	Cottage Grove, Minnesota, USA	Cohort study	*n=9,027 employees from Cottage Grove 3M Chemical Plant	APFO/ PFOA	Prostate cancer mortality & incidence, kidney cancer mortality & incidence, pancreatic cancer mortality & incidence, bladder cancer mortality & incidence, breast cancer mortality & incidence	High	*no significant finding between APFO exposure and mortality from prostate, kidney, pancreatic, bladder, or breast cancer

Steenland & Woskie 2012 (155)	West Virginia, USA	Cohort study	*n=5,791 employees from the DuPont Chemical Plant between 1952 – 2002	PFOA	Liver cancer mortality, pancreatic cancer mortality, lung cancer mortality, breast cancer mortality, prostate cancer mortality, testicular cancer mortality, kidney cancer mortality, bladder cancer mortality, mesothelioma mortality, non-Hodgkin's lymphoma mortality and leukaemia mortality	Moderate	*significant positive association between PFOA and kidney cancer mortality and mesothelioma mortality (expected confounding by asbestos exposure) *no significant association between PFOA and liver cancer mortality, pancreatic cancer mortality, lung cancer mortality, breast cancer mortality, prostate cancer mortality, testicular cancer mortality, bladder cancer mortality, non-Hodgkin's lymphoma mortality and leukaemia mortality *study was ineligible of quantitative analysis as PFOA exposure was estimated not measured
Steenland et al., 2015	West Virginia,	Cohort study	*n=4391 (73%) of 6026 workers or their next of	PFOA	Bladder cancer incidence, colorectal cancer incidence, Melanoma	Moderate	*significant trend across quartiles of

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
(185)	USA		kin from the DuPont Chemical Plant between 1952 – 2002		incidence, Prostate cancer incidence		PFOA for bladder cancer incidence. *no significant association between PFOA and colorectal cancer incidence, melanoma incidence, prostate cancer incidence *study was ineligible of quantitative analysis as PFOA exposure was estimated not measured
Vassiliadou et al.,2010 (224)	Greece	Cross sectional study	n=182 patients	PFOA, PFOS	Cancer incidence	High	* There was no significant difference in PFAS blood concentration between cancer cases and people living in Athens or rural Greece.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Vieira et al., 2013 (225)	Ohio & West Virginia, USA	Case control study	*n=25,107 patients	PFOA	Incidence of 18 cancers including bladder, kidney, liver, prostate, colorectal and breast cancer.	High	* results indicate that higher PFOA levels may be associated with testicular, kidney, prostate, and ovarian cancer, and non-Hodgkin lymphoma
Yeung et al., 2013 (226)	Melbourne, Australia	Cross sectional study	*n=104; plasma from 79 patients undergoing liver transplantation and 25 serum specimens from control patients	PFOS PFHxS EtFOSAA PFDoA PFUnDA PFDA PFNA PFOA PFHpA	Serum concentration in diseased and non-diseased persons	High	*the study demonstrates the presences of PFASs in liver of patients with liver cancer or cirrhosis

Appendix 13: Diabetes

Summary table of diabetes

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Karnes et al., 2014 (228)	USA	Cohort	* worker cohort participants with retrospective exposure estimates (n=3,713)	PFOA	Type 2 diabetes mellitus	Moderate	* no association between PFOA exposure and incidence of type 2 diabetes
Leonard et al., 2008 (221)	West Virginia, USA	Cohort study	6,027 employees from the DuPont chemical plant from 1948−2002.	PFOA	Diabetes (unspecified)	Moderate	* no significant association between PFOA and diabetes mortality compared with either US or West Virginia expected rates. .
Lin et al., 2011 (163)	Taipei, Taiwan	Cross-sectional study	* 287 school age children in grades 1 – 12 in Taipei	PFOA PFOS PFNA PFUdA	Type 2 diabetes mellitus	High	* negative association between PFNA and type 2 diabetes * no association between PFOA, PFOS and PFUdA and type 2 diabetes
Lind et al., 2014 (232)	Sweden	Cross-sectional study	* 1,016 individuals participating in a survey during 2001–04	PFHpA PFHxS PFOS PFOA PFNA PFOSA PFUdA	Diabetes	High	* PFNA was related to prevalent diabetes
Lundin et al., 2009 (159)	Cottage Grove, Minnesota, USA	Cohort study	* 807 (80% male) employees from the Cottage Grove 3M Chemical Plant	PFOA	Mortality caused by diabetes mellitus	High	* no association between PFOA and diabetes mellitus mortality
MacNeil et al., 2009 (229)	USA	Cross-sectional study	* Adults > 20 y.o. participating in a health survey in 2005–06 (n=54,468) of C8 health project	PFOA	Type 2 diabetes	High	* no association between PFOA and type 2 diabetes

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Predieri et al., 2015 (227)	Italy	Case control	* 25 children and adolescents enrolled at the onset of type1 DM and 19 healthy subjects used as control group	PFOS PFOA	Type1 diabetes mellitus	High	* high serum levels of PFOS may be considered a biomarker of susceptibility to type 1 diabetes
Shapiro et al., 2016 (230)	Canada	Cross-sectional	* women enrolled in the Maternal-Infant Research on Environmental Chemicals study with a singleton delivery (offspring born singly)	PFOA PFOS PFHxS	Gestational diabetes mellitus (GDM)	High	* no association between the chemicals and GDM
Steenland & Woskie 2012 (155)	West Virginia, USA	Cohort study	* 5,791 employees from the DuPont chemical plant 1952–02	PFOA	Mortality caused by diabetes mellitus	Moderate	* no association between PFOA and diabetes mellitus mortality
Su et al., 2016 (233)	Taiwan	Cross-sectional	* 592 participants, 20–60 y.o. at outpatient cardiology clinics in the National Taiwan University Hospital, Taipei, Taiwan from 2009 – 11	PFOA PFOS PFNA PFUdA	diabetes mellitus (DM)	Moderate	* PFOS exposure associated with DM. However, PFOA, PFNA, and PFUdA showed a potential protective effect against DM
Zhang et al., 2015 (231)	USA	Cross-sectional	* 272 pregnant women * 18-40 y.o., married, English or Spanish speaking	PFOS PFOA PFNA PFDA PFOSA Et-PFOSA-AcOH Me-PFOSA-AcOH	Gestational diabetes mellitus (GDM)	Moderate	* higher environmentally relevant concentrations of PFOA were significantly associated with an increased risk of GDM

Appendix 14: Cardiovascular

Summary table of cardiovascular health outcomes

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Geiger et al., 2014 (237)	USA	Cross-sectional study	* 1,655 children 12 – 18 y.o. or younger * from 1999-00 to 2007-08 (except 2001-02) National Health and Nutrition Examination Survey (NHANES)	PFOA PFOS	Hypertension in children	High	* no association between PFOA and PFOS and hypertension at age 12 – 18 years
Lin et al., 2013 (239)	Taipei, Taiwan	Cohort study	* n=664 * 12 – 30 y.o. from Young Taiwanese Cohort Study	PFOA PFOS PFNA PFUdA	Carotid intima–media thickness	High	* positive association between PFOS and carotid intima–media thickness * no association of PFOA, PFNA and PFUdA
Lundin et al., 2009 (159)	Cottage Grove, Minnesota, USA	Cohort study	* 807 (80% male) employees from the Cottage Grove 3M Chemical Plant	PFOA	Mortality caused by cerebrovascular disease (stroke) and coronary heart disease	High	* positive association between PFOA and cerebrovascular disease mortality * negative association between PFOA and coronary heart disease mortality
Mattsson et al., 2015 (234)	Sweden	Case-control study	* 253 case-control pairs of adults living in rural areas with and without diagnosed coronary heart disease	PFOA PFOS PFNA PFDA PFHpA PFHxS PFUdA PFDoA	Coronary heart disease in adults	High	* no association between PFAS and coronary heart disease

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Min et al., 2012 (238)	USA	Cross-sectional study	* 2,208 adults * ≥ 20 y.o. from 2003-04 and 2005-06 waves of the National Health and Nutrition Examination Survey (NHANES)	PFOA	Hypertension in adults	High	* positive association between PFOA and hypertension
Sakr et al., 2009 (235)	West Virginia, USA	Cohort study	* 105 female and 4,642 male workers employed 1948 –2002 in one industrial plant	PFOA	Mortality caused by coronary heart disease	Moderate	* no association between PFOA and coronary heart disease mortality
Shankar et al., 2012 (236)	USA	Cross-sectional study	* 1,216 adults aged ≥ 40 y.o. from the 1999-00 and 2003-04 waves of the National Health and Nutrition Examination Survey (NHANES)	PFOA	Cardiovascular disease and peripheral arterial disease in adults	High	* positive association between PFOA and cardiovascular disease and peripheral arterial disease
Steenland & Woskie 2012 (155)	West Virginia, USA	Cohort study	* 5,791 employees from the DuPont chemical plant 1952 – 2002	PFOA	Mortality caused by cerebrovascular disease (stroke) and coronary heart disease	Moderate	* no association between PFOA and coronary heart disease mortality and cerebrovascular disease (stroke)

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Winquist & Steenland 2014 (128)	Mid-Ohio Valley, West Virginia, USA	Cohort study	32,254 adults; 28,541 participants from the C8 Health Project and 6,026 participants from an occupational cohort.	PFOA	Hypertension and coronary heart disease in adults.	High	No association between PFOA and hypertension and coronary artery disease

Appendix 15: Overweight

Summary table of overweight and obesity

Author/s and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Andersen et al., 2013 (240)	Denmark	Cohort study	1,400 mother-child pairs enrolled in the DNBC.	PFOA, PFOS.	BMI and waist circumference in children aged 7 years old.	High	No association between PFOA and PFOS and BMI and waist circumference in children.
Ashley-Martin et al., 2016 (246)	Canada	Cohort study	1,301 pregnant women enrolled in the MIREC study.	PFOA, PFOS, PFHxS.	Gestational weight gain.	Moderate	Significant positive association between PFOA and PFOS and gestational weight gain.
Barry et al., 2014 (244)	West Virginia, Mid-Ohio, USA	Cohort study	8,764 adult workers or residents living near the DuPont chemical plant.	PFOA	BMI in adults.	High	No association between PFOA and BMI.
Braun et al., 2016 (241)	Ohio, USA	Cohort study	204 mother-child pairs enrolled in the HOME study.	PFOA, PFOS, PFNA, PFHxS.	BMI and overweight in children aged ≤18 years old.	Moderate	Significant positive association between PFOA and BMI of children aged 2–8 years old and overweight of children aged 8 years old only. No association of PFOS, PFNA and PFHxS.
de Cock et al., 2014 (242)	Zwolle, Netherlands	Cohort study	89 mother-child pairs from 6 midwifery clinics in Zwolle.	PFOA, PFOS.	BMI of infants in their first year of life.	Moderate	No association between PFOA and PFOS and BMI of infants.

Author/s and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Halldorsson et al., 2012 (245)	Aarhus, Denmark	Cohort study	915 mother-child pairs enrolled in the Aarhus birth cohort.	PFOA	High waist circumference and risk of overweight in adults aged 20 years old.	High	Significant positive association between PFOA and high waist circumference and risk of obesity in females only
Høyer et al., 2015 (243)	Greenland and Kharkiv, Ukraine	Cohort study	1,022 mother-child pairs enrolled in the INUENDO cohort.	PFOA PFOS	Waist-height ratio < 0.5 and risk of being overweight in children aged 5−9 years old.	Moderate	Significant positive association between PFOA and PFOS and a waist-height ratio of <0.5. No association between PFOA and PFOS and risk of being overweight in children.
Jaacks et al., 2016 (247)	Michigan and Texas, USA	Cohort study	258 pregnant women enrolled in the LIFE Study.	PFOA PFOS PFNA PFDA PFOSA Et-PFOSA-AcOH Me-PFOSA-AcOH	Gestational weight gain.	Moderate	Significant positive association between PFOS and gestational weight gain in pregnant women who had a normal BMI at the start of pregnancy only.

Author/s and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Rylander et al., 2009 (248)	South-central Vietnam	Cross-sectional study	189 pregnant women aged 14−80 years old.	PFOA PFOS PFNA PFHxS PFHpS PFHpA PFOSA	Gestational weight gain.	High	No association between PFOA, PFOS, PFNA, PFHxS, PFHpS, PFHpA and PFOSA and BMI of pregnant women.

Appendix 16: Immunological

Summary table of immunological responses

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Dong et al., 2013 (259)	Taiwan	Case-cohort study	231 children aged 10−15 years old diagnosed with asthma were recruited through the GBCA from 2009−2010. 225 non-asthmatic controls.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFBS, PFHxA, PFDoA, PFTEDA.	Asthma diagnosed in the past year.	High	Significant positive association between PFBS, PFHxS, PFOS, PFOA, PFNA, PFDoA and PFDA with asthma for children. This study presents the same results as Zhu et al. (261)
Fei et al., 2010 (257)	Aarhus, Denmark	Cohort study	1,400 mother–infant pairs randomly selected from the Danish National Birth Cohort (DNBC).	PFOA, PFOS	Any hospitalization due to infections in early childhood.	High	Prenatal exposure to PFOA and PFOS was not associated with an increased risk of hospitalization for infectious diseases during early childhood

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Goudarzi et al., 2016 (262)	Hokkaido, Japan	Cohort study	1,558 mother-infant pairs enrolled in the Hokkaido Study on Environment and Children's Health.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFTrDA.	Total allergic diseases, eczema, rhino conjunctivitis and wheezing in children aged 4 years old.	High	Significant negative association between exposure to PFDoA and PFTrDA and total allergic diseases for males only. Significant negative association between PFDoA and PFTrDA and eczema. Significant negative association between PFOA and eczema for males only Significant negative association between PFNA, PFUdA and PFDoA and rhino conjunctivitis. No significant association between PFAS and wheezing.
Grandjean et al., 2012 (249)	Tórshavn, Faroe Islands	Cohort study	587 singleton newborns enrolled in the Faroe Islands Birth Cohort between 1997−2000 and followed up in 2008.	PFOA, PFOS, PFHxS, PFNA, PFDA.	Antibody concentrations against tetanus and diphtheria in children at 5 and 7 years old.	Moderate	Significant negative association between PFOA and PFOS and diphtheria antibody concentration. Significant negative association between PFOA and PFHxS and tetanus antibody concentration.
Grandjean et al., 2016 (252)	Tórshavn, Faroe Islands	Cohort study	516 singleton newborns enrolled in the Faroe Islands Birth Cohort between 1997−2000 and followed up at 13 years old from 2010−2013.	PFOA, PFOS, PFHxS, PFNA, PFDA.	Antibody concentrations against tetanus and diphtheria in children at 13 years old.	Moderate	Significant negative association between PFOA, PFOS, PFHxS, PFNA and PFDA and diphtheria antibody concentration. No significant association between PFOA, PFOS, PFHxS, PFNA and PFDA and tetanus antibody concentration.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Granum et al., 2013 (253)	Oslo and Akershus, Norway	Cohort study	56 mother-infant pairs enrolled in the BraMat cohort from April 2007−March 2008 and followed up at 3 years old from 2010−2011. Mother-infants pairs were eligible to participate if the mother had provided a blood sample at time of delivery and infants had provided a blood sample at 3 years old.	PFOA, PFOS, PFHxS, PFNA; maternal serum.	Antibody concentration against MMR, tetanus and haemophilus influenzae (Hib), cases of middle ear infection, gastroenteritis, colds and influenza, asthma, wheezing and eczema in children at 3 years old.	Moderate	Significant negative association between PFOA, PFOS, PFHxS and PFNA and MMR antibody concentration. Significant positive association between PFOA and PFHxS and gastroenteritis. Significant positive association between PFOA and PFNA and cases of colds and influenza. No significant association between PFAS and tetanus antibody concentration, asthma, wheezing and eczema.
Humblet et al., 2014 (260)	USA	Cross-sectional study	1,877 children aged 12−19 years old who participated in the 1999−2000, 2003−2004, 2005−2006, and 2007−2008 waves of the NHANES study.	PFOA, PFOS, PFHxS, PFNA.	Asthma (current and ever) and wheezing.	High	No significant association between PFAS and asthma or wheezing in children.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Kielsen et al., 2016 (251)	Copenhagen, Denmark	Cohort study	12 healthy adult volunteers recruited by staff at the Copenhagen University Hospital Rigshospitalet with no history of a tetanus-diphtheria booster in the past 5 years.	PFHxS, PFHpA, PFOS, PFOA, PFNA, PFDA, PFUdA, PFDoA.	Antibody concentrations against tetanus and diphtheria in adults.	High	Significant negative association between PFOS, PFNA, PFDA, PFUdA and PFDoA and diphtheria antibody concentrations. Significant negative association between PFUdA and PFDoA and tetanus antibody concentrations.
Looker et al., 2014 (255)	Ohio and West Virginia	Cohort study	Subset of participants in a larger study of PFAS blood levels and association with self-reported outcomes invited to participate in vaccine study. No detail on how selected in relation to PFAS levels	PFOA, PFOS	Post-vaccination antibody titre to specific components of the flu vaccine (and self-reported colds and influenza).	High	Higher PFOA serum concentrations were associated with reduced rise in antibody titre post-influenza vaccination only to A/H3N2 (not H1N1 or B) and increased risk of not attaining protection
Mogensen et al., 2015 (250)	Tórshavn, Faroe Islands	Cohort study	464 children enrolled in the Faroe Islands Birth Cohort from 1997−2000.	PFOS, PFOA, PFHxS, PFNA, PFDA.	Antibody concentrations against tetanus and diphtheria in children at 5 and 7 years old.	Moderate	Significant negative association between PFOA and PFOS and diphtheria antibody concentrations. Significant negative association between PFOS and PFHxS and tetanus antibody concentrations.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Okada et al., 2012 (258)	Sapporo, Japan	Cohort study	504 mother-infant pairs recruited from July 2002−October 2005. Mothers were native Japanese women enrolled at 23−35 weeks gestation and were residents of Sapporo City or its surrounding areas.	PFOA, PFOS.	Eczema, wheezing and food allergies during the first 18 months of life; milk, egg rice gruel, egg-drop, shrimp, or other food.	High	No association between exposure to PFOA and PFOS and wheezing, eczema and food allergies in children.
Okada et al., 2014 (263)	Hokkaido, Japan	Cohort study	2,063 mother-infant pairs enrolled in the Hokkaido Study on Environment and Children's Health.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA, PFTrDA.	Total allergic diseases and eczema during the first 24 months of life.	High	Significant negative association between exposure to PFTrDA and eczema during the first 24 months of life for female infants only. No association between exposure to PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA and total allergic diseases and eczema during the first 24 months of life.
Steenland et al., 2015 (185)	West Virginia, USA	Cohort study	4,391 workers with ≥1 day of work from 1948−2002 at the DuPont at their West Virginia plant.	PFOA	Asthma with medication, ulcerative colitis and rheumatoid arthritis with and without a 10-year lag.	Moderate	Significant negative association between exposure to PFOA and medicated asthma for no-lag analysis only. Significant positive association between exposure to PFOA and ulcerative colitis and rheumatoid arthritis.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Steenland et al., 2013 (264)	West Virginia, USA	Cohort study	32,254 people who lived or worked in any of six PFOA-contaminated water districts and participated in the C8 Health Project baseline survey in 2005–2006.	PFOA	Ulcerative colitis, lupus, multiple sclerosis, Crohn's disease and rheumatoid arthritis with and without a 10-year lag.	High	Significant positive association between exposure to PFOA and ulcerative colitis. No association between exposure to PFOA and lupus, multiple sclerosis, Crohn's disease and rheumatoid arthritis.
Stein et al., 2016 (256)	New York, USA	Cohort study	78 healthy adult volunteers aged 18−49 years old recruited between October−December 2010 by the Mount Sinai Medical Center in New York. Participants were not eligible to participate if they had received an influenza vaccination in the 2010−2011 season.	PFOS, PFOA, PFNA, PFHxS.	Seroconversion following FluMist influenza vaccination.	Moderate	Significant positive association between PFOA, PFOS and PFNA seroconversion.
Stein et al., 2016 (254)	USA	Case-cohort	1,191 children aged 12−19 years enrolled in the 1999−2000 or 2003−2004 waves of the NHANES study were recruited for the vaccination study. 640 children aged 12−19 years enrolled in the 2005−2006 waves of the NHANES study were recruited for the asthma and allergies study.	PFNA, PFOS, PFOA, PFHxS.	Antibody concentrations against MMR, and cases of asthma and allergies.	High	Higher PFOA and PFNA concentrations were associated with increased total serum IgE, but did not state finding significance level * The pattern of association between PFOA, PFOS, PFNA and PFHxS exposures and current allergic conditions appeared null * the strongest finding, as well as the only statistically significant finding, was of increased PFOA and prevalent rhinitis

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Zhu et al., 2016 (261)	Taiwan	Case-cohort study	231 children aged 10−15 years old diagnosed with asthma were recruited through the GBCA from 2009−2010. 225 non-asthmatic controls.	PFOA, PFOS, PFHxS, PFNA, PFDA, PFBS, PFHxA, PFHpA, PFDoA, PFTEDA.	Asthma diagnosed in the past year.	High	Significant positive association between PFBS, PFHxS, PFOS, PFOA, PFNA and PFDA with asthma for males. Significant positive association between PFHxS, PFOA and PFDA with asthma for females.

Appendix 17: Skeletal

Summary table of skeletal health effects

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Innes et al., 2011 (265)	Ohio and West Virginia, USA	Cross-sectional study	49,432 adults over 21 years old who lived or worked in any of six PFOA-contaminated water districts and participated in the C8 Health Project baseline survey in 2005–2006. Participants were excluded if they had previously been diagnosed with rheumatoid arthritis	PFOA, PFOS.	Osteoarthritis.	High	Significant positive association between PFOA and osteoarthritis. Significant negative association between PFOS and osteoarthritis.
Khalil et al., 2016 (267)	USA	Cross-sectional study	1,914 people aged 12−80 years old who participated in the 2009−2010 waves of the NHANES study.	PFOA, PFOS, PFHxS, PFNA.	Total femur, femoral neck and lumbar spine bone mineral density and osteoporosis.	High	Significant positive association between PFOA, PFHxS and PFNA in women only. Significant negative association between PFNA and lumbar spine bone mineral density in post-menopausal women only. Significant negative association between PFOA and PFOS and femoral neck bone mineral density in women only. Significant negative association between PFNA and femoral neck bone mineral density in men only. Significant negative association between PFOS and total femur bone mineral density in women only.

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Lin et al., 2014 (268)	USA	Cross-sectional study	2,339 aged ≥20 years old who participated in the 2005−2006 and 2007−2008 waves of the NHANES study.	PFOA, PFOS.	Total lumbar and hip bone mineral density and odds of spine, hip and wrist bone fractures.	High	Significant negative association between PFOS and total lumbar spine bone mineral density in women not in menopause. No significant association between PFOA and total lumbar spine bone mineral density. No significant findings of PFAS and hip bone mineral density and spine, hip and wrist bone fractures.
Steenland et al., 2015 (185)	West Virginia, USA	Cohort study	4,391 workers with at least 1 day of work from 1948−2002 at the DuPont at their West Virginia plant.	PFOA	Osteoarthritis with and without a 10-year lag.	High	No association between exposure to PFOA and osteoarthritis.
Uhl et al., 2013 (266)	USA	Cross-sectional study	4,102 people aged 20−84 years old who participated in the 2003−2008 waves of the NHANES study.	PFOA, PFOS.	Osteoarthritis.	High	Significant positive association between PFOA and osteoarthritis in women only.

Appendix 18: Respiratory

Summary table of respiratory health responses

Authors and year	Location	Type of Study	Study population	PFAS Exposure	Disease Outcome	Overall risk of bias	Author Conclusions
Leonard et al., 2008 (221)	West Virginia, USA	Cohort study	6,027 employees from the DuPont chemical plant from 1948−2002.	PFOA	COPD mortality, with subanalysis of bronchitis and emphysema separately.	Moderate	No significant association between PFOA and COPD, bronchitis and emphysema mortality.
Nolan et al., 2010 (85)	Washington County, Ohio, USA	Cross-sectional study	1,548 pregnant mothers residing in Washington County, Ohio from January 1, 2003−September 1, 2005.	PFOA; Estimated maternal exposure.	Lung disease.	High	No significant association between PFOA and lung disease in pregnant women.
Steenland & Woskie 2012 (155)	West Virginia, USA	Cohort study	5,791 employees from the DuPont chemical plant from 1952−2002.	PFOA	COPD mortality.	Moderate	No significant association between PFOA and COPD mortality.