Expert Health Panel

for Per- and Poly-Fluoroalkyl Substances (PFAS)

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APPENDIX ONE – PUBLIC CONSULTATION REPORT

1. EXECUTIVE SUMMARY

1.1. Introduction

Background

Per- and poly-fluoroalkyl substances (PFAS) are a group of man-made chemicals that have been widely used since the 1950s in household and industrial products that resist heat, oil, stains, grease and water. This includes non-stick cookware, food packaging, stain protection applications to fabric, furniture and carpet, and firefighting foams. Since 1970, firefighting foams containing PFAS were used extensively in Australia and elsewhere due to their effectiveness in fighting liquid fuel fires. There are many types of PFAS, with the best-known examples being perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). PFAS have emerged as compounds of environmental interest as they can travel long distances through soil and water and can get into groundwater. These substances do not break down in the environment and can accumulate in animals, including humans.

More recently, PFAS have been found to have contaminated sites where there has been historical use of fire-fighting foams. In Australia, state and territory regulatory authorities have taken action to reduce and provide guidance on the environmental and potential public health risks at sites where there is confirmed contamination with these chemicals.

The Expert Health Panel

There is widespread community concern regarding PFAS exposure across a number of communities around Australian Defence Bases where PFAS chemicals were used. To respond to this, the Expert Health Panel (the Panel) for per- and poly-fluoroalkyl substances (PFAS) was established to advise the Australian Government on the evidence for potential health impacts associated with PFAS exposure. A complementary role was to identify priority areas for the National Health and Medical Research Council's (NHMRC's) Per- and poly-fluoroalkyl substances – National Health Research Program i.e. where further research was most likely to add important evidence.

The members of the Expert Health Panel are:

- Chair: Professor Nick Buckley (University of Sydney);
- Professor Malcolm Sim (Monash University);
- Dr Ki Douglas (Douglas Consulting Australia);
- Professor Helen Håkansson (International Representative, Karolinska Institutet).

Professor Alison Jones (University of Wollongong) was initially part of the Panel but had to withdraw from the Panel in January 2018 due to work commitments. Prof Jones was not involved in the drafting of the final report.

1.2. Methodology

Assessing the evidence

The Panel undertook a comprehensive review of recent literature reviews regarding Australian and international evidence on potential human health effects of PFAS exposure, alongside a public

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consultation. The public consultation process was able to inform the Panel of the communities' concerns regarding PFAS and their health, as well as their views on priorities for future research.

In order to provide final advice by February 2018, the Panel focussed on identifying and reviewing the *latest* systematic reviews of human epidemiological studies and (inter)national authority/intergovernmental/governmental reviews and reports on potential human health effects of PFAS exposure. This challenging timeframe was set to balance the need for well-informed expert advice on the possible effects of PFAS on human health, and the need for timely advice for the NHMRC and affected communities.

The Panel members noted that there were many systematic reviews and many government or expert reviews published or available in the last few years, and a group from the Australian National University (ANU) was already commissioned to undertake a systematic review of epidemiological studies[1]. Thus, building on existing knowledge using these systematic reviews (since 2013) and the most recent key national and international reports (since 2015) was a reasonable and appropriate mechanism to enable the Panel to meet its objectives of examining the scientific evidence within the timeframe. The Panel's review did not generally extend to reviewing the primary studies which had been included in the national and international reports and systematic reviews.

1.3. Summary of evidence for potential health effects

1.3.1. Overview of the problem, the current evidence on health effects and the need for further research

PFAS are a group of multiple related chemicals, some of which accumulate and persist in individuals over many years and also persist in the environment for even longer. The two most relevant to this review are PFOA and PFOS. These are highly persistent and were widely used in Australian fire-fighting foams until phased out around a decade ago.

Exposure is largely via oral ingestion and PFAS accumulate in people due to extremely long elimination half-lifes (many years). There are currently no known practical methods for people to speed up elimination. Decisions have been taken to phase the most persistent PFAS out of use to reduce accumulation. People have been advised to minimise excessive further exposure by not drinking contaminated water sources and consuming foods with high levels of PFAS (e.g. animals caught in certain areas). It is an ongoing important but necessary task for regulators to assess the persistence and mobility in water and lipid environments of PFAS and similar compounds (>3000 in use) and limit exposure to new PFAS compounds until there is good quality evidence that they pose no concerns. It is not practically possible to eliminate all PFAS exposure due to the extremely wide range of sources from which very low exposures may continue to occur.

International evidence shows that the general population typically have measurable PFAS concentrations in their blood, and that people in highly exposed communities (e.g. those living near PFAS manufacturing plants) typically have PFAS concentrations up to tenfold higher than those in the general population (IARC, 2016; Priestly, 2016; RIVM, 2017; FSANZ, 2017). In Australia, available evidence indicates blood concentrations of PFOS are generally higher than for PFOA for the general population (Priestly, 2016). Available evidence indicates fire fighters in Australia may have PFAS concentrations up to 10-fold higher than the general population (Priestly, 2016). Many studies related to overseas manufacturing plants have focussed more on PFOA. International evidence has

[1] This became Kirk et al., 2018.

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shown that workers in these plants often have PFAS concentrations up to 1000-fold higher than the general population (IARC, 2016; Priestly, 2016; RIVM, 2017; FSANZ, 2017).

Although the evidence on health effects associated with PFAS exposure is limited, the current reviews of health and scientific research provide fairly consistent reports of associations with several health outcomes, in particular: increased cholesterol, increased uric acid, reduced kidney function, altered markers of immunological response, levels of thyroid and sex hormone levels, later menarche and earlier menopause, and lower birth weight. Differences between those with the highest and lowest exposures are generally small, with the highest groups generally still being within the normal ranges for the whole population. There is mostly limited or no evidence for an association with human disease accompanying these observed differences. There is no current evidence that supports a large impact on an individual's health. In particular, there is no current evidence that suggests an increase in overall cancer risk. The main concerning signal for life-threatening human disease is an association with an increased risk of two uncommon cancers (testicular and kidney). These associations in one cohort were possibly due to chance and have yet to be confirmed in other studies. However, because the evidence is very weak and inconsistent in many respects, some degree of important health effects for individuals exposed to PFAS cannot be ruled out based on the current evidence.

The published evidence is mostly based on studies in just seven cohorts (see Kirk et al. 2018, page 15-16). These cohorts have generated hundreds of publications but there is a high risk that bias or confounding is affecting most of the results reported. There are very large numbers of comparisons being done in many studies, such that the risk of random variation in exposures and outcomes being interpreted as real associations is greatly increased. This is compounded by the fact that there are multiple PFAS, and other environmental or occupational hazards, so that there may be interacting toxic effects, and it is hard to isolate the association with one or two analysed compounds. Many of the biochemical and disease associations may be explainable by confounding or reverse causation (see Section 6.15). Many studies had limited power to detect important associations.

Our advice to the Minister in regards to public health is that the evidence does not support any specific biochemical or disease screening, or health interventions, for highly exposed groups (except for research purposes). Decisions to regulate or avoid specific PFAS chemicals should continue to be largely based on evidence of persistence and accumulation; they should not need to also be justified by strong evidence of adverse health effects.

1.3.2. Research priorities

The community consultation highlighted a great many concerns about PFAS exposure and several health effects; respondents were largely from those in highly exposed communities and fire-fighters. Cancer risk and risks for children and firefighters stood out as areas of very great concern but it was clear there were many potential concerns across the health spectrum. Detailed guidance on research considerations and priorities are included at the end of the sections on each health effect as part of Section 6, but there are some general comments that can be made about research priorities:

- Longitudinal studies are needed rather than cross-sectional studies to reduce the risk of bias and confounding. The best value for money for increasing the evidence for many conditions will be adding PFAS exposure analysis to existing large cohort studies (e.g. existing cohorts studying pregnancy or early life or long-term health or multiple environmental exposures or fire fighters).

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- Australia is well placed to undertake good whole-of-population studies of exposed communities/workers, due to the very high capture of linkable 'big data' on health (e.g. cancer registries, PBS/MBS data, ABS data, electronic medical records, etc.). Such studies would avoid selection biases affecting many cohort studies, and also directly address concerns of communities and firefighters that their health may be affected by PFAS.
- Better understanding of mechanisms of PFAS kinetics in humans would also be extremely useful across a range of studies. This might include longitudinal biomonitoring, but also might identify means to rapidly increase elimination which would allow for before-after design studies across many outcomes within short time frames.
- The mechanisms for toxicity and the doses at which toxicity occurs are not well defined, but animal evidence indicates PFAS can alter metabolism and gene expression in many ways via interactions with a range of nuclear receptors. Exposure is usually quantified based on the concentration of one or more compounds at one point in time. Better biomarkers of the 'net effect' of all PFAS would be extremely useful. Human-derived experimental models (i.e. human cell cultures) might be a useful adjunct to human studies. Breaking down the link from molecular mechanisms to human disease into a series of causal steps potentially allows use of a wider range of mechanistic data and facilitates complementary use of human and animal toxicology data.
- Involvement of representative(s) of the exposed occupational group and/or community in study advisory committees for future PFAS research could help to avoid perceptions of lack of fairness, transparency and control, and improve hazard and risk communication.

1.3.3. Cancer

The Expert Health Panel considered the findings and conclusions of five published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and three systematic reviews since 2014 that analysed the human epidemiological evidence regarding exposure to PFAS and cancer.

Summary of findings

With regards to the evidence of exposure to PFAS and cancer, there are:

- small numbers of studies on PFAS and cancer in manufacturing workers and communities near these manufacturing plants;
- small numbers of cancers in many studies;
- low methodological quality and high risk of bias with many studies;
- lack of consideration of important confounders;
- multiple comparisons; and
- a lack of consistency in findings between studies.

The occupational studies relate to manufacturing workers, not end users such as firefighters who are the major group of workers at risk of occupational exposure in Australia.

The suggestive evidence, although still limited, relates to two types of cancer: kidney and testicular, both uncommon tumours. Very limited evidence relates to bladder and prostate cancer and there is no suggestive or convincing evidence for any other types of cancer.

The limited amount of evidence which is available relates to PFOA and not PFOS. Findings in animal studies about tumour induction in rodents by PFOS and PFOA may not be relevant to humans.

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Advice to the Minister

The evidence does not support PFAS being a major contributor to cancer burden in workers or exposed community populations.

The evidence on cancer risk is limited, but it is possible there is increased risk of some uncommon cancers, such as kidney and testis. The limited evidence relates to PFOA, not PFOS.

Given the high concern about cancer-risk among both occupational groups, such as firefighters, and those members of the community in contaminated areas during the consultation, and the limitations of the available evidence, future research into cancer is a priority (see below). Better designed cohort studies in exposed workers, such as firefighters, and communities in contaminated areas, especially with improved exposure assessment could lead to stronger conclusions.

Research priorities

Large collaborative cohort studies are required to examine cancer associations in exposed Australian workers and community populations in exposed areas. Further studies into the relatively uncommon cancers – kidney and testes – are most indicated, based on the limited evidence in previous studies. Studies need to be adequately powered, ideally supported by some quantitative exposure data (e.g. blood concentrations), covering the majority of exposed populations, involve access to complete cancer registry and death notifications from the region and also include access to data on possible confounders.

There is also a priority for future research into cancer to investigate PFOS, rather than PFOA, because PFOS is the most highly detected PFAS in Australia, and the best previous research focussed on PFOA.

Previous studies have often been at high risk of bias due to low cohort numbers, very limited exposure data, unadjusted multiple comparisons, lack of data on confounders or effect modifiers (e.g. smoking) and selection, recall and survivor biases. Further studies subject to these same biases are unlikely to add useful evidence.

Research in specific occupational groups (e.g. firefighters) will also have to deal with confounding by the many other potentially carcinogenic chemicals that these groups are exposed to. This is also the case with general population cohort studies, where account needs to be taken of work exposures for cohort members. This can be more challenging in population cohort studies, due to the greater diversity of jobs undertaken and relevant exposures in those jobs.

1.3.4. Metabolic biomarkers: Concentrations of cholesterol and triglycerides in the blood

The Panel considered the findings and conclusions of six international authority/intergovernmental/governmental reports published between 2015 and 2017 and four systematic reviews and literature reviews since 2013, that reported on exposure to PFAS and any associations with blood cholesterol and lipid concentrations.

Summary of findings

Many studies highlighted that although there was a small statistical association between PFOA and total cholesterol levels, this is unlikely to represent important differences for individual people. However, these findings might still have some relevance for PFAS risk assessment for regulating general population exposures.

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The association of PFAS with total cholesterol does not have an established causal mechanism. One point to note is that PFAS do interact with PPAR receptors and these are involved in lipid regulation. Drugs that are PPAR-α[2] agonists (e.g. fibrates) generally lower total cholesterol; PPAR-γ agonists (glitazones) increase total cholesterol.

The current evidence is largely from cross-sectional studies, which is generally a weak study design, and stronger evidence would come from future cohort studies. Note that animal studies, including some primate studies, have found decreases in serum cholesterol levels which is the opposite effect to that observed in humans.

Advice to the Minister

An association of PFAS with cholesterol, but not other lipids, is generally observed but it is of small magnitude, although there is an exposure-response relationship. Evidence to date does not establish whether or not PFAS causes higher cholesterol, due to weak studies, inconsistencies with animal studies, limited adjustment for confounders, the possibility of reverse causation and a lack of any clear causative mechanism.

Due to the small association found and the other limitations noted above, the existing scientific evidence does not warrant any change to peoples' medical management or risk assessment for heart disease. In the clinic, established risk factors for high cholesterol and/or heart disease such as diabetes, diet, smoking, alcohol, blood pressure and kidney disease are usually of a much greater magnitude than seen in studies on PFAS.

Research priorities

Studies that look for causal evidence are the key research need. Further cross-sectional studies are unlikely to provide this information, but well-designed longitudinal studies may provide stronger epidemiological evidence. Relevant studies would (for example) investigate direct evidence for activation of causal biochemical mechanism(s) in humans, or determine whether reducing PFAS concentrations in individuals alters cholesterol measurements.

1.3.5. Liver function

The Panel considered the findings and conclusions of five published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and two systematic reviews since 2016 that analysed the human epidemiological evidence regarding exposure to PFAS and liver function.

Summary of findings

An association of PFAS with elevated levels of the liver enzyme ALT[3] was observed in many studies. This was generally of small magnitude, is not considered biologically significant and no link to clinically important liver disease was noted. Evidence to date does not establish whether or not PFAS causes a high alanine aminotransferase (ALT), and it is possible this reflects confounding by other factors.

The scientific evidence does not support an association between PFAS and specific liver conditions, such as hepatitis, cirrhosis or fatty liver.

[2] Peroxisome proliferator-activated receptors.

[3] Alanine transaminase.

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The liver is a target organ for PFAS toxicity in high dose animal toxicity studies, where hepatic steatosis (fatty liver) is observed. It is also a key organ for metabolic regulation relevant to PPAR and other nuclear receptors. It is unclear if these are activated at concentrations relevant to Australian exposures.

Advice to the Minister

There are small but inconsistent associations of LFTS and PFAS in some studies. Current standard medical tests for liver damage and function in Australians frequently show minor abnormalities such as those associated with PFAS. These can be due to underlying disease (e.g. chronic hepatitis, alcoholic liver disease, viral diseases), medications, herbal supplements and obesity, or just be a transient and reversible abnormality.

No routine medical monitoring of liver function for residents or others exposed to PFAS is required on the basis of current evidence.

Research priorities

Studies that look for causal evidence are the key research need. Further cross-sectional studies are unlikely to provide useful information. Well-designed longitudinal studies which take into account confounders (chronic hepatitis, alcoholic liver disease, viral diseases, medications, herbal supplements and obesity) may provide stronger epidemiological evidence to indicate whether long-term alteration of metabolism occurs and increases the risk of clinically important liver disease (e.g. hepatic steatosis and subsequent fibrosis). Relevant studies would also explore measurement of activation of biochemical mechanisms that disturb liver metabolism, especially those pathways relevant to lipids and cholesterol.

1.3.6. Kidney function

The Panel considered the findings and conclusions of five published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and four systematic reviews since 2013 that analysed the human epidemiological evidence regarding exposure to PFAS and kidney function, uric acid and kidney disease.

Summary of findings

There is a clear link to kidney function with consistently shown associations between PFAS and uric acid/kidney function in key reports and reviews. However, there is not strong support for a link between PFAS exposure and kidney pathology; albeit one study linked deaths from kidney disease to estimated high occupational exposures (which may have been due to confounding by other potentially toxic chemicals).

All associations could be influenced by reverse causation, as it is well known that most PFAS are eliminated by the kidney. Reduced kidney function would cause an increase in both serum uric acid and PFAS.

Advice to the Minister

An association of PFAS with impaired kidney function and higher serum uric acid is consistently shown. However, it has not been demonstrated that PFAS causes these problems or indeed is linked to human disease; people with kidney disease are expected to have impaired elimination of PFAS and thus higher levels.

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Research priorities

This will be a difficult area for researchers to propose and conduct rigorous study designs addressing causal relationships. To reduce the problem created by potential reverse causation, long-term prospective studies (not cross-sectional studies) are required, e.g. people with low and high PFAS levels with baseline normal kidney function followed over time to examine the progress of kidney function. Even these study designs might be subject to confounding due to unknown factors affecting both PFAS clearance and rate of decline in kidney function.

Kidney tissue concentrations would be expected to be higher than concentrations in most tissues due to active reuptake of filtered PFAS, thus it could be selectively causing kidney injury. Studies on mechanisms of kidney PFAS elimination and potential for damage might be useful, and these could potentially use human renal cell cultures.

Research on kidney elimination and kidney disorders might best be nested into broader studies examining mechanisms or long-term health respectively.

1.3.7. Thyroid effects

The Panel considered the findings and conclusions of five published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and five systematic reviews since 2013 that analysed the human epidemiological evidence regarding exposure to PFAS and thyroid effects.

Summary of findings

There are no consistent associations between any particular PFAS and thyroid hormones. In those studies where small associations were found, the pattern of changes in levels of the different hormones was not consistent and there were often differences within the normal range, which is of uncertain clinical significance. This applied to infants, children and adults.

For thyroid disease, there is limited evidence of an association between PFOA in women (in whom thyroid disease is much more common), but not in men.

Studies of workers involved in the manufacture of PFAS, for whom exposure levels were considerably higher than community members in population studies, were largely negative for thyroid function and thyroid disease.

If there are any causal associations, it is difficult to disentangle which PFAS is likely to be involved because of high correlations between the different exposures. Reverse causation may also be an alternative explanation.

Potential thyroid effects were not a major concern among those who responded to the community consultation.

Advice to the Minister

PFAS exposure is unlikely to be a major contributor to the burden of thyroid dysfunction or disease in the community among infants, children or adults.

Research priorities

If further studies of thyroid function and thyroid disease are to be undertaken, these would best be nested into longitudinal studies of a range of health effects and focus on groups where alterations in thyroid function would be most critical (e.g. pregnancy and early childhood).

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Studies that explored the potential causal mechanisms of associations would also be useful e.g. whether thyroid function changes PFAS elimination or whether PFAS affect thyroid hormone-related transcription.

1.3.8. Neonatal, infant and maternal outcomes from exposure during pregnancy

The Panel considered the findings and conclusions of five key international authority/intergovernmental/governmental reports published between 2015 and 2017 and six systematic reviews from 2014 that reported on exposure to PFAS during pregnancy.

Summary of findings

There are several studies on PFAS exposure associated with pregnancy, prenatal and birth outcomes, as well as infant growth. These studies are mainly cross-sectional and based on small-to-intermediate population sizes in just a few study populations. From the limited evidence available, current data on pregnancy, prenatal and birth outcomes and infant growth suggest that significant associations with increased PFAS exposure relate to small changes in end-points such as pregnancy-induced hypertension and pre-eclampsia, weight and length at birth, as well as infant growth.

However, the evidence is very limited. One major limitation is the lack of mechanistic data explaining if/how PFAS might impact on pregnancy, prenatal development and infant growth processes. Further, existing mechanistic evidence is mainly based on experimental data from cell and animal models. There is minimal human evidence linking pregnancy and/or developmental outcomes associated with PFAS exposure to demonstrable effects of PFAS on human cell biology and physiology.

Advice to the Minister

Current evidence does not support PFAS being a major cause of pregnancy-induced hypertension/ pre-eclampsia or other complications. PFAS exposure in fetal life was often associated with lower weight and length at birth in general population studies. However, these decreases in birth weight and length were mostly small and within the normal range. There was also an association with slightly slower infant growth.

The major concern about PFOA/PFOS exposure in pregnancy would be these effects at general population exposures. However, there are many other PFAS and environmental pollutants that warrant surveillance in the general population. A strategy to provide PFAS research that also supports ongoing human biomonitoring of early life exposures would be the most useful way to contribute to prevention and assessment activities by public health researchers and regulators.

Research priorities

Pregnancy, prenatal and birth outcomes and infant growth measurements associated with PFAS exposure were of high concern to those who responded in the public consultation, who generally expressed strong support for "*research into the potential health effects of PFAS exposure on vulnerable populations such as pregnant women, babies, young children and the elderly*".

Large longitudinal studies are required to provide better data on associations between PFAS and pregnancy, prenatal, birth and infant outcomes. Access to existing birth cohorts would be the most efficient way to undertake such studies. Studies need to be adequately powered and ideally supported by quantitative exposure data (e.g. blood concentrations) as well as relevant effect biomarkers. Access to disease registers, as well as registers which monitor weight/growth/length

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parameters at birth, during childhood and into young adult age, can form the basis for well-designed studies.

It is most likely that if PFAS exposure causes pregnancy, prenatal, birth and infant outcomes, this would be due to altered endocrine function and/or metabolic changes rather than direct effects on all cells. Therefore, this research should include analyses of hormones relevant to reproductive and developmental/growth processes.

As all individuals are exposed to multiple other chemicals, it would be best value to include PFAS measurement in studies that include assessment of other persistent chemicals and other environmental factors affecting normal pregnancy (e.g. smoking, alcohol).

1.3.9. Reproductive outcomes

The Panel considered the findings and conclusions of three key international authority/intergovernmental/governmental reports published between 2015 and 2016 and four systematic reviews from 2013 onwards that reported on exposure to PFAS and reproductive effects.

Summary of findings

There is very little animal evidence referred to by the reviews to support that PFAS may alter endocrine function at concentrations found in humans with environmental and occupational exposures.

There are many human studies on PFAS and reproductive effects, with most studies examining multiple biomarkers and clinical end points and multiple chemical exposures, with often a post-hoc analysis of observed associations. There is thus a substantial risk that many findings are due to bias or chance. This is reflected in the lack of consistency in the findings of studies. The reviews are not generally in direct conflict, although often highlight different measures that might be worth pursuing further.

There is a strong potential for 'reverse causation' in associations with late menarche and early menopause, as menstrual blood loss and female sex hormones might both increase elimination of PFAS (thus the absence of these would be associated with higher levels).

There is strong potential for confounding by other persistent organic pollutants with endocrine effects in studies in the general population (which is where many of these studies have found associations). There is also potential for confounding by many other factors e.g. BMI[4] and age.

Overall the human evidence is weak for a link between PFAS and clinically important reproductive effects. The reviews conclude the strongest evidence of an association is for delayed puberty and reduced sperm quality but these are of unclear significance and quite likely confounded.

The human dose-response threshold for these potential effects is very poorly characterised; the majority of studies have been with background population levels rather than highly exposed individuals.

Advice to the Minister

It is feasible that PFAS have effects on human reproduction and reproductive hormones. However, despite several studies and reviews, the rationale and evidence is deficient in most respects. Studies have generally compared average values or out-of-range values in those with higher or lower measured PFAS. While this approach works for some outcomes where it is clear what is 'normal' and

[4] Body Mass Index.

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desirable, studies of human reproductive function are more difficult to do well. This is an extremely complex and variable area of human biology and people's reproductive capacity is expected to vary greatly over time due to many other factors (e.g. age, diet, alcohol consumption, contraceptive use and obesity). Further, interpretation of laboratory results often requires both knowledge of the reproductive stage of the individual and simultaneous interpretation of several tests, to determine what is abnormal and important and what might be contributing to them. This applies in research as well as for individuals seeking specialist medical treatment.

Fertility issues were highlighted by a small number of respondents to the public consultation.

Research priorities

Studies of the effects of PFAS on reproductive health seem likely to provide useful information only if done on existing well-characterised longitudinal cohorts that are examining clinical outcomes (e.g. measuring PFAS in stored samples and whether these affected later fertility). The need for a specific reproductive cohort is that there are many potentially important factors and confounders that are unlikely to be recorded well even in general health records (e.g BMI, smoking, contraceptive use, sexual history, etc) and interpretation of laboratory tests often requires clinical analysis. The best value would come from adding this to an existing cohort, because setting a study up from scratch would take a long time and be very expensive, and the evidence to date implicating PFAS is not compelling.

Cross-sectional studies of multiple reproductive biomarkers have been done many times and further studies are likely to be largely unhelpful, unless they are combined with a method of rapidly eliminating PFAS so that a before-after design can be used to provide evidence for causal mechanisms.

1.3.10. Immunological effects

The Panel considered the findings and conclusions of seven published key (inter)national authority/intergovernmental/ governmental reports ('key national and international reports') published between 2015 and 2017 and four systematic reviews since 2016 that analysed the human epidemiological evidence regarding exposure to PFAS and immune function.

Summary of findings

There are few human studies on PFAS and immunological effects, with studies examining multiple immune biomarkers and clinical end points and multiple chemical exposures, often with a post-hoc analysis of observed associations. There is thus a substantial risk that many findings are due to bias or chance. This is reflected in the lack of consistency in the findings of studies, which in turn has led to the very diverse conclusions of the reviews summarised above. In addition, there is strong potential for confounding by other persistent organic pollutants with immune effects in studies in the general population (which is where many of these studies have found associations).

Inflammatory and immune disease also alter transporter expression, and thus it is feasible that inflammatory disease could cause reduced elimination of PFAS (i.e. reverse causation).

The strongest evidence for a link between PFAS and clinically-important immunological effects is for impaired vaccine response. However, the human dose-response threshold for potential immune effects is very poorly characterised, and the overall human evidence is weak.

However, there is animal evidence that PFAS may alter immune function at concentrations found in humans with environmental and occupational exposures.

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Advice to the Minister

PFAS are likely to alter the function of the immune system. However, it is unclear if this occurs at current exposures or has any clinically important consequences. In particular there is no consistent evidence for increased risk of infections or auto-immune disease.

Impaired vaccine response is the most consistent reported association. Internationally, most studies that have observed decreased antibody levels have not found significant increases in incidence of human disease or associations of higher blood levels of PFAS with infectious disease. However, they were generally very underpowered to detect important differences in disease incidence (given the rarity of many of these diseases).

Research priorities

Measuring vaccine response is a strong candidate for further studies as it has the advantage of prospective (post-exposure) design, and objective outcomes.

Studies of infections or auto-immune disease would be best nested within a very large study of overall health outcomes (ideally supported by data linkage to avoid recall biases).

Cross-sectional studies of multiple immune biomarkers have been done many times and further studies are likely to be largely unhelpful, unless they are combined with a method of rapidly eliminating PFAS so that a before-after design can be used to provide evidence for causal mechanisms.

1.3.11. Neurodevelopmental and neurophysiological effects

The Panel considered the findings and conclusions of three international authority/intergovernmental/governmental reports published in 2015 and 2016 and five systematic reviews from 2013 onwards that reported on exposure to PFAS and neurodevelopmental and neurophysiological effects.

Summary of findings

The area of neurodevelopment is difficult to study. There are no biomarkers (as for cholesterol). There is inconsistency in definitions and diagnostic criteria for conditions such as autism and ADHD[5]. Some studies had insufficient participants making it difficult to draw statistically valid conclusions; others relied on parental report of behaviour and diagnosis. Additionally, there is no established causal mechanism for PFAS to have an effect on neurodevelopment.

Advice to the Minister

An association with PFAS and neurodevelopmental and neurobehavioural outcomes in infants and children is not consistently observed. There are many other significant influences on infant and child development including maternal alcohol, drug and medication intake, maternal smoking, socioeconomic status, parental education level, and heavy metal exposure (e.g. lead).

Four respondents in the community submissions process identified autism (3) and ADHD (1) as a health concern.

Research priorities

Studies that provide causal evidence are the key research need. Further, cross-sectional studies are unlikely to provide useful information. Well-designed longitudinal studies which take account of

[5] Attention-deficit/hyperactivity disorder.

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confounders (alcohol, drug and medication intake, smoking, socioeconomic status, parental education level, heavy metals including lead) may provide stronger epidemiological evidence that might indicate whether PFAS affects neurological development. Any measurement of neurodevelopment should be undertaken by trained examiners using a validated assessment instrument. Such studies are expensive, and thus this means the best value for money would be to add PFAS blood sampling to other prospective birth cohort/neurodevelopment studies that are being undertaken or planned.

1.3.12. Diabetes, glycaemic control and metabolic syndromes

The Panel considered the findings and conclusions of four published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and four systematic reviews since 2013 that analysed the human epidemiological evidence regarding exposure to PFAS and diabetes, glycaemic control and metabolic syndromes.

Summary of findings

Epidemiological studies do not generally document consistent associations between PFAS and diabetes, glucose metabolism or metabolic syndrome. One of the two studies of gestational diabetes found an association. An association of PFOA concentration with increased diabetes mortality, but not diabetes incidence, was found in one study of workers. However, there was no relationship with estimated exposure to PFAS, or increased risk over the general population.

There are inconsistent associations in some selected populations, mostly based on weak study designs. Any associations in cross-sectional studies may be due to reverse causation or confounding with other conditions, such as kidney function.

Any association of PFAS with diabetes does not have an established causal mechanism. PFAS interact with PPAR receptors which leads to multiple metabolic changes, but PPAR agonist drugs generally improve glucose control.

Diabetes was not specifically raised as a concern in the community consultation.

Advice to the Minister

Consistent associations of PFAS with diabetes or metabolic syndrome have not generally been observed. The most concerning signals are for diabetes mortality (but not diabetes incidence) and gestational diabetes, but these might be explained by confounding by kidney function. The known biological effects of PFAS on metabolism do not suggest this is a likely effect of PFAS.

Research priorities

Studies on diabetes risk would best be combined with other studies of overall health effects in exposed workers or communities or pregnant women. Conversely, any studies of cholesterol, kidney, weight gain, and cardiovascular disease should include a consideration of interactions with diabetes and hyperglycemia.

Studies that look for causal evidence might also be useful. Relevant studies would (for example) investigate direct evidence for activation of causal biochemical mechanism(s) in humans, or investigate whether reducing PFAS concentrations in individuals alters glucose metabolism.

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1.3.13. Obesity, overweight and BMI

The Panel considered the findings and conclusions of four published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2016 and four systematic reviews since 2013 that analysed the human epidemiological evidence regarding exposure to PFAS and obesity and BMI.

Summary of findings

There were some inconsistent associations between PFAS and obesity in various age groups, but any associations found related to very small increases and these are unlikely to represent important differences at a clinical or population level. There was little consistent evidence for associations with PFOS or other PFAS.

Any association of PFAS with obesity does not have an established causal mechanism. However, PFAS do interact with PPAR receptors and these are involved in energy regulation; PPARγ agonists used in diabetes (rosiglitazone and pioglitazone) cause weight gain.

The current evidence is largely from cross-sectional studies, which is generally a weak study design, and stronger evidence would come from future cohort studies with standardised measures and those that could demonstrate a causal mechanism (to exclude confounding and reverse causation).

Obesity and weight gain were not a concern of those exposed to PFAS who responded in the public consultation (although cardiovascular diseases that might be affected by weight gain were a concern).

Advice to the Minister

An association of PFAS with excessive weight gain has been observed in some studies, but the relationship is conflicting across studies and poorly characterised. Evidence to date does not establish whether or not PFAS exposure is causally related to increased weight gain in any age group, but if there is a causal link, then any weight gain is likely to be small. Study limitations, such as weak study designs, limited adjustment for confounders, inconsistent measures, the possibility of reverse causation, and lack of any measured causative mechanism, hinder firm conclusions to be drawn.

Due to the limitations noted above, the existing scientific evidence does not warrant any change in obesity prevention programs or to peoples' medical management for obesity or related disorders. Established risk factors for obesity, such as poor diet, excessive alcohol, some prescription medications, and lack of exercise, are likely to be of a much greater magnitude than those potentially caused by PFAS.

Research priorities

Studies that look for causal evidence are the key research need. Further cross-sectional studies are unlikely to provide this information, but well-designed longitudinal studies in occupational groups or highly exposed community groups may provide stronger epidemiological evidence. Relevant studies would (for example) investigate direct evidence for activation of causal biochemical mechanism(s) in humans, or determine whether reducing PFAS concentrations in individuals alters weight or adipose tissue distribution.

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1.3.14. Cardiovascular effects

The Panel considered the findings and conclusions of two published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published in 2015 and 2016 and three systematic reviews since 2016 that analysed the human epidemiological evidence regarding exposure to PFAS and cardiovascular effects.

Summary of findings

Epidemiological studies do not generally document associations between PFAS and cardiovascular diseases. There are inconsistent associations, mostly based on weak study designs, with various cardiovascular risk factors (i.e. lipids, weight, hypertension). The association of PFAS with cardiovascular disease does not have an established causal mechanism. However, PFAS do interact with PPAR receptors and one potent PPARγ agonist used in diabetes (rosiglitazone) has been linked to heart failure and ischaemic heart disease. This could be a potential biological mechanism for increasing the risk of cardiovascular disease. Alternatively, the lack of a consistent association may be due to a small effect being swamped by the wide variation in intake of naturally occurring PPARγ modulators in foods.

Several studies investigated the link between PFAS and hypertension, based on self-report of hypertension or taking medication. When actual blood pressure was measured in children, there was no association with hypertension and exposure to PFOS or PFOA.

The current evidence for cardiovascular disease risks is limited, and based on studies of mortality and cross-sectional self-reported health in PFAS exposed workers and in residents exposed to PFAS in drinking water. Changed risk factors for heart disease may take decades to manifest as disease, and stronger evidence would come from very long-term cohort studies and those that could demonstrate causal mechanisms (to exclude confounding and reverse causation).

Cardiovascular disease, often linked to cholesterol, was a common concern of those exposed to PFAS who responded in the public consultation.

Advice to the Minister

Associations of PFAS with cardiovascular disease have not generally been observed but the relationship is poorly characterised. The known biological effects of PFAS on metabolism suggest this should be the primary concern from excessive exposure in adults. As noted in other sections of this report, there are consistent associations with biomarkers linked to cardiovascular disease (e.g. uric acid, cholesterol, kidney function).

Evidence to date does not establish whether PFAS at exposure levels seen in Australia might increase risks of cardiovascular disease, due to weak study designs, limited adjustment for confounders, the possibility of reverse causation, and a lack of any measured causative mechanism.

Due to the small number of studies and limitations noted above, the existing scientific evidence does not warrant any change to peoples' medical management. Established risk factors for cardiovascular disease such as smoking, poor diet, excessive alcohol, diabetes, some prescription medications, and lack of exercise are likely to be of a much greater magnitude than those potentially caused by PFAS.

Research priorities

Further cross-sectional studies are unlikely to provide useful information, but well-designed long-term cohort studies may provide stronger epidemiological evidence.

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Studies that look for causal evidence are a key research need. Relevant studies would (for example) investigate direct evidence for PFAS concentrations that activate potential causal biochemical mechanism(s) in humans (e.g. PPAR activation), or determine whether as PFAS concentrations in individuals reduce, biomarkers associated with cardiovascular risk also decrease (e.g. cholesterol, weight, insulin resistance and blood pressure).

1.3.15. Respiratory effects

The Panel considered the findings and conclusions of one published key (inter)national authority/intergovernmental/governmental report ('key national and international report') published in 2015 and one systematic review published in 2018 that analysed the human epidemiological evidence regarding exposure to PFAS and respiratory effects.

Summary of findings

There is no known direct effect of PFAS on the lungs, but effects through other pathways, such as altered immune function, may be possible. There is very limited research and none supports any associations.

The public consultation indicated respiratory effects were not a common concern of those who participated.

Advice to the Minister

An association with respiratory effects has not been demonstrated in human studies, and there is no known biological mechanism. As the main exposure pathway is through ingestion, research into respiratory disease is not considered a high priority for research.

Research priorities

Specific research on respiratory effects is not a high priority, and any research on respiratory effects should be done as part of a global health assessment, e.g. analysing whether elimination of PFAS alters biomarkers of immune function including those relevant to the respiratory system.

1.3.16. Skeletal effects

The Panel considered the findings and conclusions of one published key (inter)national authority/intergovernmental/governmental report ('key national and international report') published in 2015 and two systematic reviews since 2016 that analysed the human epidemiological evidence regarding exposure to PFAS and skeletal effects.

Summary of findings

There is a small number of cross-sectional studies on skeletal effects and PFAS exposure in a few adult study populations. Current data suggest that the limited evidence of significant associations relates to small changes in end points such as osteoarthritis, osteoporosis/bone mineral density. The small amount of evidence which is available relates to associations with PFOA, PFOS, PFHxS or PFNA exposure.

Skeletal and rheumatological effects were not a concern of those exposed to PFAS who responded in the public consultation.

Advice to the Minister

The evidence does not support PFAS being a major cause of skeletal or rheumatological diseases in highly-exposed communities, and nor was it a concern noted in the public consultation.

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Research priorities

Specific research on skeletal effects is not considered to be a high priority. Effects on bone growth would be best integrated within other studies of PFAS and childhood development, e.g. include measures of weight/growth/length from birth through childhood and into young adult age. This would be complemented by analyses of hormone levels relevant to bone formation (e.g. growth, thyroid and sex hormones). Rheumatological diseases would be best integrated with studies of overall health and/or immune function.

1.4. Limitations

Multiple limitations and issues with the human epidemiology literature were highlighted by the key (inter)national reports and systematic reviews. Limitations of the studies included study design, particularly the large number of cross-sectional studies whereby cause and effect cannot be substantiated, exposure to multiple PFAS, the small number of studies available on some health effects, issues with statistical analysis (such as multiple comparisons), confounding (whereby something else other than PFAS may be influencing the findings), the possibility of conflict of interest in studies funded by PFAS manufacturers, response issues and selection, recall and reporting biases.

1.5. Key findings from public consultation

The purpose of the consultation process was to allow the public the opportunity to provide information to the Panel on their health concerns regarding PFAS exposure and contamination, the exposure pathways that concern them, and the extent to which they feel they have been informed on various aspects of PFAS contamination. The submission form also allowed the public to express their views on which areas of human health research relating to PFAS they felt should be prioritised as part of the Australian Government's further research into the potential health effects of PFAS exposure.

The public's views on the various health effects have already been commented on under a number of the 14 health effects above. The public consultation also showed that:

- There is concern from the public, many of whom feel that PFAS exposure has already affected their health, and it may affect their health in the future.
- Overall, respondents indicated that past exposure to PFAS, occupational exposure to PFAS especially in firefighters, and skin contact with PFAS were the most concerning exposure pathways to them.
- Over half of respondents felt "*not at all informed*" or "*not informed*" about the Government's response to addressing health concerns of communities exposed to PFAS. Conversely, only 21% of respondents reported feeling "informed" or "*very informed*" about the Government's response.
- When asked about their views on what research on PFAS exposure should be prioritised, respondents reported that research on the health effects of occupational exposure to PFAS, in particular among firefighters, should be prioritised, along with further research into potential health impacts on communities that have experienced high exposure to PFAS due to contamination.

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- Thirty-one of the 109 respondents who commented on other areas of human health research they want prioritised, commented on a need for blood testing for those who have been exposed through their work or who live in or near an investigations site.

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2. INTRODUCTION

2.1. Background

The Expert Health Panel (the Panel) for per- and poly-fluoroalkyl substances (PFAS) was established to advise the Australian Government on the potential health impacts associated with PFAS exposure and to identify priority areas for further research to inform the National Health and Medical Research Council's (NHMRC's) Per- and poly-fluoroalkyl substances – National Health Research Program.

The Panel members are:

- Chair: Professor Nick Buckley (University of Sydney);
- Professor Malcolm Sim (Monash University);
- Dr Ki Douglas (Douglas Consulting Australia);
- Professor Helen Håkansson (International Representative, Karolinska Institutet).

Professor Alison Jones (University of Wollongong) was initially part of the Panel but had to withdraw from the Panel in January 2018 due to work commitments. Prof Jones was not involved in the drafting of the final report.

The Panel convened in October and December 2017, and early February 2018.

The Australian Government requested the Panel's advice be informed by:

- taking into account the recent evidence available from both Australian and international scientific research into the potential human health effects of PFAS exposure; and
- considering the views of the public and other stakeholders via a public submissions process which was open between 1 – 19 November 2017.

The Panel has been supported by the secretariat services and technical drafting services of *Allen + Clarke.*

2.2. Purpose of the report

The purpose of the Report is to provide the Minister of Health with the Panel's assessment of:

- findings of recent reviews regarding Australian and international evidence on potential human health effects of PFAS exposure;
- future research needs related to PFAS exposure and its potential impacts on health.

This Report also contains the findings from the public consultation that was targeted at Australians who were concerned about PFAS to gather information on how they perceived PFAS affected their health, and what they thought research priorities should be.

The Report also provides an overview of the methodology used by the Panel to inform its findings and advice (including its approach to the review of recent systematic reviews and key reports and the public consultation process).

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3. METHODOLOGY

3.1. Review of reviews and reports

At the first PFAS Expert Health Panel meeting, the Panel set the Terms of Reference for the literature search to inform the review of scientific research on potential human health effects of PFAS.

In order to provide final advice by March 2018, the Panel focussed on identifying and reviewing the *latest* systematic reviews of human epidemiological studies and (inter)national authority/ intergovernmental/governmental reviews and reports on potential human health effects of PFAS exposure. This challenging timeframe was set to balance the need for well-informed expert advice on the possible effects of PFAS on human health, and the need for timely advice for affected communities.

The Panel members agreed that, due to the many systematic reviews having been published and with several reviews published or available in 2017, building on existing knowledge using these systematic reviews and the most recent key national and international reports (since 2013) was a reasonable and appropriate mechanism to enable the Panel to meet its objectives of examining the scientific evidence within the timeframe.

The search terms used by the Australian National University in their PFAS Health Study Systematic Review of the Literature (2018), was used as the basis for the search for systematic reviews. The search terms included literature on a wide range of health effects among adults and children. New Zealand's Massey University's library conducted the search of the published literature for relevant reviews and reports. The results of the search were used to check that all systematic reviews published within the timeframe agreed in the Terms of Reference for this review had been identified. Two additional reviews of relevance were identified in the Massey University library search and included in the literature review (Negri et al, 2017; Saikat et al, 2013).

The titles and abstracts of the identified systematic reviews were reviewed and considered against the inclusion criteria. A search of the grey literature was also undertaken in order to identify the latest international authority and government guidance on PFAS exposure.

The Panel's review of reviews and reports was informed by the following sources of information.

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR, 2015)**. Draft toxicological profile for perfluoroalkyls;
- **United States Environmental Protection Agency (US EPA, 2016a)**. Health effects support document for perfluorooctanoic acid (PFOA);
- **United States Environmental Protection Agency (US EPA, 2016b)**. Health effects support document for perfluorooctane sulphonate (PFOS);
- **New Jersey Drinking Water Quality Institute (DWQI, Public Review draft 2016)**. Health-based maximum contaminant level support document: perfluorooctanoic acid (PFOA);
- **National Toxicology Program (NTP, 2016)**. NTP monograph immunotoxicity associated with exposure to perfluorooctanoic acid and perfluorooctane sulphonate;
- **International Agency for Research on Cancer (IARC, 2016).** Monograph on perfluorooctanoic acid, 2016;

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- **Dutch National Institute for Public Health and the Environment (RIVM, 2017)**[6]. PFOA exposure and health: a review of scientific literature;
- **Food Standards Australia New Zealand (FSANZ, 2017)**. Hazard assessment report (PFOS, PFOA, PFHxS).

Systematic reviews and reviews

- **Saikat et al. (2013).** The impact of PFOS on health in the general population: a review;
- **Chang et al. (2014).** A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and cancer risk in humans;
- **Johnson et al. (2014)**. The navigation guide – evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth;
- **Lam et al. (2014).** The navigation guide – evidence-based medicine meets environmental health: integration of animal and human evidence for PFOA effects on fetal growth;
- **Roth and Wilks (2014)**. Neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals: a systematic review of the epidemiological literature using a quality assessment scheme;
- **Bach et al. (2015).** Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: a systematic review;
- **Chang et al. (2016):** A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and immunological health conditions in humans;
- **Priestly (2016).** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS), Monash University;
- **Ballesteros et al. (2017).** Exposure to perfluoroalkyl substances and thyroid functions in pregnant women and children: a systematic review of epidemiologic studies;
- **Negri et al. (2017).** Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data;
- **Rappazzo et al. (2017).** Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature;
- **Kirk et al. (2018).** The PFAS health study: systematic literature review. Australian National University.

3.2. Literature analysis and quality assessment

The Panel requested that *Allen + Clarke* undertake a review of the main human health findings from the identified national and international reports and systematic reviews of epidemiological studies of human health effects of PFAS (mainly PFOA and PFOS), taking note of the short timeframe available. The main health outcome categories used by the Australian National University (ANU) systematic review were adopted by the Panel, and the findings and conclusions of the key reports and systematic reviews were identified for each health outcome. These health effects are: neonatal, infant and maternal outcomes, reproductive effects, metabolic biomarkers (concentrations of cholesterol and triglycerides in the blood), kidney function, liver function, thyroid effects, neurodevelopmental effects, cancers, diabetes, cardiovascular effects, overweight and obesity, immunological effects, skeletal effects, and respiratory effects.

[6] Note that in the Reference list, this report is saved under *Rijs KJ, Bogers RP (2017).*

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At the request of the Panel, a review of the quality of the national and international reports and systematic reviews, including using AMSTAR-2, the critical appraisal tool for systematic reviews, was undertaken in October 2017. No report or review was excluded on the basis of the AMSTAR-2 rating.

After the main findings and conclusions from the relevant national and international reports and systematic reviews were identified for each health outcome category, the Panel assessed consistencies and inconsistencies in the findings and conclusions of the respective reports along with potential reasons why differences may have occurred. To do this, the Panel considered factors such as the inclusion or exclusion of different studies, different criteria for levels of evidence, and different purposes of the national and international authorities (e.g. hazard assessment, risk assessment, toxicological assessment). In addition, the Panel then considered the level of evidence and assessed whether chance, bias or confounding could explain the associations found in the reports and reviews.

The Panel's review did not generally extend to reviewing the primary studies which had been included in the national and international reports and systematic reviews.

3.3. Public consultation

The purpose of the consultation process was to allow the public the opportunity to provide information to the Panel on their health concerns regarding PFAS exposure and contamination, the exposure pathways that concern them, and the extent to which they felt they had been informed on various aspects of PFAS contamination. The submission form also allowed the public to express their views on which areas of human health research relating to PFAS they felt should be prioritised as part of the Australian Government's further research into the potential health effects of PFAS exposure.

The Panel approved a submissions form for the public to use, containing a number of questions across five key areas with the opportunity to provide further comments:

1. general information on the respondent including demographic data (age, sex), and which sector best represented them as either an individual or a group;
2. exposure pathways including questions on why PFAS exposure is relevant to the respondent, and which exposure pathways concerned respondents the most;
3. concerns about potential health impacts of PFAS exposure, including questions on which potential health impacts from PFAS exposure concerned respondents the most;
4. information and understanding including questions on how informed respondents feel about research on PFAS and the government response to address health concerns;
5. future research priorities including questions on which topics related to human health should be prioritised for future research;
6. other comments, providing an opportunity for respondents to discuss other issues relevant to health concerns relating to PFAS exposure or future research priorities.

The public was invited to engage in the submissions process via four methods: using the online submission form housed in Survey Monkey; downloading a PDF version of the submission form and emailing it to a dedicated email address; by printing a hard copy and mailing it to a Department of Health postal address; or by posting or emailing a submission using their own format. All questions

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in the submissions were voluntary, and many respondents chose only to answer some of the questions.

The public consultation period ran for 19 days between 1 November and 19 November 2017. In total, 499 complete submissions were received from the public. Four hundred and ninety-one respondents completed their submission via Survey Monkey, and eight respondents emailed submissions in their own format. No postal submissions were received within the consultation timeframe. There was some criticism from some groups about the limited timeframe for the consultation process and time allowed to make submissions.

The public was notified of the consultation process using the following channels:

- advertisements in The Australian (National Newspaper);
- advertisements in local newspapers in Oakey, Williamtown, and Katherine;
- contact with Community Liaison Officers in the Department of Human Services;
- press releases to national newspapers;
- press releases to local newspapers in Investigated Areas;
- online sources, including the Department of Health's website;
- direct contact with other key stakeholders known to the Department of Health.

These communication channels were selected to ensure that the key messages were delivered so that those communities which were most affected by PFAS received the information as early as possible.

Once received by *Allen + Clarke*, all submissions were anonymised and given numerical identifiers. Email submissions were provided to the Panel in their entirety.

3.3.1. Using sub-groups for more detailed analysis

Demographic information gathered under the General Information and Exposure Pathways question areas was used to classify respondents into two sub-groups to allow for more detailed comparisons between groups of respondents. Based on the responses received, the two sub-groups created based on the number of respondents were:

- occupationally exposed: respondents who reported that they were occupationally exposed, usually through firefighting, to PFAS containing chemicals at some point in their lives (n=249), and
- living in an investigation area: respondents who reported living, or having lived, in an area being investigated for PFAS contamination (n=224).

These two sub-groups provided different perspectives regarding the health impacts and exposure pathways they were concerned about, and the research priorities they thought were most important. A small number of respondents did not fit into either of these subgroups; however, their responses were considered as part of the wider analysis of responses as a whole.

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4. INTERNATIONAL AUTHORITIES REPORTING ON PFAS AND HEALTH CONSIDERED BY THE EXPERT HEALTH PANEL

Several international agencies and organisations have assessed the risk or hazard of PFAS compounds as they relate to human health. The United States Environmental Protection Agency (US EPA) defines human health risk assessment as "*the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media*". The US EPA notes the human health risk assessment process involves four basic steps:

- **Planning:** planning and scoping, including research;
- **Step 1:** hazard identification to examine whether a stressor has the potential to cause harm to humans and/or ecological systems, and if so, under what circumstances;
- **Step 2:** dose-response assessment to examine the numerical relationship between exposure and effects;
- **Step 3**: exposure assessment to examine what is known about the frequency, timing and levels of contact with the stressor;
- **Step 4:** risk characterisation to examine how well the data support conclusions about the nature and extent of the risk from exposure to environmental stressors.

International Agency for Research on Cancer

The **International Agency for Research on Cancer (IARC)** is the intergovernmental specialised cancer agency of the World Health Organization of the United Nations. The IARC Monographs Programme is a core element of IARC's portfolio of activities, with international expert working groups evaluating the evidence of carcinogenicity of specific exposures. The Monographs represent the first step in carcinogen risk assessment (hazard identification) which involves examination of all relevant information (exposure data, studies of cancer in humans and in experimental animals and mechanistic and other relevant data), in order to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans. In the Monographs, an agent is termed 'carcinogenic' if it is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity. IARC's classification of human carcinogenicity ranges from: carcinogenic to humans (Group 1); probably carcinogenic to humans (Group 2A); possibly carcinogenic to humans (Group 2B); not classifiable as to its carcinogenicity to humans (Group 3); and probably not carcinogenic to humans (Group 4).

United States Environmental Protection Agency

The **United States Environmental Protection Agency (US EPA)** is an agency of the federal government of the United States whose stated mission is to 'protect human health and the environment'. In 2016 the US EPA published health advisories for PFOA and PFOS based on the Agency's assessment of the latest peer-reviewed science (the US EPA's Health Effects Support Documents on PFOA and PFOS, 2016a and 2016b). These advisories were established "*to provide drinking water system operators, and state, tribal and local officials who have the primary responsibility for overseeing these systems, with information on the health risks of these chemicals, so they can take the appropriate actions to protect their residents*".

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The US EPA states that for the Agency's Health Effects Support Documents of PFOA and PFOS the following criteria were utilised in determining inclusion for the review:

1. The study examines a toxicity end point or population that had not been examined by studies already present in the draft assessment.
2. Aspects of the study design, such as the size of the population exposed or quantification approach, make it superior to key studies already included in the draft document.
3. The data contribute substantially to the weight of evidence for any of the toxicity end points covered by the draft document.
4. Elements of the study design merit its inclusion in the draft document based on its contribution to the mode of action (MoA) or the quantification approach.
5. The study elucidates the mode of action for any toxicity end point or toxicokinetic property associated with PFOA/ PFOS exposure.
6. The effects observed differ from those in other studies with comparable protocols.

In the 'Background' section of both reports, the US EPA states the studies included in the final draft were determined to provide the most current and comprehensive description of the toxicological properties of PFOS or PFOA and the risk they pose to humans exposed to them in their drinking water. Development of the hazard identification and dose-response assessment for PFOS and PFOA followed the general guidelines for risk assessment put forth by the National Research Council (1983) and US EPA's Framework for Human Health Risk Assessment to Inform Decision Making (US EPA 2014a).

Food Standards Australia New Zealand

Food Standards Australia New Zealand (FSANZ) is a statutory authority in the Australian Government Health portfolio. FSANZ develops food standards for Australia and New Zealand. In 2017, FSANZ prepared the 'Hazard assessment report for PFOS, PFOA and PFHxS', to provide advice to the Department of Health, Australia on appropriate health-based guidance values for these chemicals.

FSANZ reports the scope of its assessment included using the comprehensive international assessments of mammalian toxicology of PFOS and PFOA previously undertaken by the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (UNCOT), the European Food Safety Authority (EFSA), the Swedish Environmental Protection Agency (Swedish EPA), the Danish Environmental Protection Agency (Danish EPA), and the US Agency for Toxic Substances and Disease Registry (ATSDR) to determine Health Based Guidance Values for PFOA and PFOS. For human data, FSANZ presented the major conclusions from the EFSA (2008), US EPA (2016) and ATSDR (2015) assessments. FSANZ notes: "*A detailed consideration of individual epidemiological studies is beyond the scope of this review.*" FSANZ also reviewed the available epidemiological data relating to PFOS and PFOA exposure and serum cholesterol, and relating to PFOS and PFOA exposure and birthweight.

Dutch National Institute for Public Health and the Environment

The **Dutch National Institute for Public Health and the Environment (RIVM)** is a knowledge and research institute that is an independent agency of the Dutch Ministry of Health, Welfare and Sport. It is dedicated to promoting public health and a healthy and safe living environment. RIVM collects

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and collates knowledge and information from various sources, both national and international, works to prevent and control outbreaks of infectious diseases, promotes public health and consumer safety and helps to protect the quality of the environment. RIVM published its report, 'PFOA exposure and health: a review of the scientific literature', in 2017, following questions raised by residents who live in the vicinity of the DuPont/Chemours factory in Dordrecht concerning possible health effects of PFOA emissions by the factory.

RIVM reports that it used reviews previously performed by recognised national and international organisations to determine which biological and physiological parameters and diseases are associated with PFOA. The reviews RIVM included were undertaken by the US EPA (PFOA, 2016), NTP (2016), IARC (2016), DWQI (2016), ECHA-RAC (2015a), ATSDR (2015), Health Council of the Netherlands (2013), C8 Science Panel (2011,2012). RIVM then selected epidemiological studies from those previous reviews and from an additional search of the literature to determine the exposure levels at which associations were observed.

National Toxicology Program – United States Department of Health and Human Services

The **National Toxicology Program (NTP)** is a United States interagency program established to evaluate agents of public health concern by developing and applying tools of modern toxicology and molecular biology. The NTP's Office of Health Assessment and Translation (OHAT) conducted a systematic review to evaluate the evidence as to whether exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS) is associated with immune-related health effects.

The NTP initiated the review in response to studies reporting immune-related health effects of PFOA and PFOS in both humans and animals, and observations from the CDC in 2015 that the general US population has detectable blood levels of these chemicals despite actions that have substantially reduced emissions.

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

The NTP Monograph, 'Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid and Perfluorooctane Sulphonate', was published in September 2016.

New Jersey Drinking Water Quality Institute

The **New Jersey Drinking Water Quality Institute (DWQI)** Health Effects Subcommittee (the Subcommittee) develops Maximum Contaminant Levels (MCL) or standards for hazardous contaminants in drinking water. The DWQI reports it voted to pursue development of a Maximum Contaminant Level (MCL) recommendation for PFOA in 2009, based on its potential health effects and its occurrence in New Jersey public water supplies. The Subcommittee published the 'Health-based maximum contaminant level support document: perfluorooctanoic acid (PFOA)' in September 2016.

The Subcommittee reviewed 54 human epidemiology studies for the following end points:

- serum cholesterol/lipids;
- liver enzymes/bilirubin and liver disease;
- uric acid;

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- thyroid function and thyroid disease antibody concentrations following vaccination.

Reviews of other end points were also reported on, including fetal growth (e.g. birth weight by Johnson et al. 2015), and cancer (US EPA 2005, IARC 2015).

Agency for Toxic Substances and Disease Registry

The **Agency for Toxic Substances and Disease Registry (ATSDR)**, based in Atlanta, Georgia, is a federal public health agency of the U.S. Department of Health and Human Services. ATSDR published the Draft Toxicological Profile for Perfluoroalkyls in 2015. ATSDR reports it used a weight-of-evidence approach to evaluate whether available data supported a link between perfluoroalkyl exposure and a particular health effect.

The ATSDR stated: "*This weight-of-evidence approach takes into consideration the consistency of the findings across studies, the quality of the studies, dose-response, and plausibility. It should be noted that although the data may provide strong evidence for an association, it does not imply that the observed [association] is biologically relevant, because the magnitude of the change is within normal limits or not indicative of an adverse health outcome. Plausibility depends on experimental toxicology studies that establish a plausible biological mechanism for the observed effects.*"

C8 Science Panel

The Expert Health Panel acknowledge that many of the key international reports and systematic reviews referred to the C8 Science Panel and their conclusions. The Expert Health Panel did not review the C8 Science Panel reports published in 2011 and 2012, because the Panel focused on reviewing the most recently published international reports, particularly those in the last three years, and these reports and systematic reviews included considerable information about the C8 Science Panel findings. It is worth noting that the C8 Science Panel were often reviewing multiple studies that members of the C8 Science Panel had co-authored when coming to their overall findings.

The following information about the C8 Science Panel and its conclusions is taken from the Unites States Environmental Protection Agency health effects support document for PFOA (US EPA 2016a):

"*C8 Science Panel conclusions. As part of the C8 Health Project, the C8 Science Panel used epidemiological and other data available to them to assess probable links between PFOA exposure and disease (C8 Science Panel 2012). Analyses conducted by the C8 Science Panel used historical serum PFOA estimates over time, which were developed based on estimated intake of contaminated drinking water. The panel concluded that a probable link existed between PFOA exposure and ulcerative colitis, high cholesterol, pregnancy-induced hypertension, and thyroid disease. The C8 Science Panel found no probable link between PFOA exposure and multiple other conditions, including birth defects, other autoimmune diseases (e.g., rheumatoid arthritis, lupus, type 1 diabetes, Crohn's disease, MS), type II diabetes, high blood pressure, coronary artery disease, infectious disease, liver disease, Parkinson's disease, osteoarthritis, neurodevelopmental disorders in children (e.g., ADHD, learning disabilities), miscarriage or stillbirth, chronic kidney disease, stroke, asthma or COPD, and preterm birth or low birth weight (C8 Science Panel 2012).*"

"*In 2012, the C8 Science Panel concluded that there is a probable link between exposure to PFOA and testicular and kidney cancer, but no other types of cancers. Their conclusion was based on the studies presented above, other epidemiology studies on cancer incidence in the mid Ohio population, worker cohorts, and published data. Panel studies addressed 21 different categories of cancer and looked for positive trends with increasing exposure as measured by cumulative serum levels*".

http://www.c8sciencepanel.org/index.html

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5. SYSTEMATIC REVIEWS REPORTING ON PFAS AND HEALTH CONSIDERED BY THE EXPERT HEALTH PANEL

Saikat et al. (2013). The impact of PFOS on health in the general population: a review.

Saikat et al. conducted a search of the literature to investigate the association between PFOS exposure and a range of health-related outcomes in the general population.

Based on the selection criteria, 15 relevant studies were included in the review out of 477 potentially relevant papers. The review included 10 cross-sectional studies, three cohort studies and two case-control studies.

The authors noted that the design of the studies included in this review was a key limitation for attributing significance to the findings. Eight of the studies assessed health end points and the remaining studies looked at surrogate makers (e.g. cholesterol levels) to investigate any associations between PFOS exposure and health outcomes.

Chang et al. (2014). A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and cancer risk in humans.

Chang et al. systematically and critically reviewed 18 epidemiologic studies looking at the association between PFOA and PFOS exposure and cancer risk in humans.

The epidemiological studies (including eight of PFOA, four of PFOS, and six of both PFOA and PFOS) have estimated associations of exposure to these chemicals with cancer incidence or mortality.

The authors noted that observed associations are evaluated with regard to whether they were likely to be causal or due to bias, taking into consideration the probable direction and magnitude of bias. However, individual associations must be interpreted in light of the results from other studies, especially to assess whether chance may explain inconsistent findings. Therefore, the weight of evidence regarding possible causal relationships of PFOA and PFOS exposure with human cancer risk has been assessed in accordance with the Bradford Hill guidelines of strength of association, consistency, biological gradient, plausibility, and coherence with toxicological evidence.

It was also noted the work of all authors was supported by the 3M Company[7].

Johnson et al. (2014). The navigation guide – evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth.

Johnson et al. reviewed the literature to determine whether developmental exposure to perfluorooctanoic acid (PFOA) affects fetal growth in humans.

[7] 3M is a former manufacturer and user of long-chain perfluorooctanyl chemistry, mostly phasing-out the use of these chemicals by the end of 2002 (source: https://www.3m.com/3M/en_US/sustainability-us/policies-reports/3m-and-fluorochemicals/).

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The authors applied the first three steps of the Navigation Guide methodology to human epidemiology data: 1) specify the study question; 2) select the evidence; 3) rate the quality and strength of the evidence. Eighteen studies (with a total of 19 data sets) were identified that met the inclusion criteria, and 10 of these were combined through meta-analysis. The studies covered the years 1988-2009, from populations located in nine counties, and ranged from 17 to 11,737 study subjects. The authors evaluated each study for risk of bias and conducted meta-analyses on a subset of the studies. The authors report they "*rated quality and strength of the entire body of human evidence*". Using the Navigation Guide methodology, the authors determined there was a low risk of bias across the studies and assigned a "*moderate*" quality rating to the overall body of human evidence. The meta-analysis estimated a decrease in birth weight in relation to PFOA exposure using a 95% confidence interval.

Lam et al. (2014). The navigation guide – evidence-based medicine meets environmental health: integration of animal and human evidence for PFOA effects on fetal growth.

Lam et al. reviewed the human and nonhuman literature to determine whether developmental exposure to perfluorooctanoic acid (PFOA) affects fetal growth in humans.

The authors used the first three steps of the Navigation Guide: 1) specify the study population; 2) select the evidence; 3) rate the quality and strength of the evidence. The fourth and final step of the Navigation Guide (grade the strength of the recommendation – to determine the final recommendation for public health protection) was not addressed in the review. The authors stated this was due to resource constraints. A PECO[8] framework was used to develop the research questions and determine the eligibility criteria for the review.

The authors reported they developed and applied prespecified criteria to systematically and transparently rate the quality of the scientific evidence as "*high*", "*moderate*" or "*low*"; rate the strength of human and nonhuman evidence separately as "*sufficient*", "*limited*", "*moderate*", or "*evidence of lack of toxicity*"; and c) integrate the strength of the human and nonhuman evidence ratings into a strength of evidence conclusion.

In terms of the human evidence, the studies looked at participants before and/or during pregnancy or development in relation to their exposure to PFOA. The review considered effects on foetal growth, birth weight, and/or other measures of size, such as length.

Eighteen human epidemiological studies and 21 animal toxicology studies were identified as being relevant to the study question. The human and nonhuman mammalian evidence were both rated as "*moderate*" quality and "*sufficient*" strength using the Navigation Guide systematic review methodology. The ratings for the epidemiological studies were combined with the nonhuman mammalian evidence to produce an overall evidence rating, in which the authors concluded that PFOA is "*known to be toxic*" to human reproduction and development.

[8] PECO (Population, Exposure, Comparator, Outcomes)

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Roth and Wilks (2014). Neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals: a systematic review of the epidemiological literature using a quality assessment scheme.

Roth and Wilks developed a checklist-type quality assessment scheme based on the STROBE guidelines and the proposed HONEES[9] criteria, and conducted a systematic review of the epidemiological peer-reviewed literature published since 2006 on neurodevelopmental and/or neurobehavioural effects (such as adverse birth outcomes, cognitive deficits, developmental delay and attention deficit hyperactivity disorders) following prenatal and postnatal exposure to polybromo diphenylethers (PBDEs) and perfluorinated chemicals (PFCs). The authors noted that the epidemiological literature lacks comparability across studies in terms of design, conduct, methodology and reporting.

Based on the checklist quality assessment criteria, seven of the 18 studies were rated as being of high quality, seven of moderate quality and four of low quality. Frequently observed shortcomings were the lack of consideration of confounding factors; uncertainties regarding exposure characterisation; inadequate sample size; the lack of a clear dose-response; and the representativeness/ generalisability of the results.

Bach et al. (2015). Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: a systematic review.

Bach et al. reviewed 14 studies published between 2004 and 2013 to summarise the evidence of an association between exposure to PFASs, particularly PFOS and PFOA, and human fetal growth. Birth weight and other related measures were used as proxies for fetal growth.

A PICOS[10] framework was used to establish the selection criteria for the review. The review included original studies on pregnant women with measurements of PFOA or PFOS in maternal blood during pregnancy or the umbilical cord (from both populations with background exposure and high exposure). Birth weight was the primary outcome, and other related outcomes were also measured, according to individual PFAS levels. The methodology included assessing the completeness of reporting as well as the risk of bias and confounding.

Chang et al. (2016). A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and immunological health conditions in humans.

Chang et al. systematically and critically reviewed 24 studies looking at the relationship between exposure to PFOA and PFOS and several immune-related health conditions. The review included 10 studies of immune biomarker levels or gene expression patterns, 10 studies of atopic or allergic disorders, five studies of infectious diseases, four studies of vaccine responses, and five studies of chronic inflammatory or autoimmune conditions (with several studies evaluating multiple end points). Asthma, the most commonly studied condition, was evaluated in seven studies.

The authors reported the overarching question of interest for the review is whether PFOS and PFOA are causally related to adverse health conditions in humans. Following data extraction, the quality of

[9] HONEES: "Harmonisation of Neurodevelopmental Environmental Epidemiology Studies"
[10] PICOS criteria (Participants, Interventions/exposures, Comparators, Outcomes, Study design)

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individual epidemiologic studies was evaluated based on the validity and reliability of outcome assessment, control of confounding, potential for selection bias, and appropriateness of the statistical approach.

It was also noted that the manuscript was supported by the 3M Company.

Priestly (2016). Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS), Monash University.

This report, commissioned by the Victorian Department of Health & Human Services, provided independent evidence-based advice and an update to the review originally prepared in October 2015, to assist various Australian government agencies to respond to concerns relating to the possible health effects of PFAS- mainly PFOS. The methodology for the review included identifying some key reviews undertaken by international authorities and tracking back relevant references cited in those reviews. This was supplemented by a PubMed search using key terms 'PFOS/PFOA/perfluoroalkyl substances health effects, 'perfluoroalkyl biomonitoring' and 'PFAS human exposures'.

The review of the literature summarised some of the key animal studies on the toxicology of PFAS but put greater focus on the human epidemiological evidence of potential associations between PFAS exposures and adverse health outcomes. The review included 301 studies or reviews.

Priestly identified key reviews undertaken by international authorities and the references cited in these reviews to examine the potential associations between PFAS exposures and adverse health outcomes.

Priestly (2017). Literature Review and Report on the Potential Health Effects of Perfluoroalkyl Compounds, Mainly Perfluorooctane Sulfonate (PFOS)

During the later stages of drafting this report, the Panel became aware that the Priestly (2016) report had been updated (December 2017) and has recently become publicly available. This updated report summarises about 50 new studies identified since the November 2016 report.

Priestly, in the executive summary of the December 2017 report, stated of the human epidemiology studies:

"*The new epidemiology studies have not added any substantially new or concerning information on the potential health effects of PFOS. There have been some papers addressing end points that received only passing attention in my previous reviews (metabolic dysfunctions, including effects on glycaemic controls), some papers that expand on the previously covered main associations with adverse health effects (thyroid disease, reproductive and fertility changes, neurodevelopmental effects, effects on blood lipids, and immunomodulation), along with 1-2 papers on some new indicators (coronary heart disease, endometriosis and effects on bone and lung disease). In the main, these studies report inconsistent findings, with associations (not necessarily causal) between individual PFAS varying in strength from study to study, and for some end points, a range of positive and negative findings for these same PFAS.*

Papers dealing with immunomodulatory effects and cancer have received additional attention in this 2017 update, because these are end points that commonly feature in media reports that cause some degree of alarm in communities living around point sources of (mainly) PFOS contamination associated

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with the legacy use of Aqueous Film-Forming Foams (AFFFs) used to fight fuel fires. There is currently no consensus on whether there are causative associations between exposure to any PFAS, and these end points. There have been international reviews; for example, an oft-cited (in the media) International Agency for Research on Cancer (IARC) evaluation of PFOA-related carcinogenicity (categorised as possibly causing human cancer), while other reviews have reached even less certain categorisations, or even a conclusion for a lack of evidence. One recent Italian study, outlining an increased relative risk (RR) for deaths (from cancers and other diseases) in communities exposed over time to known PFAS water pollution, has been analysed in more detail, with some methodological issues pointing to a reduction in the strength of the evidence that should be accorded the rather startling conclusions from this study.

Similarly, for immunomodulatory effects, some reviews (e.g. US NTP 2016 and FSANZ 2016) have reached consensus on the strength of the animal studies, but varying degrees of consensus on the strength of the epidemiological evidence. The lack of consensus on the epidemiology outcomes is largely due to disparities, even within the same study, on which immune marker has been affected, and by which PFAS. In some cases, the inconsistency may be confounded by an inability to rule out concurrent exposure to other Persistent Organic Pollutants (POPs) known to influence immune responses, and by conflicting findings for the same, or related end points, across different studies."

Ballesteros et al. (2017). Exposure to perfluoroalkyl substances and thyroid functions in pregnant women and children: a systematic review of epidemiologic studies.

Ballesteros et al. reviewed 10 studies to examine the association between PFOS, PFOA, PFHxS and thyroid outcomes in prenatal life and childhood (<19 years).

The authors report they developed a protocol and performed a systematic review in accordance with the general principles recommended in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. They used a PICOS framework to establish the criteria for selecting the studies to be included in the review. Ten studies were identified that met the inclusion criteria and did not achieve low scores according to the nine items included in the 'Methods' section of the STROBE statement checklist (Strengthening the Reporting of Observational Studies in Epidemiology Statement).

Studies selected for the review were carried out in populations of pregnant women or children up to 19 years old. One study looked at pregnant mothers and children from a community living near a fluoropolymer manufacturing facility, and the remaining studies focused on women and children from general populations. Studies were conducted in Asia, Europe, and North America with a sample size varying between 40 to >10,000 participants, and published between 2011 and 2015. The design of the studies was either cross-sectional (n=3), case-control (n=1), or cohort (n=6).

Negri et al. (2017). Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data.

Negri et al. reviewed the literature to assess the association between perfluorooctanoic acid (PFOA) or perfluorooctane sulfonic acid (PFOS) and birth/fetal weight.

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The review used the (PRISMA) guidelines for reporting and the meta-analyses of Observational Studies in Epidemiology (MOOSE). A PICOS[11] approach was used to determine eligibility criteria. The authors undertook a risk of bias appraisal, assessing the methodological aspects of each study using a modification of the 'Newcastle-Ottawa Quality Assessment Scale' (NOS).

The review included women who were enrolled in studies before or during pregnancy or at delivery who were exposed to PFOA or PFOS, as determined by maternal or umbilical cord serum, plasma or whole blood, or maternal milk.

The authors identified 16 papers, published between 2007 and 2015, which met the inclusion criteria. The study designs were cross-sectional (n=4), case-control (n=3), and cohort (n=9). The studies were conducted in North America (n=5), Asia (n=5), and Europe (n=6).

Rappazzo et al. (2017). Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature.

Rappazzo et al. reviewed 64 studies on the relationships between prenatal and/or childhood exposure to PFAS and health outcomes in children and provided a risk of bias analysis of the literature.

For inclusion in this review, the reviewers required serum, blood, or breast milk concentrations of PFAS that were measured concomitantly with the health outcome (e.g. serum PFAS and triglyceride concentrations) or early in life and associated with a later health outcome (e.g. PFAS in cord blood and behavioural outcomes in children); however measurements primarily used serum levels.

The study designs of included papers were primarily cohort or cross-sectional.

A risk of bias analysis was performed to evaluate the methodological design and implementation of the studies included. Seven criteria for risk of bias were considered: selection bias, exposure assessment, outcome assessment, confounding, missing data, conflict of interest, and 'other'. The studies included in the review were assigned a risk of bias score for each of the seven categories of interest. Risk of bias score were assigned as "*low risk*", "*probably low risk*", "*moderate or unclear risk*", "*probably high risk*", or "*high risk*".

Kirk et al. (2018). The PFAS Health Study: Systematic Literature Review. Australian National University.

The PFAS Health Study Systematic Literature Review by the National Centre for Epidemiology and Population Health, Research School of Population Health, the Australian University, was commissioned by the Commonwealth Department of Health in 2016. The authors systematically reviewed 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.

The authors conducted a comprehensive search of the health and grey literature published up until January 2017 with the search strategy following the PRISMA flow design.

[11] PICOS (Participants/population, Intervention/Exposures, Comparator(s)/controls, Outcome(s), and Study Design)

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The authors identified 221 papers that met the systematic review criteria then used a systematic framework to review each paper, extract data and rate risk of potential bias (using a multi-domain risk of bias tool). The authors considered whether it was possible to pool study results in a meta-analysis when five or more studies on a particular health outcome were identified. The authors noted the majority of studies included in this review were evaluated to have a moderate to high risk of bias that could have influenced published findings. The authors reported only 3.6% (8/221) of studies evaluated were considered to be at low risk of bias.

To evaluate the strength of evidence for each health effect, Kirk et al. adapted the criteria that the International Agency for Research on Cancer (IARC) uses to evaluate the evidence for carcinogenicity, and classified the evidence relevant to each separate health effect into 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.

Notes

- *In the following sections, not all of the above reports and reviews are referred to, only those which contain findings relevant to the outcome(s) considered in that section.*
- *Throughout the report, all instances of PFAS concentrations in the literature have been converted to consistent units (ng/mL). This is in order to facilitate comparisons across studies and reviews and with Australian biomonitoring data.*

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6. HEALTH EFFECT FINDINGS FROM THE REVIEW

6.1. Cancer and PFAS exposure

The World Health Organization advises[12] cancer is one of the leading causes of morbidity and mortality worldwide, with around one third of deaths from cancer being due to the five leading behavioral and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol use. Tobacco use is the most important risk factor for cancer and is responsible for approximately 22 percent of cancer deaths. Several of the key international reports and systematic reviews reviewed the human evidence on exposure to PFAS and cancer.

6.1.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of five published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and three systematic reviews since 2014 that analysed the human epidemiological evidence regarding exposure to PFAS and cancer:

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR, 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **International Agency for Research on Cancer (IARC, 2016).** Monograph on Perfluorooctanoic Acid, 2016;
- **United States Environmental Protection Agency (US EPA, 2016a)**. Health effects support document for Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA, 2016b)**. Health effects support document for Perfluorooctane Sulphonate (PFOS);
- **Dutch National Institute for Public Health and the Environment (RIVM, 2017)**. PFOA exposure and health: A review of scientific literature.

Systematic reviews

- **Chang et al. (2014).** A critical review of perfluorooctanoate and perfluorooctanesulphonate exposure and cancer risk in humans;
- **Priestly (2016).** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS) **(Monash University)**;
- **Kirk et al. (2018).** The PFAS Health Study. Systematic Literature Review. (Australian National University).

The National Toxicology Programme (NTP) Monograph on PFOA and PFOS was not considered by the Panel for this section as the Monograph did not report on cancer.

The Panel acknowledges the New Jersey Drinking Water Quality Institute (DWQI, 2016) commented on the carcinogenicity of PFAS. However, this report has not been included in this section because the DWQI did not review cancer epidemiological studies in detail, instead reporting on end points evaluated by other authoritative groups, notably the US EPA and IARC.

[12] http://www.who.int/mediacentre/factsheets/fs297/en/

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The Panel also acknowledges that FSANZ considered the evidence on cancer in the 'Hazard assessment report for PFOA, PFOS and PFHHx. FSANZ primarily reported on the findings of other international authority reports, notably EFSA 2008, Bull et al. 2014; ATSDR (2015), US EPA 2016 (2016a,b) and IARC. For this reason, the FSANZ report is not considered further in this section. Based on the review of these reports, FSANZ concluded in the 'Executive Summary': "*Epidemiological studies have not provided convincing evidence of a correlation between PFOS and PFHxS and any cancer type in human beings. Although associations between PFOA and some human cancers have been suggested from some epidemiological studies, results have often been contradictory, and a causal relationship cannot be established with reasonable confidence.*"

6.1.2. Key national and international reports

Agency for Toxic Substances and Disease Registry (ATSDR)

Studies reviewed

The ATSDR reviewed 13 studies on exposure to PFAS and cancer, including:

- nine inhalation exposure route studies that investigated the possible association between occupational exposure to perfluoroalkyls and increased cancer risk: (Gilliland and Mandel, 1993; Lundin et al. 2009; Leonard et al. 2008; Leonard et al. 2006 (unpublished); Steenland and Woskie, 2012; Alexander and Olsen, 2007; Grice et al. 2007; Alexander et al. 2003; Olsen et al. 2004);
- seven oral exposure route studies, including:
  - four studies that investigated the potential carcinogenicity of perfluoroalkyls in communities living near a facility releasing PFOA: (Barry et al. 2013; Innes et al. 2014; MDH 2007; Vieira et al. 2013); and
  - three studies in the general population: Bonefeld- Jørgensen et al. 2011; Eriksen et al. 2009; Hardell et al. 2014).

The ATSDR advised: "*No studies were located regarding cancer effect effects in humans or animals following dermal exposure to perfluoroalkyl compounds.*"

Considerations and conclusions

The ATSDR made two major statements on exposure to PFAS and cancer in humans.

In the 'Public Health Statement for Perfluoroalkyls', it noted: "*There is limited information on whether perfluoroalkyls can cause cancer in humans. Some increases in prostate, kidney, and testicular cancers have been found in workers or in community members living near a PFOA facility. These results should be interpreted cautiously because the effects were not consistently found and most studies did not control for other potential factors such as smoking. Feeding PFOA and PFOS to rats caused them to develop tumors. Some scientists believe that, based on the way this happens in rats and the differences between rats and humans, humans would not be expected to get cancer. Others believe that it is possible for perfluoroalkyls to cause cancer in humans, and the studies in rats should not be dismissed. More research is needed to clarify this issue.*"

In the 'Relevance to Public Health' section, the ATSDR stated: "*A number of studies have examined the carcinogenicity of PFOA and PFOS in humans. Occupational exposure studies have found significant increase in deaths from several cancer types, including prostate cancer at one facility and kidney cancer at a second facility. An increase in the risk of kidney cancer was also found in residents living near the second facility. An increased risk of testicular cancer was also found in the highly*

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exposed residents living near the second facility. Other occupational exposure studies have not found significant increases in cancer risks. Although several studies have found significant increases in cancer risk, the results should be interpreted cautiously since most studies did not control for potential confounding variables (particularly smoking), the number of cancer cases was low, and a causal relationship between perfluoroalkyls and cancer cannot be established from these studies. Additionally, the lack of consistency across facilities may be suggestive of a causative agent other than PFOA or PFOS."

In the section titled 'How perfluoroalkyls can affect your health?', the ATSDR noted: "*There is limited information on whether perfluoroalkyls can cause cancer in humans. Some increases in prostate, kidney, and testicular cancers have been found in workers or in community members living near a PFOA facility. These results should be interpreted cautiously because the effects were not consistently found and most studies did not control for other potential factors such as smoking.*"

On page 321, 'Identification of Data Needs', the ATSDR noted: "*Occupational exposure studies, studies of the general population, and studies of communities living in areas with known perfluoroalkyl contamination have examined the potential association between cancer and perfluoroalkyl compounds. Studies of highly exposed individuals have found increases in several cancer types; however, the results are not consistent across studies. Increases in the risk of prostate cancer (Gilliland and Mandel 1993; Lundin et al. 2009), kidney cancer (Steenland and Woskie 2012; Vieira et al. 2013), and testicular cancer (Barry et al. 2013; Vieira et al. 2013) have been reported in some groups of workers or residents living near a facility and exposed to high levels of PFOA in the drinking water. The lack of consistent findings across studies may be due to the lack of control for potential confounders, especially exposure to non-perfluoroalkyl compounds. Follow-up assessments of perfluoroalkyl workers and highly exposed populations living near manufacturing facilities are needed; these studies should attempt to control for potential confounding variables, particularly smoking, which has been associated with an increased risk of kidney and testicular cancer.*"

Summaries of studies reviewed

Inhalation exposure

The ATSDR reviewed eight studies under inhalation exposure: (Gilliland and Mandel, 1993; Lundin et al. 2009; Leonard et al. 2008; Leonard, 2006 (unpublished); Steenland and Woskie, 2012; Alexander and Olsen, 2007; Grice et al. 2007; Alexander et al. 2003; Olsen et al. 2004).

The ATSDR reviewed Gilliland and Mandel (1993), and noted that they undertook "*a retrospective cohort mortality evaluation of 2,788 male and 749 female workers employed for at least 6 months between 1947 and 1983 at a plant that produced PFOA. Workers employed ≥1 month in the Chemical Division of the plant were categorized as exposed and those who either never worked or worked for <1 month in the Chemical Division formed the unexposed group. The effects of latency, duration of employment, and work in the Chemical Division were examined using stratified SMR analyses.*" The ATSDR reported the results of the study as showing "*No significant increases in SMRs were observed in the male and female workers for all cancer types and for individual types of cancer as compared to U.S. and Minnesota mortality rates. However, there was a nonsignificant increase in the SMR for prostate cancer (2.03, 95% CI 0.55–4.59) in the Chemical Division group. Ten years of employment in the Chemical Division was associated with a 3.3-fold increase (95% CI 1.02–10.6) in the relative risk of prostate cancer mortality, as compared to no employment in PFOA production areas. The investigators noted that the prostate cancer findings are based on a small number of cases and could have resulted from chance or unrecognized confounding from exposure to other factors.*"

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The ATSDR reviewed Lundin et al. (2009), who did an update of the study of workers at the 3M Company facility in Cottage Grove, Minnesota by Gilliland and Mandel (1993), and noted that: "*Unlike the Gilliland and Mandel (1993) study, the eligibility criterion was a minimum of 365 days of cumulative employment prior to 1997. The cohort consisted of 807 deceased workers (80% male) followed through 2002. The cohort was divided into three exposure categories of APFO exposure: definite occupational exposure (high exposure), probable occupational exposure (jobs where APFO exposure was possible, but likely lower or transient) (moderate exposure), and no or minimal occupational exposure (low exposure).*" The ATSDR report the results as showing: "*No increases in deaths from all cancer types, biliary or liver cancers, pancreatic cancer, respiratory cancer, or bladder and other urinary organ cancers were found, as compared to the Minnesota general population. A nonsignificant increase in prostate cancer deaths (SMR 2.1, 95% CI 0.4–6.1) was found in the workers with definite PFOA exposure. When the cohort was divided into the three exposure categories, increased HRs for prostate cancer were found in the moderate- and high-exposure categories (HR=3.0, 95% CI 0.9–9.7 and HR=6.6, 95% CI 1.1–37.7) and in the combined moderate- and high-exposure categories (HR=3.2, 95% CI 1.0–10.3), as compared to the low-exposure category.*"

The ATSDR reviewed Leonard et al. (2008), who undertook a study of DuPont employees (n=6,027; 81% male) who worked at the at the Washington Works, West Virginia, polymer-manufacturing facility at any time between January 1, 1948 (plant start-up) and December 31, 2002.The ATSDR noted that "*Approximately one-half of the employees at the site had been assigned to APFO areas at some time in their careers.*" ATSDR also noted that this study was also reported in an unpublished report by Leonard 2006. The ATSDR summarised the results: "*No significant increases in deaths from all cancer types were found when the workers were compared to U.S. and West Virginia population mortality rates and to workers at other DuPont facilities in the region. An increase in the number of deaths from kidney cancer relative to the DuPont regional population was observed; however, the SMR was not significantly elevated (SMR 185, 95% CI 95–323). No other elevations in specific cancer risk were found.*"

The ATSDR reported that a follow-up study was undertaken by Steenland and Woskie (2012), who followed a cohort of 5801 workers through 2008. The ATSDR outlined the methodology: "*Using blood samples collected from 1979 to 2004 from 1,308 workers participating in a health survey, serum PFOA levels over time were estimated for eight job categories/job group categories. Serum PFOA levels were estimated for each worker based on job history and the estimated serum PFOA levels for each job category/job group category. The mean cumulative exposure to PFOA was 7.8 ppm-years and the estimated average annual serum concentration was 350 ng/mL.*" The ATSDR detailed the findings from the study, "*Deaths from all cancer types were not significantly increased when compared to the U.S. population or to the DuPont regional population. A significant increase in deaths from mesothelioma was found when compared to the U.S. population (SMR 4.83, 95% CI 1.77–10.52) and the DuPont regional population (SMR 2.85, 95% CI 1.05–6.20); the investigators noted that this was likely due to asbestos exposure. Among workers with the highest exposure to PFOA, there was a significant increase in kidney cancer as compared to the DuPont regional population (SMR 2.66, 95% CI 1.15–5.24 for no lag, SMR 2.82, 95% CI 1.13–5.81 with a 10-year lag, and SMR 3.67, 95% CI 1.48–7.57 with a 20-year lag); a positive exposure-response trend was also observed for kidney cancer at all three lag times. Steenland and Woskie (2012) noted that tetrafluoroethylene, a rodent kidney carcinogen, is used in the manufacture of a variety of fluoropolymers; tetrafluoroethylene is well controlled due to its volatile and explosive properties.*"

The ATSDR reviewed Alexander et al. (2003), who undertook a retrospective mortality study of a cohort of 2083 employees (83% male) at a perfluorooctanesulphonyl fluoride (PFOSF) based

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fluorochemical production facility in Decatur, Alabama. The employees had at least one year of cumulative employment at the facility. The reported results showed that: "*The geometric mean serum PFOS levels were 900 ng/mL in a randomly selected group of 126 workers in the chemical plant and 100 ng/mL in a group of 60 workers in the film plant. Biomonitoring conducted at this facility indicates that the workers also had elevated serum PFOA levels; in a 2000 survey, the mean serum PFOA level was 1,780 ng/mL (Olsen et al. 2003a). Based on job history and serum PFOS levels, workers were assigned to one of three groups: high exposure (n=982), low exposure (n=289), or no exposure (n=812). No significant increases in the SMR for all types of cancer or specific types of cancer were observed, as compared to mortality rates in the state of Alabama. There was an increased risk of death from bladder cancer for the entire cohort, 3 observed and 0.62 expected; however, the 95% CI included the null value (SMR 4.81, 95% CI 0.99–14.05). All three cases of bladder cancer occurred in workers from the high exposure group (0.19 expected) (SMR 16.12, 95% CI 3.32–47.41) and all of them had worked in high exposure jobs for at least 5 years.*"

The ATSDR reported that Alexander and Olsen (2007) undertook a reanalysis of workers at this facility, which included all current, retired, and former employees (total=1,895) who had at least 365 days of cumulative exposure prior to 1998 and information from 188 deceased workers. The ATSDR provided an overview of the study: "*The NIOSH Surveillance Epidemiology and End Results (SEER) referent data were used to calculate the standardized incidence ratios. Bladder cancer incidence was collected via a self-administered questionnaire; for subjects self-reporting bladder cancer, an attempt was made to verify the diagnosis with medical records. The exposure assessment followed the method used in the previous study; workers were assigned to a high-exposure (serum PFOS 1,300–1,970 ng/mL), low-exposure (390–890 ng/mL), and no direct exposure (110–290 ng/mL) groups.*" The ATSDR report the results as showing "*Eleven cases of bladder cancer were identified from surveys (n=6) and death certificates (n=5). Only two of the six self-reported bladder cancer diagnosis were confirmed via medical records; the other four subjects declined to give consent for medical verification. The standardized incidence ratios (SIRs) were 1.28 (95% CI 0.64–2.29) for the entire cohort and 1.74 (95% CI 0.64–3.79) for those ever working in a high-exposure job. When compared with those in the lowest cumulative exposure category, the high-exposure workers had a 1.5–2.0-fold increased risk but the Cis included unity; for example, the relative risk in the workers exposed for 5–<10 years was 1.92 with a 95% CI of 0.30–12.06.*"

The ATSDR made the following observations about this study: "*Although the study did not adjust for smoking, the investigators noted that 83% of the living bladder cancer cases (five of the six subjects) reported cigarette use, as compared to 56% reported in the noncases. An additional limitation of the study is inclusion of four cases of bladder cancer that were not verified by medical records. The results of this study do not appear to confirm the findings of increased bladder cancer in the mortality study (Alexander et al. 2003).*"

The ATSDR also reviewed Grice et al. (2007), who examined the potential carcinogenicity of PFOS in 1,400 workers (81% male) at the Decatur, Alabama manufacturing facility via a self-administered health questionnaire. The ATSDR noted that: "*Attempts were made to validate the self-reported diagnoses of prostate cancer, colon cancer, breast cancer, and melanoma through medical records. Exposure to PFOS was evaluated based on the job-specific exposure categories established in the Alexander et al. (2003) study. As noted previously, these workers were also likely exposed to elevated levels of PFOA. The risks of colon cancer, melanoma, and prostate cancer were not associated with any of the PFOS-exposure categories for analyses that included all self-reported or only validated cancers.*"

The ATSDR reviewed the study by Olsen et al. (2004), which investigated episodes of care in workers at the Decatur facility. The ATSDR reported: "*An episode of care is defined as a series of*

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events related to a particular health problem. Among the 211 long-term workers in the chemical plant, there was a nonsignificant increase in the number of episodes of care for malignant neoplasm of the prostate (risk ratio episodes of care [RREpC] of 8.2, 95% CI 0.8–>100), a nonsignificant increase in malignant neoplasms of the colon (RREpC of 12, 95% CI 0.8–>100), and a significant increase in benign colonic polyps (RREpC of 2.4, 95% CI 1.3–-4.5), as compared to 345 longterm workers in the film plant. No significant increases in the risk ratio episodes of care were found for liver, rectum, or respiratory tract..."

The ATSDR made the following comments and conclusion about the inhalation exposure studies in occupationally exposed workers: "*Consistent findings regarding the association between occupational exposure to PFOA and PFOS and cancer have not been found. Among workers with longer-term exposure to higher PFOA levels, an increased risk of prostate cancer deaths was found (Gilliland and Mandel 1993; Lundin et al. 2009), but this was not found in studies of workers at a different PFOA facility (Leonard et al. 2008; Steenland and Woskie 2012). The increases in kidney cancer mortality were observed at the second facility (Leonard et al. 2008; Steenland and Woskie 2012), but not at the first facility (Gilliland and Mandel 1993; Lundin et al. 2009). For PFOS, one study reported an increase in bladder cancer (Alexander et al. 2003), but a follow-up study did not confirm this finding (Alexander and Olsen 2007). The inconsistent results across studies may be due to differences in exposures or to exposure to other compounds.*"

Oral exposure route

High-exposure community studies

The ATSDR reported four studies which examined the potential carcinogenicity of perfluoroalkyls in communities living near a facility releasing PFOA (Barry et al. 2013; Innes et al. 2014; MDH 2007; Vieira et al. 2013).

The ATSDR reported on Barry et al. (2013), which examined the cancer incidence in 32,254 adults living near the DuPont Washington Works chemical plant in West Virginia and participating in the C8 Health Project and C8 Health Panel or who ever worked at the DuPont facility (11% of the cohort). The ATSDR reported the details of the study as: "*Cumulative serum PFOA levels for community members were estimated based on environmental levels, residential history, drinking water source, tap water consumption, work place water consumption, and PFOA toxicokinetic properties. Serum PFOA levels in the workers were estimated based on job histories and data from a health survey that linked job titles to serum PFOA levels; these estimated serum PFOA were combined with estimated serum PFOA levels from residential exposure. Measured PFOA levels (measured in 2005–2006) were 24.2 ng/mL for community members and 112.7 ng/mL for workers. Estimated median annual PFOA serum levels were 19.4 and 174.4 ng/mL for the community and workers, respectively. Cancer incidence data were obtained from questionnaires and cancer diagnosis verified through review of medical records or from Ohio/West Virginia cancer registry.*" With regards to the results, the ATSDR noted "*Although increases in the risk of thyroid, kidney, and testicular cancer were found, only the HRs for testicular cancer (HR=1.34, 95%CI 1.00–1.79 with no lag) was statistically significant. When serum PFOA levels were stratified, a significant positive trend across quartiles was found for testicular cancer.*"

The ATSDR reported on Vieira et al. (2013), which also examined the possible association between PFOA exposure and cancer risk in Ohio and West Virginia residents living near the Washington Works DuPont facility in West Virginia. The authors identified cancer cases for 18 cancer types from the Ohio Cancer Incidence Surveillance System and West Virginia Cancer Registry. The final data set included 7,869 Ohio cases and 17,238 West Virginia cases. The ATSDR reported that: "*Serum PFOA levels were estimated for the Ohio residents using estimated environmental levels, exposure*

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assumption, and PBPK modeling. The residents were grouped by water districts with the Little Hocking district having the highest levels of PFOA (estimated serum PFOA level of 125 ng/mL) and Mason having the lowest level (5.3 ng/mL)." The ATSDR reported on the results from the study as showing "*Significant adjusted odds ratios (AORs) were found for testicular cancer in the Little Hocking district (AOR=5.1, 95% CI 1.6– 15.6), kidney cancer in Tuppers Plain district (AOR=2.0, 95% CI 1.3–3.1; estimated serum PFOA level of 23.9 ng/mL), and lung cancer in Mason district (AOR=1.3, 95% CI 1.1–1.5). When analysed based on estimated serum PFOA levels, significantly elevated AOR were found in the very high (serum PFOA levels of 110–655 ng/mL) and high (30.8–100 ng/L) annual PFOA serum level groups for kidney cancer (AOR=2.0, 95% CI 1.0–3.9 and AOR=2.0, 95% CI 1.3–3.2, respectively), compared with cases living in unexposed areas. The AOR for testicular cancer was 2.8 (95% CI 0.8–902) in the very high PFOA group, which was based on six cases; the investigators noted there was an inverse association between testicular cancer and the lower exposure groups. Elevated AORs were also found for prostate and ovarian cancer and Non-Hodgkin's lymphoma in the very high exposure group; however, the CIs included the null value.*"

The ATSDR reported on a third study of the communities near the Washington Works facility which was undertaken by Innes et al. (2014). It examined the possible association between serum PFOA and PFOS levels and the risk of colorectal cancer in over 47,000 adults. The ATSDR reported that the mean (and range) serum PFOA and PFOS levels in this cohort were 86.6 ng/mL (<0.5- 22,412 ng/mL) and 23.4 ng/mL (0.5–759.2 ng/mL) respectively, concluding that: "*The investigators noted that the PFOS levels were similar to those in the U.S. general population. Statistically significant inverse associations were found between the risk of colorectal cancer and serum PFOA and PFOS levels with the least likelihood of colorectal cancer in residents with PFOA and PFOS serum levels in the fourth quartile. Individuals with the highest PFOS serum level were 80% less likely to receive a diagnosis of colorectal cancer and those with the highest serum PFOA levels were 40% less likely to be diagnosed with colorectal cancer.*"

The ATSDR also reported on a study by the Minnesota Department of Health (MDH 2007), which examined cancer incidence in residents living in Washington and Dakota Counties. The ATSDR noted that: "*Elevated PFOA, PFOS, and PFBA levels have been measured in municipal and private drinking water wells in these counties.*" The ATSDR reported on the study conclusions: "*As compared to statewide cancer rates, no significant increases in specific cancers were found in Washington County. In Dakota County, significant increases in liver and breast cancer rates were observed in females; no significant increases in cancer rates were found in males. The study also examined cancer incidence in eight communities in these counties: Cottage Grove, Hastings, Lake Elmo, Newport, Oakdale, South St. Paul, St Paul Park, and Woodbury. Some statistically significant increases in a specific cancer type were found; however, the results were not consistent across communities or between males and females.*"

General population studies

The ATSDR reviewed three studies on PFAS exposure and cancer undertaken in the general population (Eriksen et al. 2009; Hardell et al. 2014; Bonefeld- Jørgensen et al. 2011).

The ATSDR reported on the study by Eriksen et al. (2009), which examined the possible association between blood PFOA and PFOS levels in 1,240 Danish men and women with prostate (n=713), bladder (n=332), pancreatic (n=128), or liver cancer (n=67) enrolled in a prospective cohort study, but who did not have cancer prior to enrollment. The study used a group of 772 men and women without cancer also enrolled in the prospective study as a comparison group. The ATSDR reported the results: "*The respective median plasma PFOA and PFOS levels were 6.8 and 35.1 ng/mL in the cancer group and 6.9 and 35.0 ng/mL in the comparison group. No significant associations between serum*

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PFOA or PFOS levels and the risk of prostate, bladder, pancreatic, or liver cancer were found. Although 31–38% increases in prostate cancer was found in the second, third, and fourth serum PFOS quartiles, there was no difference between the quartiles and the 95% CI included unity."

The ATSDR reviewed Hardell et al. (2014) which examined the possible association between prostate cancer and perfluoroalkyls among 201 cases with 186 age-matched controls living in Sweden. The ATSDR reported on the results as showing: *"No significant increases in the risk of prostate cancer were associated with serum PFOA, PFOS, PFHxS, PFNA, PFDeA, or PFUA levels; similarly, there were no associations with Gleason score or prostate-specific antigen (PSA) levels. When serum perfluoroalkyl levels greater than the median were combined with heredity as a risk factor (firstdegree relative with prostate cancer), significant increases in the ORs were found for PFHxS (OR 4.4, 95% CI 1.7–12), PFOS (OR 2.7, 95% CI 1.04–6.8), PFOA (OR 2.6, 95% CI 1.2–6.0), PFDeA (OR 2.6, 95% CI 1.1–6.1), and PFUA (OR 2.6, CI 1.1–5.9)."*

The ATSDR also reviewed Bonefeld-Jørgensen et al. (2011), which examined 31 breast cancer cases among Inuit women in Greenland to evaluate a possible association with blood PFOA and PFOS levels. The comparison group consisted of 115 matched controls. The ATSDR reported on the results: *"Blood levels of PFOS and PFOA was significantly higher in the cancer group, as compared to the comparison group. The median levels of PFOS and PFOA were 45.6 and 2.5 ng/mL in cancer group and 31.1 and 1.6 ng/mL in the comparison group. A significant increase in the likelihood of breast cancer (OR 1.03, 95% CI 1.001–1.07) was only found for PFOS. The study also looked for possible associations between breast cancer and other persistent pollutants. No significant difference in polychlorinated biphenyls (PCBs), organochlorine pesticides, selenium, cadmium, mercury, or lead blood levels were found between the two groups; when the PCB levels were divided into quartiles, the fourth quartile blood PCB levels were significantly higher in the cancer group than in the comparison group. Zinc levels were significantly higher in the cases."*

United States Environmental Protection Agency (US EPA)

The Panel considered the US EPA's 'Health Effect's Support Documents on PFOA and PFOS which the US EPA had used to establish health advisories on PFOA and PFOS for drinking water officials.

Studies reviewed

For PFOA, the US EPA cited:

- five studies in occupational settings (Raleigh et al. 2014; Steenland and Woskie, 2012; Steenland et al. 2015; Olsen et al. 1998a; Olsen et al. 2000);
- three studies in high exposure communities (Vieira et al. 2013; Barry et al. 2013; Innes et al. 2014);
- five general population studies (Eriksen et al. 2009; Bonefeld-Jørgensen et al. 2014; Hardell et al. 2014; Vassiliadou et al. 2010; Yeung et al. 2013).

The US EPA also referred to the systematic review by Chang et al. (2014).

For PFOS, the US EPA cited:

- four studies on occupational exposure (Alexander et al. 2003; Alexander and Olsen 2007; Mandel and Johnson 1995; Grice et al. 2007);
- one study in high exposure communities (Innes et al. 2014);
- five studies on general exposure (Bonefeld-Jørgensen et al. 2014; Bonefeld-Jørgensen et al. 2011; Eriksen et al. 2009; Hardell et al. 2014; Vassiliadou et al. 2010).

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Of the studies on PFOA, six have been reviewed by the ASTDR, and are covered in the section above (Steenland and Woskie, 2012; Vieira et al. 2013; Barry et al. 2013; Innes et al. 2014; Eriksen et al. 2009; Hardell et al. 2014).

Of the studies on PFOS, six have been reviewed by the ASTDR, and are covered in the section above (Alexander et al. 2003; Alexander and Olsen 2007; Grice et al. 2007; Bonefeld-Jørgensen et al. 2011; Eriksen et al. 2009; Hardell et al. 2014).

Considerations and conclusions

PFOA

In their 'Executive Summary' for PFOA, The US EPA stated that: "*Human epidemiology data report associations between PFOA exposure and ... cancer (testicular and kidney).*"

Also, in the Executive Summary, the US EPA noted: "*Under EPA's Guidelines for Carcinogen Risk Assessment (USEPA 2005a), there is "suggestive evidence of carcinogenic potential" for PFOA. They note further: "Epidemiology studies demonstrate an association of serum PFOA with kidney and testicular tumors among highly exposed members of the general population. Two chronic bioassays of PFOA support a positive finding for its ability to be tumorigenic in one or more organs of rats, including the liver, testes, and pancreas. EPA estimated a cancer slope factor (CSF) of 0.07 (mg/kg/day)-1 based on testicular tumors. As a comparative analysis, the concentration of PFOA in drinking water that would have a one-in-a million increased cancer risk was calculated using the oral slope factor for testicular tumors, assuming a default adult body weight of 80 kg and a default drinking water intake (DWI) rate of 2.5 liter per day (L/day) (USEPA 2011). This concentration is lower than the concentration for cancer (also derived with adult exposure values), indicating that a guideline derived from the developmental end point will be protective for the cancer end point.*"

In the 'Summary and conclusion's from the 'Human cancer epidemiology studies' section, the US EPA stated that: "*Evidence of carcinogenic effects of PFOA in epidemiology studies is based on studies of kidney and testicular cancer. These cancers have relatively high 5-year survival rates of 73% for kidney cancer and 95% for testicular cancer (based on National Cancer Institute [NCI] Surveillance, Epidemiology, and End Results data for 2005–2011). Thus studies that examine cancer incidence are particularly useful for these types of cancer. The high-exposure community studies also have the advantage for testicular cancer of including the age period of greatest risk, as the median age at diagnosis is 33 years. The two occupational cohorts in Minnesota and West Virginia (most recently updated, respectively, in Raleigh et al. 2014 and Steenland and Woskie 2012) do not support an increased risk of these cancers, but each of them is limited by a small number of observed deaths and incident cases. Two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrollment of 24 ng/mL) and kidney and testicular cancers (Barry et al. 2013; Vieira et al. 2013). There is some overlap in the cases included in these studies. None of the general population studies examined kidney or testicular cancer, but no associations were found in the general population between mean serum PFOA levels up to 86.8 ng/mL and colorectal, breast, prostate, bladder, or liver cancer (Bonefeld-Jørgensen et al. 2014; Eriksen et al. 2009; Hardell et al. 2014; Innes et al. 2014)*".

The US EPA noted the findings of the C8 Science Panel on cancer, and also referred to the systematic review by Chang et al. (2014), noting: "*A group of independent toxicologists and epidemiologists critically reviewed the epidemiological evidence for cancer based on 18 studies of occupational exposure to PFOA and general population exposure with or without coexposure to PFOS. The project was funded by 3M, but the company was not involved in the preparation or approval of the report. The authors evaluated the published studies based on the study design, subjects, exposure*

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assessment, outcome assessment, control for confounding, and sources of bias. They followed the Bradford Hill guidelines on the strength of the association, consistency, plausibility, and biological gradient in reaching their conclusion. They found a lack of concordance between community exposures and occupational exposures one or two magnitudes higher than those for the general population. The discrepant findings across the study populations were described as likely due to chance, confounding, and/or bias (Chang et al. 2014)."

PFOS

The US EPA stated in the 'Executive Summary' of the Health Effects support document for PFOS that: "*Several human epidemiological studies evaluated the association between PFOS and cancers including bladder, colon and prostate but these data present a small number or cases and some are confounded by failure to adjust for smoking.*"

Also in the 'Executive Summary', the US EPA stated: "*Applying the U.S. EPA Guidelines for Carcinogen Risk Assessment, there is suggestive evidence of carcinogenic potential for PFOS (USEPA 2005a). ...Human epidemiology studies did not find a direct correlation between PFOS exposure and the incidence of carcinogenicity in worker-based populations. Although one worker cohort found an increase in bladder cancer, smoking was a major confounding factor, and the standardized incidence ratios were not significantly different from the general population. Other worker and general population studies found no statistically-significant trends for any cancer type. Thus, the weight of evidence for the carcinogenic potential to humans was judged to be too limited to support a quantitative cancer assessment.*"

In the 'Summary and Conclusions from the Human Cancer Epidemiology Studies' for PFOS, the US EPA reported: "*A small number of epidemiology studies of PFOS exposure and cancer risk are available. While these studies do report elevated risk of bladder and prostate cancers, limitations in design and analysis preclude the ability to make definitive conclusions. While an elevated risk of bladder cancer mortality was associated with PFOS exposure in an occupational study (Alexander et al. 2003), a subsequent study to ascertain cancer incidence in the cohort observed elevated but statistically insignificant incidence ratios that were 1.7- to 2-fold higher among workers with higher cumulative exposure (Alexander and Olsen 2007). The risk estimates lacked precision because the number of cases was small. Smoking prevalence was higher in the bladder cancer cases, but the analysis did not control for smoking because data were missing for deceased workers, and therefore positive confounding by smoking is a possibility. Mean PFOS serum levels were 941 ng/mL. No elevated bladder cancer risk was observed in a nested case control study in a Danish cohort with plasma PFOS concentrations at enrollment of 1–130.5 ng/mL (Eriksen et al. 2009).*"

"*Elevated odds ratios for prostate cancer were reported for the occupational cohort examined by Alexander and Olsen (2007) and the Danish population-based cohort examined by Eriksen et al. (2009). However, the confidence intervals included the null, and no association was reported by another case-control study in Denmark (Hardell et al. 2014). A case-control study of breast cancer among Inuit females in Greenland with similar serum PFOS levels to those of the Danish population (1.5–172 ng/mL) reported an association of low magnitude that could not be separated from other perfluorsulfonated acids, and the association was not confirmed in a Danish population (Bonefeld-Jørgensen et al. 2011, 2014). Some studies evaluated associations with serum PFOS concentration at the time of cancer diagnosis, and the impact of this potential exposure misclassification on the estimated risks is unknown (Bonefeld-Jørgensen et al. 2011; Hardell et al. 2014). No associations were adjusted for other perfluorinated chemicals in serum in any of the occupational and population-based studies.*"

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Summaries of studies reviewed

PFOA

Occupational exposure settings

The US EPA reviewed five studies in occupational settings: Raleigh et al. 2014; Steenland and Woskie, 2012; Steenland et al. 2015; Olsen et al. 1998a; and Olsen et al. 2000.

The US EPA reported on Raleigh et al. (2014), as being the latest update of the analyses of mortality in the 3M Cottage Grove workers (which was previously analysed in Lundin et al. 2009, and Gilliland and Mandel 1993). The US EPA outlined the details of the study: "*Raleigh et al. (2014) followed 4,668 Cottage Grove workers through 2008, using an improved exposure reconstruction method and adding a nonexposed worker referent group from a different 3M plant. In addition to the mortality data, incidence data based on state cancer registries also were included. Exposure estimates for inhalation exposures were calculated from work history records and industrial hygiene monitoring data; blood levels were not included. No associations were found between PFOA exposure and the risk of dying from any cancer type (see Table 3-12 for bladder, kidney, and testicular cancer results). The mean age of the workers was 29 years at the start of employment and 63 years at the end of follow-up.*"

The most recent report on the same cohort (same as Steenland and Woskie (2012) who updated the cohort study by Leonard et al. (2008) of employees at the DuPont Washington Works plant in West Virginia) was undertaken by Steenland et al, 2015. The US EPA reported that this study "*included 6,026 workers evaluated for disease incidence, based on self-report with validation from medical records (Steenland et al. 2015). Lifetime serum cumulative dose was estimated by combining occupational and nonoccupational exposures. Median measured serum level was 113 ng/mL based on samples collected in 2005. Bladder cancer incidence (n = 29 cases) decreased with increased PFOA levels (RR 1.0, 0.55, 0.47, and 0.31 across quartiles, trend p = 0.03). Prostate cancer risk increased in Q1 compared to Q2 (n = 1.92), and remained at this level in the remaining quartiles (RR 1.89 and 2.15 in Q3 and Q4, respectively, trend p = 0.10).*"

The US EPA reported on the study of Bonefeld-Jørgensen et al. (2014), which evaluated a subset of females enrolled in the Danish National Birth Cohort for an association between plasma PFOA levels (as well as 15 other PFASs) measured during pregnancy and risk of breast cancer during a follow-up period of 10–15 years. The US EPA provided the following details about the study: "*A total of 250 females diagnosed with breast cancer were matched for age and parity with 233 controls. The mean PFOA level in the controls was 5.2 ng/mL while levels in the cases were divided into quintiles ranging from 6.5 ng/mL. No association was found between PFOA levels and breast cancer risk. A weak positive association was found only with perfluorooctane sulphonamide.*"

High-exposure communities

The US EPA reviewed three studies in high exposure communities (Vieira et al. 2013; Barry et al. 2013; Innes et al. 2014). The ATSDR reviewed these studies, and summaries are provided above.

General population studies

The US EPA reviewed five general population studies (Eriksen et al. 2009; Bonefeld-Jørgensen et al. 2014; Hardell et al. 2014; Vassiliadou et al. 2010; Yeung et al. 2013). The studies by Eriksen et al. (2009) and Hardell et al. (2014) were reviewed by the ATSDR, with summaries provided above.

The US EPA reported on two studies (Vassiliadou et al. 2010; and Yeung et al. 2013), which "*found no differences in blood and tissue PFOA levels between cancer and noncancer patients; the types of cancer in the patients were not defined*".

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The US EPA noted that: "*Vassiliadou et al. (2010) found that median serum PFOA concentrations among 40 cancer patients (2.27 ng/mL in males; 1.85 ng/mL in females) were similar to two control groups (3.14 and 1.81 ng/mL in males; 1.7 and 1.71 ng/mL in females).*"

Of the study by Yeung et al. (2103), the US EPA reported: "*Yeung et al. (2013) found similar PFOS levels in serum and liver tissue between controls and those with hepatocellular carcinoma. Median serum levels in controls (n = 25) and patients with liver cancer (n = 24) were 2.34 and 2.5 ng/mL, respectively, and liver tissue were 0.506 (n = 9) and 0.495 (n = 12) ng/g, respectively.*"

Of the study by Bonefeld-Jørgensen et al. (2014), the US EPA reported: "*A subset of females enrolled in the Danish National Birth Cohort was evaluated for an association between plasma PFOA levels (as well as 15 other PFASs) measured during pregnancy and risk of breast cancer during a follow-up period of 10–15 years (BonefeldJørgensen et al. 2014). A total of 250 females diagnosed with breast cancer were matched for age and parity with 233 controls. The mean PFOA level in the controls was 5.2 ng/mL while levels in the cases were divided into quintiles ranging from 6.5 ng/mL. No association was found between PFOA levels and breast cancer risk. A weak positive association was found only with perfluorooctane sulphonamide.*"

PFOS

Occupational exposure studies

The US EPA reviewed four studies on occupational exposure (Alexander et al. 2003; Alexander and Olsen 2007; Mandel and Johnson 1995; Grice et al. 2007). The only study not previously reviewed by the ATSDR was the study by Mandel and Johnson et al. (1995). The US EPA reported the following about this study: "*Several analyses of various health outcomes have occurred on cohorts of workers at the 3M Decatur, Alabama plant (Alexander et al. 2003; Alexander and Olsen 2007; Mandel and Johnson 1995). Cause-specific mortality was examined in a cohort of 2,083 workers employed for at least 1 year among workers grouped into three PFOS exposure categories: nonexposed, low exposed, and high exposed. Exposure classifications were determined using PFOS serum concentrations measured in a subset of workers linked to specific jobs and work histories. Cumulative exposures were also estimated by applying a weight to each of the exposure categories and multiplying by the number of years of employment for that job for each individual. The geometric mean serum PFOS levels were 941 ng/mL for chemical plant employees and 136 ng/mL for non-exposed workers. Results of these studies are summarized in Table 3-8. A total of 145 deaths were identified with 65 of them in high-exposure jobs. Standardized mortality ratios (SMRs) were calculated using the state of Alabama reference data and when analyzing the entire cohort, SMRs were not elevated for most of the cancer types and for nonmalignant causes. SMRs that were above 1 included cancer of the esophagus, liver, breast, urinary organs, bladder, and skin. However, the number of cases was very small (1–3), resulting in wide confidence intervals. The SMRs for these causes (except breast cancer) were also elevated when the cohort was limited to the 65 employees ever employed in a high exposure job. The SMR for bladder cancer was 4.81 (95% CI: 0.99–14.06). Three male employees in the cohort died of bladder cancer (0.62 expected). All were employed at the Decatur plant for > 20 years and had worked in high exposure jobs for at least 5 years. The SMR for bladder cancer for workers who were ever employed in a high exposure job was 12.77 (0.23 expected, CI: 2.63–37.35). When the data were analyzed for workers with > 5 years of employment in a high exposure job, the SMR was 24.49. This effect remained when the data were analyzed using county death rates. While the three deaths from bladder cancer were greater than the expected number observed in the general population, the small number of deaths (especially for females in all categories) precludes a definitive conclusion regarding an association with PFOS exposure. In*

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addition, six death certificates were not obtained, and smoking status was not known for the cohort increasing the uncertainty with regard to the estimated risk."

High-exposure communities

The US EPA reviewed the study by Innes et al. (2014). This study was reviewed by the ATSDR, with a summary of the study provided in that section.

General population studies

The US EPA reviewed five studies on general population exposure (Bonefeld-Jørgensen et al. 2014; Bonefeld-Jørgensen et al. 2011; Eriksen et al. 2009; Hardell et al. 2014; Vassiliadou et al. 2010). The ATSDR did not review the studies by Bonefeld-Jørgensen et al. 2014.

The US EPA reported on the study undertaken by Bonefeld-Jørgensen et al. 2014, in which a subset of females enrolled in the DNBC were evaluated for an association between plasma PFOS levels (as well as 15 other perfluoroalkylated substances) measured during pregnancy and risk of breast cancer during a follow-up period of 10–15 years. The US EPA provided the following detail about the study: *"A total of 250 females diagnosed with breast cancer were matched for age and parity with 233 controls. The mean PFOS level in the controls was 30.6 ng/mL while levels in the cases were divided into quintiles ranging from < 20.4 up to > 39.1 ng/mL. No association was found between PFOS levels and breast cancer risk in logistic regression models adjusted for age at blood draw, BMI before pregnancy, gravidity, use of oral contraceptives, age at menarche, smoking, alcohol consumption, maternal education and physical activity. A weak positive Relative Risk (1.04; 95% CI: 0.99–1.08) was found only with perfluorooctane-sulfonamide."*

The US EPA refers to Vassiliadou et al. 2010 as *"a small study"*, and noted that it *"found no differences in blood PFOS levels between cancer and non-cancer patients; the types of cancer in the patients were not defined. [the authors] found median serum PFOS concentrations among 40 cancer patients (11.3 ng/mL, males; 8 ng/mL, females) were similar to two control groups (10.5 and 13.7 ng/mL, males; 7 and 8.5 ng/mL, females)."*

International Agency for Research on Cancer (IARC)

In 2016, the IARC published its evaluation of the cancer hazard of PFOA as a Monograph, based on the conclusions of a Working Group comprising invited scientists from relevant disciplines. The IARC Monograph examined studies, dating back to the 1980s on exposure data, studies of cancer in humans, studies of cancer in experimental animals and mechanistic and other relevant data.

Studies reviewed

IARC examined epidemiological studies on the occurrence of cancer in humans exposed to PFOA in three different types of populations:

- five cohort studies of workers in chemical plants producing or using PFOA in the US: two in plants in West Virginia (Leonard et al. 2008; Steenland and Woskie, 2012); and three in Minnesota (Gilliland and Mandel, 1993; Lundin et al. 2009; Raleigh et al. 2014);
- three community-based studies (high-exposure setting) (Vieira et al. 2013b; Barry et al. 2013; Innes et al. 2014);
- three case-control studies of cancer of the bladder, liver, prostate, pancreas, or breast in the general population with background exposures (Eriksen et al. 2009; Hardell et al. 2014; Bonefeld-Jørgensen et 2011).

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IARC also cited one study of workers producing tetrafluorethylene (Consonni et al. 2013), which it stated "*also provides some potentially relevant information but was not included in the tables because the study population overlapped with other studies, and the assessment of exposure to PFOA was limited. This study is reviewed in detail in the Monograph on tetrafluoroethylene, in the present volume.*"

IARC also cited two other studies that examined workers at a plant producing perfluorooctanesulfonyl fluoride in a plant in Alabama, USA (Alexander et al. 2003; Alexander & Olsen, 2007). IARC stated: "*The manufacturing process produced PFOA as a by-product, and PFOA was also used in some other production processes and was manufactured at the plant beginning in 1998. The focus of the studies in this plant has been on perfluorooctanesulfonate (PFOS) exposure measures, which are higher than, but correlated with PFOA exposures (Olsen et al. 2003a); these studies are not discussed further here.*"

Considerations and conclusions

In the 'Evaluation' section of the Monograph, IARC stated for cancer in humans: "*There is 'limited evidence' in humans for the carcinogenicity of perfluorooctanoic acid (PFOA). A positive association was observed for cancers of the testis and kidney*", and for cancer in experimental animals "*There is limited evidence in experimental animals for the carcinogenicity of perfluorooctanoic acid (PFOA).*"

The IARC provided this overall evaluation: "*Perfluorooctanoic acid (PFOA) is possibly carcinogenic to humans (Group 2B).*"

In the 'Human carcinogenicity data' section, IARC stated: "*The literature on the epidemiology of cancer in relation to PFOA is relatively small and includes studies in three different types of populations: workers exposed in chemical plants producing or using PFOA, high-exposure communities (i.e. areas surrounding a plant with documented release of PFOA and contamination of public and private water supplies), and studies in the general population with background exposures.*"

For 'Cancer of the testis', IARC stated: "*The only informative results on risk of cancer of the testis were from two studies of cancer incidence in a high-exposure community setting in West Virginia and Ohio, USA; there was some overlap in the cases examined in these studies. Both publications, using different study designs (i.e. a cohort study of incidence and a population-registry case–control study), observed an increased risk of incidence of cancer of the testis. In the highest quartile of exposure in both studies, the observed increase in risk was approximately threefold, with a significant trend in increasing risk with increasing exposure in the cohort study (no trend test was reported in the case–control study). The evidence for cancer of the testis was considered credible and unlikely to be explained by bias and confounding, however, the estimate was based on small numbers.*"

For 'Cancer of the kidney', IARC stated: "*There were several publications that have examined PFOA and risk of cancer of the kidney. Three of these were conducted in West Virginia, USA, and included occupational and community exposure, and the fourth was conducted in a different occupational setting. In the exposure– response analysis of workers in West Virginia, 8 of the 12 deaths from cancer of the kidney were seen in the highest quartile of exposure, with an elevated standardized mortality ratio and a significant trend in increasing risk with increasing exposure. The other occupational cohort study reported no evidence for increased incidence. A modestly increased risk of incidence of cancer of the kidney was seen in a community population with high exposure. A study in a somewhat overlapping population also found elevated relative risks in the groups with high and very high exposure compared with the group with low exposure. The evidence for cancer of the kidney was considered credible; however, chance, bias, and confounding could not be ruled out with reasonable confidence.*"

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For 'Other cancer sites', IARC stated: "*The evidence regarding other cancer sites, including the urinary bladder, thyroid, prostate, liver, and pancreas was also evaluated. Some positive associations were observed for cancers of the bladder, thyroid, and prostate, but the results were inconsistent among studies and based on small numbers. The evidence for carcinogenicity for all of these sites was judged to be inadequate.*"

In its Monograph, IARC also provided exposure data on PFOA for the general population, for people living near industrial sources of PFOA and for workers with occupational exposure[13]. IARC has 299 agents currently classified as Group 2B.

Summaries of studies reviewed

Occupational exposure studies

IARC reviewed five cohort studies (Leonard et al. 2008; Steenland and Woskie, 2012; Gillaland and Mandel, 1993; Lundin et al. 2009; Raleigh et al. 2014). Three of these studies were reviewed by the ATSDR and US EPA (2016a). The US EPA reviewed the study by Raleigh et al. 2014, which the ATSDR did not review. Additional details about these studies, included in the IARC Monograph, are reported below.

IARC provided a general overview of these cohorts, noting: "*For each of these cohorts, plant operations began around 1950; the study in West Virginia included individuals who had worked at least 1 day (Steenland & Woskie, 2012), while the Minnesota cohort required at least 365 work days for inclusion (Raleigh et al. 2014). The proportion of women was approximately 20%, and each was a relatively young cohort. The studies included a cumulative-exposure indicator based on a job-exposure matrix developed using serum PFOA concentrations in workers or air-monitoring data,but differed in terms of the extent of available samples and modelling of exposure, with consideration of changes in exposure over time. Standardized mortality ratios (SMR) for all causes, all cancers, and heart disease ranged from 0.7 to 1.0.*"

Of the study by Steenland and Woskie (2012), IARC noted: "*Trends of increasing risk of cancer of the kidney and mesothelioma with increasing exposure to PFOA (P = 0.02) were observed, with standardized mortality ratios of 2.66 (95% CI, 1.15–5.24; 8 cases) and 6.27 (95% CI, 2.04–14.63; 5 cases), respectively, in the highest quartile of PFOA exposure. There was no indication of increased risk for cancers of the bladder, liver, pancreas, breast, or prostate...*"

IARC commented about this study: "*A strength of this study was the detailed exposure analysis, while a limitation was the small numbers. The Working Group interpreted the association between PFOA exposure and risk of mesothelioma to be an indication of exposure to asbestos in these workers.*"

IARC provided additional details to those provided by the US EPA, above, about the study by Raleigh et al. (2014): "*Raleigh et al. (2014) examined mortality risk in 4668 workers (1125 deaths) in a plant manufacturing ammonium perfluorooctanoate in Minnesota, USA, with a mean follow-up of 34 years. Exposure assessment was based on 205 personal air samples and 659 area samples collected from production areas in 1977–2000; exposures before 1977 were estimated based on variation in annual production levels; procedures and tasks had not changed over this period. The exposure data were combined with job-history data (department, job title, work area, equipment, task and year) to estimate*

[13] Serum levels of PFOA measured in the general population worldwide are less than about 10 ng/mL. For people living near industrial sources of PFOA, mean concentrations of PFOA have ranged from near-background concentrations to > 200 ng/mL. The IARC notes that the predominant route of exposure was drinking water. In groups of workers with occupational exposure to PFOA, through inhalation and dermal contact occurring during fluoropolymer production, mean serum concentrations were measured as > 1000 ng/mL.

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time-weighted average exposures, which were then used to estimate cumulative exposure estimates for individual workers. Mortality was analysed for the period 1960–2008. Incidence data, based on Minnesota and Wisconsin state cancer registries were also included, but were limited to cases occurring since 1988, when both of these registries were in operation. Workers at another plant in the area, manufacturing tape and abrasive products, were used as the referent group (n = 4359) for internal analyses of mortality and incidence. For mortality from cancer of the bladder, the relative risk estimate for the combined upper two quartiles of exposure (compared with unexposed referents) was 1.96 (95% CI, 0.63–6.15; 5 cases); in the analysis of incidence of cancer of the bladder (40 exposed cases), the pattern across the four quartiles of cumulative exposure was 0.81, 0.78, 1.50, and 1.66, respectively (Table 2.1). Cancer of the kidney was not associated with exposure to PFOA in analyses of mortality (6 exposed cases) or incidence (16 exposed cases). Examination of incidence and mortality data in relation to cumulative exposure revealed little or no evidence of increased risk of cancer of the liver, pancreas, prostate, or breast. Risks were not analysed for cancers of the thyroid or testes."

IARC made the following comments about this study: "*The Working Group noted the reasonable quality of the exposure data. Another strength of this study was the use of incidence data, but this analysis covered only a 20-year period, which limited the number of observed cases for some cancers.*"

IARC provided the following summary of the studies it reviewed on cancer and PFOA exposure in occupationally-exposed workers: "*In summary, these studies conducted in two different occupational cohorts included some evidence of an association between PFOA exposure and cancer of the kidney (Steenland & Woskie, 2012) or bladder (Raleigh et al. 2014), with elevated risks seen at higher exposures in one (but not both) of the studies. Elevated risk of cancer of the liver, pancreas, or breast in relation to higher exposure was not seen in either study, and the initial report of an increased risk of cancer of the prostate (Lundin et al. 2009) was not substantiated in subsequent analyses (Steenland & Woskie, 2012; Raleigh et al. 2014). These studies did not provide a basis for examining cancer of the testes or thyroid, since an analysis of incidence data was not available for these cancers.*"

High-exposure community studies

IARC reviewed three high-exposure community-based studies on PFOA and cancer (Vieira et al. 2013b); Barry et al. 2013; Innes et al. 2014).

IARC provided the following background and comment about these studies: "*An area along the Ohio River in West Virginia and Ohio, USA, surrounding one of the fluoropolymer production plants described in the previous section has been the site of a series of community health studies. Emissions from this plant resulted in contamination of public water systems and private wells with PFOA. Three studies examined cancer risk for multiple cancer types (Barry et al. 2013; Vieira et al. 2013b) or specifically for cancer of the colon (Innes et al. 2014). The Working Group noted that Barry et al. (2013) and Vieira et al. (2013b) were overlapping, rather than independent studies, in that the same geographical areas and some of the same cases are included in both analyses.*"

IARC provided additional details, to those by the ATSDR, above, about the study by Vieira et al. (2013): "*Using a case–control design, Vieira et al. (2013b) examined incident cancers occurring in 1996–2005, using West Virginia and Ohio state cancer registries. Cases living in 13 counties around the fluoropolymer production plant were identified; analyses were limited to 18 cancer types that were of a-priori interest, or that had at least 100 cases in each state. The controls for each analysis were all other cancer types, excluding cancers of the kidney, liver, pancreas, and testes. In one set of analyses, residence at time of diagnosis was used to assign study participants to specific water districts in Ohio and West Virginia (Vieira et al. 2010, 2013a). A more robust exposure assessment was used in the second set of case–control analyses, restricted to the Ohio data, where exposure was estimated based*

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on street-level data. This information was combined with emission data, environmental characteristics, and pharmacokinetic data to estimate annual exposure from 1951 to date of diagnosis, assuming that residence at time of diagnosis was the residence for the previous 10 years (Shin et al. 2011a, b). Residence in a contaminated water district was not associated with a notable increase in the risk of any cancer. In analyses of cancer incidence in relation to estimated serum PFOA concentrations, elevated risks of cancer of the kidney (2.0; 95% CI, 1.0–3.9; 9 cases) and testes (2.8; 95% CI, 0.8–9.2; 6 cases), and more modestly increased risks for cancer of the prostate (1.5; 95% CI, 0.9–2.5; 31 cases), and breast (1.4; 95% CI, 0.9–2.3; 29 cases) were observed in the upper 10% of the exposure distribution. There was no indication of an increased risk of cancers of the bladder, liver, pancreas, or thyroid..."

IARC commented about this study: "*A strength of this study was its use of incidence data. A limitation was that for the part of the sample residing in West Virginia, it was not possible to conduct the more detailed exposure assessment based on street addresses, reducing the sample size for these analyses. Another limitation was that the residential data were limited to only one residence (i.e. residence at time of diagnosis), rather than a more complete residential history.*"

IARC also reviewed the study by Barry et al. (2013), and provided additional details to those reported under the ATSDR (above): "*Barry et al. (2013) included exposure–response analyses based on cumulative exposure measures for cancers of the kidney, testes, and thyroid. In analyses with no exposure lag, the relative risks for cancer of the kidney (n = 105 cases) were 1.23, 1.48, and 1.58 in quartiles 2, 3, and 4, respectively, compared with the lowest quartile of exposure (P for trend, based on continuous variable measure, 0.10). For cancer of the testes (n = 17 cases), relative risks of 1.04, 1.91, and 3.17 across quartiles of exposure were observed (P for trend, 0.05). The trend P using another test (i.e. using median values of quartiles) was 0.04, and the two P values for trend in the 10-year lagged analysis were 0.02 and 0.10, respectively, for quartile and continuous analysis. For cancer of the thyroid, the relative risks by quartile were 1.54, 1.48, and 1.73 (P for trend, 0.20). Similar results were obtained with a 10-year exposure lag. There was no indication of increased risk for the other cancer sites (liver, pancreas, prostate, and breast)...*"

IARC's comment about this study was: "*The strengths of this study included its use of incidence data and individual-level exposure modelling using lifetime residential history, and the validation of the exposure modelling.*"

IARC reported the following detail about the study by Innes et al. (2014): "*Innes et al. (2014) conducted a case–control study of prevalent cases of cancer of the colorectum among 47 359 participants in the C8 Health Project (see Barry et al. 2013), using medical history and blood samples collected in the 2005–2006 survey. Self-reported cases of cancer of the colorectum, verified by chart review (n = 208) were compared to the 47 151 participants who did not report a history of any type of cancer. An inverse association was seen between serum PFOA concentrations and risk of cancer of the colorectum, including in analyses restricted to cases diagnosed within the past 6 years who had lived in the same residence for the previous 10 or 15 years...*"

IARC made the following comment about this study: "*A limitation of this study was that the PFOA measurements were taken after diagnosis, and so may not have reflected the etiologically relevant exposure to PFOA.*"

General population studies

IARC reviewed three case-control studies of cancer of the bladder, liver, prostate, pancreas, or breast in the general population with background exposures (Eriksen et al. 2009; Hardell et al. 2014; Bonefeld-Jørgensen et 2011).

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IARC provided the following background to these studies: "*Three population-based case–control studies were available that examined PFOA serum concentrations in relation to various types of cancer (Eriksen et al. 2009; Bonefeld- Jørgensen et al. 2011; Hardell et al. 2014). Exposure levels in these studies were considerably lower than those seen in the community studies of high exposure or occupational studies described previously.*"

Of the study by Eriksen et al. (2009), IARC provided more detail than the ATSDR, including: "*There was no association between variation in PFOA exposure in this population and risk of cancers of the bladder or liver (Table 2.3). For cancer of the pancreas, the rate ratio in the highest quartile was 1.55 (95% CI, 0.85–2.80), and for cancer of the prostate the corresponding rate ratio was 1.18 (95% CI, 0.84–1.65). PFOS was also measured in the blood samples; the correlation between PFOA and PFOS was r = 0.70. PFOS was not associated with cancers of the bladder, liver, or pancreas. For cancer of the prostate, however, the rate ratio for the highest quartile of PFOS exposure was 1.38 (95% CI, 0.99–1.93).*"

IARC commented about this study: "*A strength of this study was that the PFOA measurements were based on samples collected before diagnosis, and thus are likely to reflect an etiologically relevant time-window of exposure; however, the number of cases of cancer of the liver was relatively small. Another limitation was the relatively high correlation between PFOA and PFOS, which hampered interpretation of the association with cancer of the prostate seen with each of these exposures.*"

For the study by Hardell et al. (2014), IARC provided the following detail: "*PFOA concentration was measured in whole blood samples collected after enrolment (i.e. after diagnosis for cases); among controls, the median PFOA concentration was 1.9 ng/mL (range, 0.35–8.4 ng/mL). There was no association between PFOA concentration and cancer of the prostate in the analysis of the full sample, but a relative risk of 2.6 (95% CI, 1.2–6.0) was seen among individuals who reported a first-degree relative with cancer of the prostate, and who had a serum PFOA concentration that was above the median for controls (compared with individuals with no family history of cancer of the prostate and serum PFOA concentration that was greater than the median for controls) ...*"

Of this study IARC commented: "*A limitation of this study was that the PFOA measurements were taken after diagnosis, and so may not reflect a relevant time-window of exposure.*"

Of the study by Bonefeld-Jørgensen et al. (2011), IARD reported: "*Serum PFOA concentrations were measured in samples taken at the time of diagnosis for cases, and at enrolment for controls; among controls, the median PFOA concentration was 1.6 ng/L (95% CI, 2.11–2.90). Only 7 cases and 69 controls were included in analyses adjusting for covariates (age, body mass index, pregnancy, cotinine, breastfeeding, and menopausal status) because of missing data (Table 2.3).*"

IARC raised the following issue about this study: "*The Working Group considered this study to be uninformative because of the small sample size resulting from the high proportion of missing covariate data.*"

Dutch National Institute for Public Health and the Environment (RIVM)

The **RIVM** (2017) reported on PFOA and testicular and kidney cancer.

Studies reviewed

The RIVM reviewed:

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- eight international reviews and reports (C8 Science Panel, 2012b; Health Council Netherlands, 2013; ATSDR, 2015; ECHA-RAC, 2015a; ECHA-RAC 2015b; DWQI 2016; IARC 2016;US EPA, 2016a);
- one systematic review (Chang et al. 2014); and
- four epidemiological studies (Barry et al. 2013; Vieira et al. 2013; Lundin et al. 2009; Steenland and Woskie, 2012).

Considerations and conclusions

RIVM reported specifically on PFOA exposure and cancer of the testis and kidneys, concluding that for these cancers: "*the evidence is 'less clear'*" and "*Indications have also been found for a higher risk of ...testis and kidney cancer.*"

In the 'Discussion and conclusions' section, the RIVM stated: "*In summary, four out of seven international organizations have concluded that an association potentially exists between PFOA exposure and testicular and kidney cancer, but the epidemiological studies have some limitations. It should also be noted that the number of epidemiological studies that have investigated testicular and/or kidney cancer is limited and were performed only in study populations that are part of the C8 Health Study.*"

Summaries of studies reviewed

The four studies reviewed by the RIVM were reviewed by the ATSDR, US EPA and IARC, with summaries provided above.

RIVM noted the following about PFOA exposure levels in the two occupational studies: "*In both studies performed in occupational study populations (Lundin et al. 2009; Steenland and Woskie, 2012) there were insufficient cases or no cases to examine testicular cancer. Lundin et al. (2009) found no association with kidney cancer. Steenland and Woskie (2012) observed that kidney cancer was more likely to occur in DuPont workers in Parkersburg, who had estimated cumulative blood concentrations of 1,819 ppm-years (ppm=parts per million; 1 ppm=1000 ng/mL). To illustrate what ppm years entail, Steenland and Woskie (2012) reported that, for example, 100 ppm over five years would be equal to 500 ppm-years.*"

6.1.3. Systematic reviews

Chang et al. (2014)

Chang et al. (2014) undertook a critical review of the human epidemiological literature on PFOS and PFOA and cancer, noting in the Abstract that: "*PFOS and PFOA are ubiquitous synthetic chemicals with no known effect on human cancer development.*"

Studies reviewed

Chang et al. (2014) examined 18 epidemiologic studies in all (Ubel et al. 1980; Gilliland and Mandel, 1993; Leonard et al. 2008; Eriksen et al. 2009; Lundin et al. 2009; Vassiliadou et al. 2010; Bonefeld-Jorgenson et al. 2011; Steenland and Woskie 2012; Barry et al. 2013; Consonni et al. 2013; Vieira et al. 2013; Yeung et al. 2013; Hardell et al. 2014; Innes et al. 2014; Alexander et al. 2003; Olsen et al. 2004; Alexander and Olsen 2007; Grice et al. 2007) looking at the association between PFOA and PFOS exposure and cancer risk in humans.

Of these studies:

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- 14 studies had evaluated the association between PFOA exposure and human cancer (Barry et al. 2013, Bonefeld- Jørgensen et al. 2011, Consonni et al. 2013, Eriksen et al. 2009, Gilliland and Mandel 1993, Hardell et al. 2014, Innes et al. 2014, Leonard et al. 2008, Lundin et al. 2009, Steenland and Woskie 2012, Ubel et al. 1980, Vassiliadou et al. 2010, Vieira et al. 2013, Yeung et al. 2013);
- 10 studies had evaluated the association between PFOS exposure and human cancer (Alexander and Olsen 2007, Alexander et al. 2003, Bonefeld- Jørgensen et al. 2011, Eriksen et al. 2009, Grice et al. 2007, Hardell et al. 2014, Innes et al. 2014, Olsen et al. 2004, Vassiliadou et al. 2010, Yeung et al. 2013).

Note, some studies examined both exposures.

Of these studies, nearly all were reviewed by either ATSDR or the US EPA, with the exception of Consonni et al. 2013 which was reviewed by IARC, and Ubel et al. which only Chang et al. reviewed.

Considerations and conclusions

In the 'Executive Summary', Chang et al. stated: "*Although some statistically significant positive associations have been reported, for example, with cancers of the prostate, kidney, testis, and thyroid, the majority of relative risk estimates for both PFOA and PFOS have been between 0.5 and 2.0 (with 95% confidence intervals including 1.0), inconsistently detected across studies, counterbalanced by negative associations, not indicative of a monotonic exposure-response relationship, and not coherent with toxicological evidence in animals, in which the primary target organs are the liver, testis (Leydig cells), and pancreas (acinar cells). Many positive associations with PFOA exposure were detected in community settings without occupational exposure and were not supported by results in exposed workers. Given that occupational exposure to PFOA and PFOS is one to two orders of magnitude higher than environmental exposure, the discrepant positive findings are likely to be due to chance, confounding and/or bias.*"

The authors concluded: "*Taken together, the epidemiological evidence does not support the hypothesis of a causal association between PFOA or PFOS exposure and cancer in humans.*"

Chang et al. reported that the work of all authors was funded by the 3M Company, but that "*the findings and conclusions are those of the authors and do not necessarily represent the views of 3M.*"

Summaries of studies reviewed

Of Ubel et al. (1980), Chang et al. reported: "*The first study of health outcomes in PFOA production workers was published by Ubel et al. (1980), who reported qualitative results of a cross-sectional analysis and retrospective cohort mortality study of employees at the 3M facility in Cottage Grove (Table 1). This plant consists of several divisions, with PFOA production limited to the chemical division, which produced PFOA from 1947 to 2000. The chemical division also manufactured small amounts of fluorochemicals involving PFOS, but PFOA was the predominant fluorochemical product. Starting in 1976, voluntary medical surveillance examinations, which included measurement of total serum fluorine levels, were offered to fluorochemical workers. The authors reported that based on three annual health evaluations of approximately 300 employees per year beginning in late 1976 (~ 90% of plant workers in each year, with ~ 50% participating during all 3 years), " [n]o health problems related to exposure to fluorochemicals were encountered among those examined " (Ubel et al. 1980). They added that: "a review of absenteeism and illness patterns in these employees does not suggest any work related problems." As described by Ubel et al. (1980), an independent research group conducted a retrospective cohort mortality study among 3,688 workers employed at the Cottage Grove facility for at least 6 months between 1948 and 1978, a period during which 180 deaths (177 with death certificates obtained)*

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were identified. Among the male workers, analyses revealed "no disagreement between the observed mortality and that expected. This was true of all the various causes of death and also of various specific causes of death due to cancer" (Ubel et al. 1980). In analyses restricted to chemical division workers, there were also "no disagreements between observed and expected mortality for any cause of death." Due to the brevity of the study description and the absence of quantitative results, the strengths and limitations of the study methods cannot be thoroughly evaluated. Although this study provides limited evidence regarding the association between PFOA and cancer risk, its findings suggest no notable increase in cancer mortality among fluorochemical workers at the Cottage Grove plant."

Consonni et al. (2013) was reviewed by IARC; however Chang et al. reported the following details about the methodology of the study: "*Consonni et al. (2013) conducted a retrospective cohort mortality study that combined 5,879 male workers (excluding 778 female workers with 16 deaths) at six of the seven TFE production sites in Europe and the United States (excluding a small plant in North Carolina that employed only 31 workers in TFE processes starting in 1979). Although TFE exposure was the main focus of this study, the authors separately analyzed associations with PFOA exposure, which was highly correlated with TFE exposure. The minimum employment tenure varied by facility; all employees at three plants in Italy, England, and New Jersey were included, employees for at least 6 months at the Parkersburg plant were included, and employees for at least 1 year at two plants in Germany and the Netherlands were included in the analysis. The period of follow-up was 1960– 2008 at the Italian site, 1952– 2008 at the English site, 1969 – 2007 at the New Jersey site, 1950 – 2002 at the Parkersburg site, 1965 – 2001 at the German site, and 1967 – 2002 at the Dutch site. Ascertainment of vital status was conducted through linkages to population registries or other statistical or health databases, and death certificates and/ or cause-of-death codes were obtained for 98.8% of known decedents from company-wide, local, state, or national health departments or databases. Time-varying cumulative exposure to PFOA and TFE was estimated semiquantitatively by using a job-exposure matrix with annual PFOA and TFE values for each relevant job title at each production site. The presence or absence of asbestos or vinyl chloride monomer at each plant was also recorded. Expected numbers of cause-specific deaths were calculated based on national age- and calendar-period specific mortality reference rates for males (white males in the United States), with regional or state mortality rates used in sensitivity analyses."*

The findings of this study were reported by Chang et al. as: "*After an average of 25 years of follow-up, significantly fewer than expected deaths from cancer occurred among the 4,205 male workers ever occupationally exposed to PFOA (SMR 0.79 [0.67 – 0.92]), and no site-specific cancer SMRs were significantly elevated (Table 2) (Consonni et al. 2013). When estimated cumulative exposure to PFOA was categorized according to tertiles among observed all-cause deaths in PFOA-exposed workers, no significant excess mortality from total cancer, leukemia, or esophageal, liver, pancreatic, lung, or kidney/other urinary organ cancer was detected in the highest tertile of cumulative exposure, nor was a significant exposure-response trend observed for any of these outcomes. When cumulative exposures to TFE and PFOA were cross-classified, no deaths from any cause were observed (0.8 expected) among workers with high cumulative PFOA exposure and low cumulative TFE exposure, and only three deaths from cancer were observed (6.0 expected) among those with medium cumulative PFOA exposure and low TFE exposure. Thus, associations with PFOA exposure independent of TFE exposure could not be estimated robustly. In general, results were similar when regional mortality rates were used as the reference."*

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Weight of evidence for PFOA/PFOA and cancer in humans

Chang et al. used the main Bradford Hill criteria[14] as a framework to consider the weight of evidence for or against the hypothesis of a causal effect of PFOA or PFOS on human cancer risk. For PFOA, Chang et al. noted: "*Here, the community-based case-control studies (Bonefeld- Jørgensen et al. 2011, Hardell et al. 2014) and cross-sectional studies (Vassiliadou et al. 2010, Yeung et al. 2013), which yielded generally statistically null results, are not considered because their methodological limitations render them largely uninformative for addressing the hypothesis of interest. The cross-sectional study of colorectal cancer in the C8 Health Project (Innes et al. 2014) is included because of its relevance to communities exposed to higher environmental levels of PFOA.*"

For PFOS, Chang et al. excluded the "*lower-quality studies (Bonefeld-Jørgensen et al. 2011, Hardell et al. 2014, Vassiliadou et al. 2010, Yeung et al. 2013) from consideration*".

Below are some of the comments, determinations or conclusions from the Bradford Hill framework reported in Chang et al. (2014).

Strength of association – PFOA

On the strength of association of PFOA, Chang et al. noted: "*Exposure misclassification in these studies may not be nondifferential between cancer cases and noncases and independent of other errors. Exposure misclassification is especially likely to be differential in cross-sectional and casecontrol studies, where exposure status is classified after or simultaneously with disease status, but differential misclassification may also occur in cohort studies, resulting in an unpredictable direction of bias on RR estimates. For example, in a cohort study using a job-exposure matrix to classify exposure, differential error might occur if job title were associated with both the degree of exposure misclassification and the probability of developing or being ascertained with cancer via socioeconomic status (i.e., apart from its role as a surrogate for exposure level). Moreover, even in the presence of nondifferential exposure misclassification, reported associations are not necessarily underestimated. Additional conditions must be satisfied for the bias to be toward the null, and even when all such conditions are met, a given estimate may by chance be biased away from the null (Jurek et al. 2005, Jurek et al. 2008). Thus, it cannot be assumed that more accurate classification of PFOA exposure would necessarily have led to stronger associations in these studies.*"

Strength of association – PFOS

On the strength of association of PFOS, Chang et al. noted: "*As shown in Table 4, most estimated associations between PFOS exposure and cancer have been in the range of 0.5 to 2.0. Except for the striking inverse association between serum PFOS and colorectal cancer prevalence (Innes et al. 2014), RR estimates falling outside this range were typically based on five or fewer cases, with correspondingly imprecise 95% CIs consistent with no association. Confounding, bias, and chance could readily explain such observed associations.*"

Consistency of association – PFOA

In terms of the consistency of association of PFOA, Chang et al. noted: "*Overall, there was no consistent finding across all or even most studies. Perhaps the only positive association that showed some consistency across multiple studies is that with kidney cancer. However, it should be recognized that all of the studies that observed a positive association between estimated PFOA exposure and kidney cancer risk or mortality were based at the Parkersburg plant or in the community surrounding the*

[14] Hill, Austin Bradford (1965). The Environment and Disease: Association or Causation? *Proceedings of the Royal Society of Medicine*. **58** (5): 295–300.

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Parkersburg plant [or, in the case of Consonni et al. (2013), in a study cohort that comprised largely Parkersburg workers] (Barry et al. 2013, Consonni et al. 2013, Leonard et al. 2008, Steenland and Woskie, 2012, Vieira et al. 2013). The three occupational study groups overlapped substantially (Consonni et al. 2013, Leonard et al. 2008, Steenland and Woskie, 2012), as did the two community study groups (Barry et al. 2013, Vieira et al. 2013), in which the same exposure estimation model was applied. Thus, the results of these studies do not constitute independent replications. The only study that reported on kidney cancer outside of the Parkersburg region (Lundin et al. 2009) found that kidney cancer mortality was nonsignificantly lower than expected among workers who were probably directly exposed to PFOA, with no kidney cancer deaths among definitely exposed workers. These findings call into question the consistency and generalizability of the observed kidney cancer association."

Consistency of association – PFOS

In terms of the consistency of association of PFOS, Chang et al. noted: "*Given that all four occupational studies of PFOS exposure and cancer were conducted at the Decatur facility (Alexander and Olsen, 2007, Alexander et al. 2003, Grice et al. 2007, Olsen et al. 2004), one might have expected to find consistent associations in these workers, despite the major differences in outcome ascertainment and classification across the studies. The fact that findings were inconsistent among these studies, as well as across the community-based studies of PFOS and cancer, underscores the tenuousness of reported associations with estimated PFOS.*"

Exposure response gradient – PFOA

When considering exposure response gradients for PFOA, Chang et al. noted that: "*It is important to recognize that the magnitude of probable exposure to PFOA differs substantially among occupational and community groups. As shown in Figure 1, median serum PFOA levels among directly exposed fluorochemical workers at the Parkersburg plant in 1979– 2004 (Woskie et al. 2012), the Cottage Grove plant in 1993 – 1997 (Olsen et al. 2000), the Decatur, Alabama, plant in 1998 (where levels were reported as the geometric mean, which is generally close to the median in studies that reported both) (Olsen et al. 2003b), and the Cottage Grove, Decatur, and Antwerp, Belgium, plants in 2000 (Olsen and Zobel, 2007) ranged from approximately 1,000 to 2,880 ng/mL (1– 2 .88 ppm). By contrast, median serum PFOA levels were approximately 15 – 30% as high among intermittently directly exposed workers and 5– 1 0% as high among indirectly (background) exposed workers in Parkersburg (Woskie et al. 2012), and geometric mean levels were 5% as high among background-exposed film division workers in Decatur (Olsen et al. 2003b). Median serum PFOA concentrations among residents of the six PFOA-contaminated public water districts in Ohio and West Virginia near the Parkersburg plant in 2005 – 2006 were generally between 20 and 40 ng/mL, depending on age group and sex (Frisbee et al. 2009), a level comparable to the background exposure level at the Decatur plant. Median serum PFOA levels were an order of magnitude lower among participants in the US population-based National Health and Nutrition Examination Survey (NHANES) in 1999– 2008 (Kato et al. 2011) and among American Red Cross adult volunteer blood donors in 2000 – 2010 (Olsen et al. 2012), with declining levels over time. Thus, average exposure to PFOA differed by up to two orders of magnitude between directly exposed workers and nonoccupationally exposed community members, and by another order of magnitude between directly exposed workers and indirectly exposed workers or residents near the Parkersburg plant (Figure 1). However, many of the positive associations with cancer outcomes were observed with environmental rather than occupational exposures to PFOA (Barry et al. 2013, Vieira et al. 2013). This pattern might be explained by greater statistical power in the community based studies, or by chance, confounding, and/or bias. In light of the fact that most SMR and RR point estimates in occupational studies were close to unity, insufficient statistical power cannot be the only reason for the generally null findings.*

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Instead, chance, confounding, and bias (with an unknown degree and direction of impact) are more plausible explanations for the apparently stronger associations in less exposed study groups."

Exposure response gradient – PFOS

When considering exposure response gradients for PFOS, Chang et al. noted that: "*As with PFOA, biomonitoring studies of serum PFOS levels show major differences among occupational and community groups (Figure 2). The geometric mean level was 941 ng/mL (0.941 ppm) among fluorochemical workers at the Decatur plant in 1998 (Olsen et al. 2003b) and the median was 1,000 ng/mL at the same plant in 2000 (Olsen and Zobel, 2007). At the Antwerp and Cottage Grove plants, the median levels were 550 and 450 ng/ mL, respectively (Olsen and Zobel, 2007), while the geometric mean level among background-exposed film division workers at the Decatur plant was 136 ng/mL (Olsen et al. 2003b). By contrast, median serum PFOS levels were up to two orders of magnitude lower in Ohio and West Virginia residents near the Parkersburg plant (approximately 20 ng/mL in 2005–2006), where industrial use of PFOS did not occur (Frisbee et al. 2009). Median serum PFOS levels were comparable in US general population participants in NHANES (30.2 ng/mL in 1999– 2000 and 13.6 ng/mL in 2007– 2008) (Kato et al. 2011), and in American Red Cross adult volunteer blood donors (35.8 ng/mL in 2000– 2001 and 8.6 ng/ mL in 2010) (Olsen et al. 2012). Again, these differences must be considered when contemplating the plausibility of observed positive associations in community, but not in occupational, settings."*

Plausibility and coherence with toxicological evidence – PFOA

With regards to plausibility and coherence with toxicological evidence for PFOA, Chang et al. included the following statements: "*Although animal toxicology data on PFOA are not readily translated to humans, a causal interpretation of an observed association may be better justified if it is coherent with laboratory evidence (Hill, 1965). Such evidence can also support the biological plausibility of a causal hypothesis (Hill, 1965)."*

"*A priori, based on the results of experimental animal studies, the organs of greatest concern with respect to a potential carcinogenic effect of PFOA are the liver, testis (Leydig cells), and pancreas (acinar cells). However, no convincing associations with malignancies affecting any of these organs have been observed in epidemiologic studies of humans. Only testicular cancer has been associated with PFOA exposure in any of these studies (Barry et al. 2013, Vieira et al. 2013), with ambiguous exposure-response trends. On the other hand, given the relatively poor site concordance between animals and humans for many known human carcinogens, the lack of associations between PFOA exposure and liver, testicular, and pancreatic cancers among humans does not constitute evidence against human carcinogenicity of PFOA; rather, it provides no evidence to support such an effect. Of note, nearly all testicular cancers in humans are of germcell origin, with Leydig cell tumors constituting only an estimated 1 – 3% of testicular malignancies (Sarma et al. 2006). Therefore, it is questionable whether a positive association between PFOA exposure and testicular cancer risk in humans, even if well established, could accurately be described as being coherent with the finding of excess Leydig cell adenomas in rats fed with PFOA. Likewise, pancreatic acinar cell carcinomas account for only approximately 1% of pancreatic exocrine tumors in humans (Klimstra et al. 1992), and mammary fibroadenomas [which were not significantly increased in rats fed with PFOA (Hardisty et al. 2010)] are not precursors of breast cancer or indicators of increased breast cancer risk in humans (Fitzgibbons et al. 1998). TFE – which was used to manufacture fluoropolymers in the Parkersburg plant (Steenland and Woskie, 2012) and five European plants (Consonni et al. 2013), but not the Cottage Grove plant – is a kidney, liver, hematopoietic, and possibly testicular carcinogen in rodents. Specifically, 2-year wholebody inhalation exposure resulted in significant increases in renal tubule adenoma, renal tubule*

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adenoma and carcinoma combined, hepatocellular adenoma, HCC, liver hemangiosarcoma, and mononuclear cell leukemia, as well as slight increases in testicular interstitial cell adenoma, in F344/N rats (National Toxicology Program, 1997). In B6C3F 1 mice, the same exposure resulted in significant increases in liver hemangioma, liver hemangiosarcoma, hepatocellular adenoma, HCC, and histiocytic sarcoma of the liver, lung, spleen, lymph nodes, bone marrow, and kidney (National Toxicology Program, 1997). Thus, although epidemiologic data on TFE are inconclusive, animal toxicology data are coherent with the hypothesis that TFE, which was highly correlated with PFOA at the Parkersburg facility and at the six combined US and European facilities in the pooled analysis (Consonni et al. 2013, Steenland and Woskie, 2012), was responsible for the apparent positive association between PFOA exposure and kidney cancer mortality in these study groups. As stated by Consonni et al. (2013), toxicological evidence in animals suggests that TFE could also have contributed to the modest, statistically nonsignificant excesses of liver cancer, testicular cancer, and leukemia mortality observed in the pooled TFE cohorts, as well as in some comparisons in the Parkersburg cohort (Leonard et al. 2008, Steenland and Woskie, 2012). Given that the Cottage Grove facility manufactured PFOA but did not use it for polymer production, TFE probably was not used in Cottage Grove, and its absence could plausibly explain the lack of excess kidney cancer mortality in that worker cohort (Lundin et al. 2009)."

Plausibility and coherence with toxicological evidence – PFOS

With regards to plausibility and coherence with toxicological evidence for PFOS, Chang et al. included the following statement: "*Toxicological studies in animals clearly pinpoint the liver as the main target organ for a potential carcinogenic effect of PFOS. Although Alexander et al. (2003) reported elevated SMRs for liver cancer among workers with low or high potential PFOS exposure, these estimates were based on only one death each and, therefore, highly unstable. Olsen et al. (2004) reported no episodes of care for liver cancer among chemical division workers, compared with one such episode among film division workers. The inverse RR estimates for liver cancer in association with higher quartiles of plasma PFOS concentration reported by Eriksen et al. (2009) in Denmark also are not consistent with a hepatocarcinogenic effect of PFOS in humans, at least at relatively low concentrations. The 2-year rat feeding study of PFOS detected a potentially spurious increase in thyroid follicular cell adenoma among male rats fed with PFOS for 1 year and followed for a 2nd year, but not among those fed with PFOS for the full 2 years (Seacat et al. 2002). Only Olsen et al. (2004) reported on thyroid cancer as an outcome, with one episode of care (versus 1.0 expected) in a short-term and/or low-exposure chemical division worker and none among long-term, high-exposure chemical division workers or film division workers. Thus, although concordance of sites of carcinogenesis across species is not a requirement for establishing human cancer hazards, a comparison of results from animal and human studies offers little to no support for a causal relationship between PFOS exposure and human cancer.*"

Conclusion

Chang et al. reported the following in the Conclusion of the review: "*The vast majority of reported associations with cancer mortality, incidence, or prevalence have been consistent with the null hypothesis of no effect. The few observed positive associations have not met the Bradford Hill guidelines, that is, they are weak, inconsistent, off set by negative associations, not in keeping with a positive exposure-response gradient, and not coherent with the toxicological findings of liver, testicular Leydig cell, and pancreatic acinar cell tumors in animals exposed to PFOA and liver tumors in those exposed to PFOS. Moreover, confounding, bias, and chance (especially in light of multiple comparisons) cannot be ruled out as explanations for the reported positive associations, many of which were observed in studies of environmentally exposed communities, but not in occupational settings where exposure to PFOA and PFOS was one to two orders of magnitude higher. Toxicological and mechanistic data in animals do not conflict with the epidemiologic data in humans and may even be interpreted as offering*

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evidence against a carcinogenic effect of PFOA and PFOS in humans, given that the mechanisms by which these chemicals induce tumors in rodents may not be involved in human carcinogenesis."

Chang et al. then reported on the classification of the Health Council of the Netherlands, and continued: "*This classification is consistent with our conclusion that the existing epidemiologic evidence does not support the hypothesis of a causal association between PFOA or PFOS exposure and cancer in humans. However, further research on this topic is warranted. Quantitative exposure assessment in previously unstudied occupational settings – for example, at industrial facilities in Asia that continue to produce or use PFOA and/ or PFOS (Lim et al. 2011) – could provide the basis for future cohort studies once sufficient follow-up time has accrued. More readily, continued follow-up of existing cohorts and linkage to cancer registries to ascertain cancer incidence might provide additional insight into whether these compounds affect cancer risk in humans."*

Priestly (2016)

Studies reviewed

Priestly reviewed five studies on PFAS and cancer in humans (Barry et al. 2013; Alexander et al. 2003; Alexander and Olsen, 2007; Hardell et al. 2014; Bonefeld-Jørgensen et al. 2011; Ghisari et al. 2014; Innes et al. 2014).

All of these studies were reviewed by either the ATSDR, US EPA, RIVM, Chang et al. except Ghisari et al. (2014).

In addition, Priestly reported on the findings of the review by Chang et al. (2014), and also noted the IARC Monograph for PFOA published in 2016, and the three studies on testicular and renal cancer in a fluoropolymer production plant and in the highest exposed nearby residents IARC based its classification on (Steenland and Woskie, 2012; Vieira et al. 2013; Barry et al. 2013).

Considerations and conclusions

Priestly stated in the 'Executive Summary' under 'Carcinogenicity': "*PFOA (but not PFOS) was evaluated for carcinogenicity by the International Agency for Research on Cancer (IARC) in 2014. It was classified in Group 2B – possibly carcinogenic to humans, on the basis of limited evidence of testicular and renal cancer, in workers in a fluoropolymer production plant and in the highest exposed nearby residents. The animal data was also considered to be limited. Since the mode of action data was considered moderate, there was insufficient evidence to upgrade the classification. An independent review of 18 epidemiological studies of cancer incidence reached the conclusion that the evidence does not support an association between cancer and either PFOS or PFOA. The U.S. Agency for Toxic Substances and Disease Registry (ATSDR 2015) has also concluded that: "There is no conclusive evidence that perfluoroalkyls cause cancer in humans". Despite these findings, media reports of the consequences of off-site contamination by PFOS have commonly referred to the 'cancer-causing' properties of the contaminants, no doubt unduly raising the level of concern among nearby residents."*

Priestly also noted in the 'Carcinogenicity' section that: "*IARC has not evaluated PFOS for carcinogenicity at this time.*"

Summaries of studies reviewed

Of the study by Ghisari et al. (2014), Priestly reported: "*In a small case-control study investigating the relationship between POPs exposure and breast cancer risk in Greenlandic Inuit women, higher ($p<0.05$) median blood levels of PFOA (2.5 vs 1.6 ng/mL) and PFOS (45.6 vs 21.9 ng/mL) were found in cases, compared to controls (Bonefeld-Jørgensen et al. 2011). This study was considered by the IARC Working*

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Group to be uninformative because of the small sample size and missing covariate data. In a follow-up study, the increase risk associated with PFAS exposure was assessed to be higher in subjects with a variant metabolic enzyme polymorphism involving at least one of CYP1A1; COMT and CYP19 (Ghisari et al. 2104)."

Of people's concerns about thyroid cancer, Priestly noted: "*While some affected communities in Australia have expressed concerns about a link with thyroid cancer, possibly based on reports suggesting links with thyroid disease or thyroid hormone disruption (see section 5.1 of this report), neither the epidemiological studies nor toxicological studies in animals provide any confirmatory evidence of a link with thyroid cancer. The most definitive evidence is from the study of mid-Ohio Valley residents exposed to PFOA in water supplies around the DuPont plant (Barry et al. 2013), where the relative risks (RR) for thyroid cancer were not significantly increased across the three highest exposure quartiles based on PFOA blood levels – 1.54 (95% CI 0.77 – 3.12); 1.48 (0.74 – 2.93); 1.73 (0.85 – 3.54). These RR estimates were slightly higher when a 10y lag was introduced in to the analysis, but still did not achieve statistical significance."*

Kirk et al. (2018)

Studies reviewed

Kirk et al. (2018) evaluated 20 papers investigating PFAS exposure and cancer:

- Eleven studies were reviewed on bladder cancer, with five studies evaluating bladder cancer mortality (Alexander et al. 2003; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012) and seven studies evaluating bladder cancer incidence (Alexander and Olsen, 2007; Barry et al. 2013; Eriksen et al. 2009; Olsen et al. 2004; Raleigh et al.2014; Steenland et al. 2015; Vieira et al. 2013).
- Five studies were reviewed on kidney cancer, three of which evaluated the incidence of kidney cancer (Barry et al. 2013; Raleigh et al. 2014; Vieira et al. 2013) while three studies evaluated mortality (Leonard et al. 2008; Raleigh et al. 2014; Steenland and Woskie 2012).
- Nine studies were reviewed on liver cancer, with five studies evaluating liver cancer incidence (Barry et al. 2013; Eriksen et al. 2009; Raleigh et al. 2014; Vieira et al. 2013; Olsen et al. 2004) and four studies investigating liver cancer mortality (Leonard et al. 2008; Steenland and Woskie, 2012; Alexander et al. 2003; Raleigh et al. 2014). Kirk et al. also reviewed the study by Yeung et al. (2013) on liver transplant patients.
- Thirteen studies were reviewed on prostate cancer, with five studies evaluating prostate cancer mortality (Gilliland and Mandel, 1993; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie, 2012) and nine studies evaluating prostate cancer incidence (Barry et al. 2013; Ducatman et al. 2015; Eriksen et al. 2009; Grice et al. 2007; Hardell et al. 2014: Olsen et al. 2004; Raleigh et al. 2014; Steenland et al. 2015; Vieira et al. 2013).
- Eight studies were reviewed on colorectal cancer, including two studies on colorectal cancer mortality (Leonard et al.2008; Gilliland and Mandel, 1993) and six studies on colorectal cancer incidence (Barry et al. 2013; Innes et al. 2014; Steenland et al. 2015; Grice et al. 2007; Vieira et al. 2013; Olsen et al.2004).
- Nine studies were reviewed on breast cancer, including six studies on breast cancer incidence (Barry et al. 2013; Bonefeld- Jørgensen et al. 2011; Bonefeld-Jørgensen et al. 2014; Grice et al. 2007; Raleigh et al. 2014; Vieira et al.2013) and four studies on breast cancer mortality (Alexander et al. 2003; Leonard et al. 2008; Raleigh et al. 2014; Steenland and Woskie., 2012).

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- Five studies were reviewed on testicular cancer, including three studies on mortality from testicular cancers (Gilliland and Mandel, 1993; Alexander et al. 2003; Steenland and Woskie ,2012), and two studies on incidence of testicular cancer (Barry et al. 2013; Vieira et al. 2013);
- Four studies were reviewed on thyroid cancer, including three studies on thyroid cancer incidence (Barry et al. 2013; Grice et al. 2007; Vieira et al. 2013), and one on thyroid cancer mortality (Leonard et al. 2008).
- For other cancers, eight cohort studies were reviewed (Alexander et al, 2003; Barry et al. 2013; Leonard et al. 2008; Lundin et al. 2009; Olsen et al. 2004; Raleigh et al. 2014; Steenland and Woskie, 2012; Steenland et al. 2015) and one case-control study (Vieira et al. 2013) that investigated a range of cancers in addition to those specifically reported on in the above sections.

All of the above studies were reviewed by either the ATSDR or the US EPA, with the exception of Ducatman et al. 2015.

Considerations and conclusions

In the 'Plain language summary', Kirk et al. stated that: "*We found limited evidence in a small number of relevant studies that PFAS exposure caused kidney and testicular cancers*". In the 'Executive Summary', Kirk et al. provided more detail, stating: "*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.*"

In the 'Discussion – overview of results', Kirk et al. again commented on their evaluation of the evidence on kidney and testicular cancer, and then commented on their evaluation of the evidence on other cancers: "*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 [International Agency for Research on Cancer (IARC) 2017]. There was inadequate evidence of an association between PFAS and other cancers studied.*"

In the 'Cancers' section, the authors noted that: "*Most of the studies 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 studies only examined exposure to a single PFAS.*"

Kirk et al. reported on cancer of the bladder, kidney, liver, prostate, pancreas, colorectum, breast, testes and thyroid and other cancers.

The Table below has been reproduced from Kirk et al. (pg. 111) and reports their evaluation of the evidence for each cancer by PFAS chemical.

Associations at a glance: Evidence for each cancer

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

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Health outcome	PFAS exposure	Evaluation of evidence
Breast cancer	PFOA, PFOS, PFNA, PFHxS	Inadequate evidence
Testicular cancer	PFOA	Limited evidence
Thyroid cancer	PFOA	Inadequate evidence

Bladder cancer

Kirk et al. reviewed eleven studies on bladder cancer, with five studies evaluating bladder cancer mortality (Alexander et al. 2003; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012) and seven studies evaluating bladder cancer incidence (Alexander and Olsen, 2007; Barry et al. 2013; Eriksen et al. 2009; Olsen et al. 2004; Raleigh et al.2014; Steenland et al. 2015; Vieira et al. 2013).

Of these studies, Kirk et al. reported that: "*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. [2003] examined mortality in a cohort of workers at a DuPont facility manufacturing POSF—a precursor 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 incidence 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. [2004] 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 and Olsen [2007] 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. [2008] did not observe an association between PFOS exposure and bladder cancer mortality in an occupational cohort. Eriksen et al. [2009] did not observe an association between PFOS and bladder cancer incidence in the general Danish population.*

Steenland et al. [2015] 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 [2012]. They observed a significant negative trend for bladder cancer across quartiles of PFOA with analysis without a lag period (p=0.04) [2015]. Lundin et al. [2009] did not observe an association between PFOA and mortality from bladder cancer, which was consistent with an earlier analysis of this cohort [Gilliland and Mandel 1993]. In a combined analysis of the C8 Health Project cohort and a nearby DuPont occupational cohort, Barry et al. [2013] did not observe an association between PFOA and bladder cancer incidence. Similarly, Vieira et al. [2013] 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

Kirk et al. reviewed five studies on kidney cancer; three of these studies evaluated the incidence of kidney cancer (Barry et al. 2013; Raleigh et al. 2014; Vieira et al. 2013) and three evaluated mortality (Leonard et al. 2008; Raleigh et al. 2014; Steenland and Woskie 2012).

All of these studies have been previously reviewed earlier under ATSDR, US EPA, IARC, Chang et al. above.

Kirk et al. reported that: "*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 [2012] updated mortality data for an occupational cohort study of workers exposed to PFOA originally conducted by Leonard, et al. [2008] and found elevated risks for kidney cancer (SMR (Q4-Q1) (95% CI);*

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2.82 (1.13, 5.81)). Barry et al. [2013] 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. [2013] 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. [2014] studied mortality and incidence and Leonard et al. [2008] studied mortality among AFPO workers and found no association between PFOA and kidney cancer."

Liver cancer

Nine studies were reviewed by Kirk et al. on liver cancer, with five studies evaluating liver cancer incidence (Barry et al. 2013; Eriksen et al. 2009; Raleigh et al. 2014; Vieira et al. 2013; Olsen et al. 2004) and four studies investigating liver cancer mortality (Leonard et al. 2008; Steenland and Woskie, 2012; Alexander et al. 2003; Raleigh et al. 2014). Kirk et al. also reviewed the study by Yeung et al. (2013) on liver transplant patients.

Kirk et al. reported for these studies that: "*None of the nine papers investigating the association between PFAS and liver cancer incidence and mortality reported statistically significant findings. Leonard et al. [1998] and Steenland & Woskie [2012] examined mortality from liver cancer in an occupational cohort, which was not associated with PFOA exposure. Barry et al. [2013] found no association between PFOA and liver cancer in the C8 Health Project. In a geographic analysis as part of the C8 Health Project, Vieira et al. [2013] found no association between PFOA and liver cancer incidence. Alexander et al. [2003], in an occupational cohort study found no association between PFOS and liver cancer mortality. From the same cohort, Olsen et al. [2004] found no association between episodes of care for liver cancer with PFOS exposure. Raleigh et al. [2014] found no association between PFOA exposure and mortality or incidence of liver cancer. Eriksen et al. [2009] investigated PFOS and PFOA in the Danish population and found no association with liver cancer. In a small cross-sectional study, Yeung et al. [2013] 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

Kirk et al. reviewed 13 studies on prostate cancer, with five studies evaluating prostate cancer mortality (Gilliland and Mandel, 1993; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie, 2012) and nine studies evaluating prostate cancer incidence (Barry et al. 2013; Ducatman et al. 2015; Eriksen et al. 2009; Grice et al. 2007; Hardell et al. 2014: Olsen et al. 2004; Raleigh et al. 2014; Steenland et al. 2015; Vieira et al. 2013).

Kirk et al. reported of the studies they reviewed that: "*An association between prostate cancer and PFAS exposure was identified in two of six papers evaluating mortality and none of the nine papers evaluating incidence. In an occupational cohort study of 3,993 employees, Lundin et al. [2009] 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. [2008] 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 [2012] did not observe an association between PFOA and mortality from prostate*

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cancer from the same cohort. In another occupational cohort, Raleigh et al. [2014], Lundin et al. [2009] and Gilliland & Mandel [1993] did not observe an association between prostate cancer mortality and PFOA exposure [Lundin et al. 2009; Gilliland and Mandel 1993; Raleigh et al. 2014]. In the Danish birth cohort, Eriksen et al. [2009] 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. [2015] 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. [2014] 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. [2015] did not identify an association between PFOA and incidence of prostate cancer among exposed workers in Ohio. Barry et al. [2013] 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. [2013] did not observe an association between PFOA and prostate cancer. Grice et al. [2007] found no association between self-reported prostate cancer and PFOS exposure in exposed workers. Olsen et al. [2004] did not observe an association between PFOS and episodes of care for prostate cancer."

Colorectal cancer

Kirk et al. reviewed eight studies on colorectal cancer, including two studies on colorectal cancer mortality (Leonard et al.2008; Gilliland and Mandel, 1993) and six studies on colorectal cancer incidence (Barry et al. 2013; Innes et al. 2014; Steenland et al. 2015; Grice et al. 2007; Vieira et al. 2013; Olsen et al.2004).

With regards to colorectal cancer mortality, Kirk et al. concluded: "*Neither of the two papers examining mortality identified an association between colorectal cancer and exposure to PFAS. Gilliland & Mandel [1993] did not identify an association between occupational exposure to PFOA and colorectal cancer. Similarly, Leonard et al. [2008] did not observe an association between occupational exposures to PFOA and colorectal cancer.*"

Kirk et al. reported the following on the colorectal cancer incidence studies they reviewed: "*Among six papers examining incidence, there were two papers that identified an association between PFAS and colorectal cancer. Innes et al. [2014] 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. [2013[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. [2007] did not identify an association between self-reported colorectal cancer and PFOS among exposed workers. In a similar occupational cohort, Olsen et al. [2004] did not identify an association between occupational exposure to PFOS and episodes of care for colorectal cancer.

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Steenland et al. [2015] did not identify an association between colorectal cancer incidence and PFOA exposure. Barry et al. [2013] did not identify an association between colorectal cancer incidence and PFOA in the C8 Health Project."

Breast cancer

Nine studies were reviewed on breast cancer, including six studies on breast cancer incidence (Barry et al. 2013; Bonefeld- Jørgensen et al. 2011; Bonefeld-Jørgensen et al. 2014; Grice et al. 2007; Raleigh et al. 2014; Vieira et al.2013) and four studies on breast cancer mortality (Alexander et al. 2003; Leonard et al. 2008; Raleigh et al. 2014; Steenland and Woskie, 2012).

In summarising the studies on breast cancer, Kirk et al. reported that: "*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. [2014] 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. [2011] 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. [2007] did not find an association between self-reported breast cancer and PFOS exposure at work. In another occupational cohort, Raleigh et al. [2014] did not find any association between breast cancer and occupational exposure to PFOA. Barry et al. [2013] 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. [2013] did not observe an association between breast cancer and PFOA exposure."

Testicular cancer

Kirk et al. reviewed five studies on testicular cancer, including three studies on mortality from testicular cancers (Gilliland and Mandel, 1993; Alexander et al. 2003; Steenland and Woskie ,2012), and two studies on incidence of testicular cancer (Barry et al. 2013; Vieira et al. 2013).

With regards to testicular cancer mortality and incidence, Kirk et al. reported that: "*None of three papers evaluating mortality from testicular cancer and both of the two papers evaluating incidence found an association with PFAS [Steenland and Woskie 2012; Gilliland and Mandel 1993; Leonard et al. 2008]. Two overlapping papers investigating testicular cancer in the C8 Health Project identified associations with PFOA. Barry et al. [2013] 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) [Vieira et al. 2013]. However, there was no overall association with testicular cancer in this study."*

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Thyroid cancer

Kirk et al. reviewed four studies on thyroid cancer, including three studies on thyroid cancer incidence (Barry et al. 2013; Grice et al. 2007; Vieira et al. 2013), and one on thyroid cancer mortality (Leonard et al. 2008).

With regards to thyroid cancer mortality and incidence, Kirk et al. reported that: "*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 [Grice et al. 2007; Barry et al. 2013; Vieira et al. 2013]. Leonard et al. [2008] 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

Kirk et al. evaluated eight cohort studies (Alexander et al, 2003; Barry et al. 2013; Leonard et al. 2008; Lundin et al. 2009; Olsen et al. 2004; Raleigh et al. 2014; Steenland and Woskie, 2012; Steenland et al. 2015) and one case-control study (Vieira et al. 2013) that investigated a range of cancers in addition to those specifically reported on in the above sections.

Kirk et al. reported that: "*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. [2013] 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 [2012] 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.*"

Differing conclusions

Chang et al. (2014) come to a differing conclusion to IARC and US EPA. In looking at the specific studies reviewed by IARC and Chang et al., there are 11 studies that were reviewed by both. Chang reviewed two studies (Ubel et al. 1980 and Yeung et al. 2013) that IARC did not include. IARC, on the other hand, reviewed two papers by Shin et al. (both 2011), and papers by Woskie et al. (2012), and Raleigh et al. (2014).

6.1.4. Summary of key national and international reports and systematic reviews

Recent key national and international reports:

- ATSDR concluded that the occupational mortality studies found no overall excess in cancer but inconsistent increases in bladder and prostate cancer deaths, most of which were not statistically significant. For the studies of cancer in communities with PFAS contamination, overall cancer was not elevated, while there was very limited evidence of an increase in

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testicular cancer, based on very few cases. General population studies found no evidence of increase in any type of cancer. The ATSDR concluded that a causal relationship between fluoroalkyls and cancer cannot be established from the few studies reporting significant increases in specific types of cancer risk and lack of consistency across facilities where workers were occupationally exposed may be suggestive of a causative agent other than PFOS or PFOA.

- The US EPA concluded there is *"suggestive evidence of carcinogenic potential"* for PFOA (kidney and testis). The EPA found the evidence was less convincing for PFOS, based on findings for bladder and prostate cancers, but EPA concluded that there were important limitations in the methodology, which precluded any firm conclusions.
- IARC classified PFOA as *"possibly carcinogenic to humans (Group 2B)"*, based on limited evidence of associations with kidney cancer in occupational and high exposed community studies, testicular cancer in high-exposure community studies and limited evidence from animal studies. For the kidney findings, chance, bias and confounding could not be ruled out. IARC concluded there was inadequate evidence for carcinogenicity of PFOA for all other cancer sites considered. IARC did not evaluate any studies relating to PFOS exposure and cancer.
- RIVM noted that some studies have found associations between PFOA and testis and kidney cancer, but the evidence is *'less clear'*.
- FSANZ concluded that epidemiological studies have not provided convincing evidence of a correlation between PFOS and PFHxS and any type of cancer in humans, and for PFOA, a causal relationship cannot be established with reasonable confidence.

Systematic reviews:

- Chang et al. concluded that while there were some associations between cancers of the prostate, kidney, testis, and thyroid and PFOA and PFOS exposure, the epidemiological evidence does not support the hypothesis of a causal association between PFOA or PFOS exposure and cancer in humans. Chang et al. considered that for the few associations found, chance, bias and confounding could not be ruled out. They also applied the Bradford Hill causal criteria in coming to their conclusions and noted that findings in community studies which were not found in occupational studies were not credible, due to the much lower exposure levels in community populations. Chang et al. also considered that the mechanism by which tumours are induced in rodents by PFOS and PFOA may not be relevant to humans.
- Priestly specifically addressed the small number of community studies of breast cancer and thyroid cancer and found no convincing evidence of any associations with PFOA.
- Kirk et al. conducted a quality review and reported that most cancer studies were at high risk of bias, including limitations in the exposure assessment. They reported on nine cancer sites and concluded that, while overall cancer was not elevated in thee occupational or general population groups, there was limited evidence of an association between PFOA exposure and two of the nine specific types of cancer: kidney cancer and testicular cancer. While these two associations were based on relatively weak evidence from studies with methodological limitations, Kirk et al. noted their conclusion is broadly consistent with the IARC evaluation of PFOA as being *'possibly carcinogenic to humans'*. For the other seven cancers types Kirk et al. reviewed, they concluded there was inadequate evidence of an association between PFAS and other cancers studied.

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6.1.5. Expert Health Panel synthesis to support advice to the Minister

- There are small numbers of studies on PFAS and cancer in manufacturing workers and communities in contaminated areas, small numbers of cancers in many studies, low methodological quality and high risk of bias with many studies, lack of consideration of important confounders, multiple comparisons and a lack of consistency in findings between studies.
- The occupational studies relate to manufacturing workers, not end users such as firefighters who are the major group at risk of occupational exposure in Australia.
- The suggestive evidence, although still limited, relates to two types of cancer – kidney and testicular – both uncommon tumours. Very limited evidence relates to bladder and prostate cancer and there is no suggestive or convincing evidence for any other types of cancer.
- The limited amount of evidence which is available relates to PFOA and not PFOS.
- Findings in animal studies about tumour induction in rodents by PFOS and PFOA may not be relevant to humans.

6.1.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and cancers:

- The evidence does not support PFAS being a major contributor to cancer burden in workers or exposed community populations.
- The evidence on cancer risk is limited, but it is possible there is increased risk of some uncommon cancers, such as kidney and testis.
- The limited evidence relates to PFOA, not PFOS.
- Given the high concern about cancer-risk among both occupational groups such as firefighters and those members of the community in contaminated areas during the consultation, and the limitations of the available evidence, future research into cancer is a priority (see below). Better designed cohort studies in exposed workers such as firefighters, and communities in contaminated areas, especially with improved exposure assessment, could lead to stronger conclusions.

To further investigate the association between PFAS exposure and cancer in an Australian setting, the Panel suggests the following research priorities:

- Large collaborative cohort studies are required to examine cancer associations in exposed Australian workers and community populations in exposed areas. Further studies into the relatively uncommon cancers – kidney and testes – are most indicated, based on the limited evidence in previous studies. Studies need to be adequately powered, ideally supported by some quantitative exposure data (e.g. blood concentrations), covering the majority of exposed populations, access to complete cancer registry and death notifications from the region and access to data on possible confounders.
- There is also a priority for future research into cancer to investigate PFOS, rather than PFOA, because PFOS is the most highly detected PFAS in Australia, and the best previous research focussed on PFOA.
- Previous studies have often been at high risk of bias due to low cohort numbers, very limited exposure data, unadjusted multiple comparisons, lack of data on confounders or

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effect modifiers (e.g. smoking) and selection, recall and survivor biases. Further studies subject to these same biases are unlikely to add useful evidence.

- Most previous studies have been cancer mortality studies, but Australia has the advantage of complete and high quality national cancer registration data and the ability to link cohorts to determine cancer incidence rates, which is a better measure of cancer occurrence than cancer mortality. Occupational or population cohort studies undertaking such cancer linkage are a priority.
- Research in specific occupational groups (e.g. firefighters) will also have to deal with confounding by the many other potentially carcinogenic chemicals that these groups are exposed to. This is also the case with general population cohort studies, where account needs to be taken of work exposures for cohort members. This can be more challenging in population cohort studies, due to the greater diversity of jobs undertaken and relevant exposures in those jobs.

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6.2. Metabolic biomarkers: Concentrations of cholesterol and triglycerides in the blood

The World Health Organization states that: "*Raised total cholesterol is a major cause of disease burden in both the developed and developing world as a risk factor for Ischemic heart disease and stroke*"[15]. In reality, cholesterol is not a single substance being measured, but a sum of key components of several families of lipoproteins. Thus, many studies focus on different lipoprotein fractions (e.g. LDL, VLDL, or HDL). A doubling or halving of cardiovascular risk might be roughly expected for every 20-30% change in total cholesterol or LDL cholesterol. Diet and medication have large effects on LDL cholesterol of this magnitude or greater. There is no threshold for LDL or total cholesterol above which there is a disproportionate change in risk. Thus, the references in studies to examining changes in the number of people with 'high risk' cholesterol can be seen to refer to several different cholesterol levels. Differences in the mean cholesterol are more reliably interpreted.

All of the key (inter)national authority reports and several of the systematic reviews evaluated the human evidence regarding exposure to PFAS and cholesterol and triglycerides in the blood.

6.2.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of the following six international authority/intergovernmental/governmental reports published between 2015 and 2017 and four systematic reviews and literature reviews from 2013 to 2018 that reported on exposure to PFAS and any associations with blood cholesterol and lipid concentrations.

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR, 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **New Jersey Drinking Water Quality Institute (DWQI, Public Review draft 2016)**. Health-based maximum contaminant level support document: Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA, 2016a)**. Health effects support document for Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA, 2016b)**. Health effects support document for Perfluorooctane Sulphonate (PFOS);
- **Dutch National Institute for Public Health and the Environment (RIVM, 2017).** PFOA exposure and health: A review of scientific literature;
- **Food Standards Australia New Zealand (FSANZ, 2017).** Hazard Assessment report (PFOS, PFOA, PFHxS).

The National Toxicology Programme (NTP) Monograph on PFOA and PFOS was not considered by the Panel for this section as the Monograph did not report on cholesterol and triglycerides.

Systematic reviews

- **Saikat et al. (2013).** The impact of PFOS on health in the general population: a review;
- **Priestly (2016).** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS) (Monash University);

[15] http://www.who.int/gho/ncd/risk_factors/cholesterol_text/en/

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- **Rappazzo et al. (2017).** Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature;
- **Kirk et al. (2018).** The PFAS Health Study. Systematic Literature Review (Australian National University).

This section contains a range of terminology used in the various systematic reviews and key national and international reports when reporting on cholesterol and triglycerides in the blood. The statements have been reproduced verbatim to maintain the integrity of the reported information.

6.2.2. Key national and international reports

Agency for Toxic Substances and Disease Registry (ATSDR, 2015)

The ATSDR in its draft toxicological profile for perfluoroalkyls considered the human evidence on cholesterol in the 'Summary of health effects,' 'Inhalation exposure – systemic effects' and 'oral exposure – systemic effects' sections.

Studies reviewed

The ATSDR reviewed 19 studies on the effect on cholesterol after exposure to PFAS. These studies included:

- 10 occupational exposure studies (Costa 2004; Costa et al. 2009; Olsen et al. 1999; Olsen et al. 2000; Olsen and Zobel 2007; Sakr et al. 2007a; and Sakr et al. 2007b; Mundt et al. 2007; Olsen et al. 2003a; and Olsen et al. 2012);
- Five studies in high-exposure communities (Emmett et al. 2006; Wang et al. 2012; Frisbee et al. 2010; Fitz-Simon et al. 2013; Steenland et al. 2009);
- Four studies in the general population (Château-Degat et al. 2010; Eriksen et al. 2013; Fisher et al. 2013; Nelson et al. 2010).

The ATSDR did not report any human studies for dermal exposure and cholesterol.

Considerations and conclusions

The ATSDR stated in the 'Public Health Statement for Perfluoroalkyls – How perfluoroalkyls can affect your health?' section: "*Most human studies have looked for a relationship between levels of perfluoroalkyls in the blood and a health effect. It is difficult to interpret the results of these studies because they are not consistent; some studies have found associations, but others looking at the same health effect have not found these associations. Even though some studies have found significant associations between serum perfluoroalkyl levels and adverse health effects, it does not mean that perfluoroalkyls caused these effects. The effects may have been due to other factors that were not considered by the researchers. The available studies suggest that increases in blood cholesterol levels are associated with higher PFOA or PFOS blood levels in workers inhaling PFOA and/or PFOS as well as in people ingesting these compounds.*"

In the 'Relevance to public health – summary of health effects in humans' section, the ATSDR stated: "*Epidemiology studies have found statistically significant associations between serum perfluoroalkyl levels (particularly PFOA and PFOS) and a wide range of health effects. When the subjects were categorized by serum perfluoroalkyl levels, dose-response relationships were found for most of the effects. However, findings were not always consistent across studies. However, consistent findings were found for association of serum PFOA and PFOS with increases in serum lipid levels.*"

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Also in this section, the ATSDR reported: "*Studies of workers, highly exposed individuals, and the general population have reported significant associations between serum perfluoroalkyl levels and serum lipid levels. However, because a number of factors can influence serum lipid levels, many of the studies adjusted for some of these potential confounders such as age, body mass index (BMI), and the use of cholesterol-lowering medication. The most consistently found alteration in serum lipid levels was increased serum total cholesterol levels. Statistically significant associations between serum PFOA levels and total cholesterol levels have been found in workers, residents of communities with high levels of PFOA in the drinking water, and the general population. Serum PFOS levels were also significantly associated with serum total cholesterol levels in workers, residents exposed to high levels of PFOA, and the general population. However, some studies of workers, highly-exposed residents, or the general population have not found associations between perfluoroalkyl exposure and total cholesterol levels. Studies in which the subjects were distributed into groups based on serum perfluoroalkyl levels typically found that subjects with the highest serum PFOA or PFOS levels had significantly higher total cholesterol levels than subjects with lowest serum PFOA or PFOS levels. A study of children and adolescents living in an area with high PFOA contamination also found an increased risk of high cholesterol levels (≥170 mg/dL). Similarly, an increased odds of high cholesterol (≥240 mg/dL) was observed in highly exposed adults with high serum PFOA and PFOS levels. Evidence of associations between serum perfluoroalkyl levels and other serum lipids is not as strong. Although increases in serum low-density lipoprotein (LDL)-cholesterol and triglyceride levels have been found in studies of workers and highly exposed individuals, a number of other studies have not found significant alterations. The relationship between perfluoroalkyl exposure and increases in serum lipid levels from longitudinal studies conducted in workers and highly exposed residents provide some evidence of an association. Serum PFOA levels were found to be a significant predictor of serum cholesterol levels in workers examined at least twice in a ≥5-year period. Similarly, a study of highly-exposed residents examined twice with approximately 4 years between examinations found that there were 3.6 and 1.7% decreases in serum LDL-cholesterol and total cholesterol levels, respectively, in subjects whose serum PFOS levels decreased by 50% between examinations. A 50% decrease in serum PFOS levels was associated with 5.0 and 3.2% decreases in LDL-cholesterol and total cholesterol. In addition, a greater change in cholesterol level per unit change in serum PFOA level was found at lower ranges of PFOA.*"

In the 'Minimal risk levels' subsection of the 'Relevance to public health' section, the ATSDR stated the following about PFOS/PFOA and serum cholesterol: "*The epidemiology studies lack environmental monitoring data; however, most studies used serum perfluoroalkyl levels as a biomarker of exposure. A wide range of effects have been statistically associated with serum perfluoroalkyl levels; however, there is a lack of consistency of the findings across studies and across types of studies. Based on the weight of evidence, there is support for identifying several health effects in humans that appear to be related to perfluoroalkyl exposure: increases in serum lipid levels.*"

The ATSDR continued: "*It could be proposed that serum perfluoroalkyl levels associated with increased risks of high serum cholesterol levels or hyperuricemia be used as the basis for developing an MRL. Of the two end points, the increased risk of high cholesterol is the stronger given the well-established association between serum cholesterol levels and the risk of heart disease. However, there are a number of factors that should be considered. Although 11 studies found significant associations between serum perfluoroalkyl levels and serum cholesterol levels, several studies of workers (Olsen and Zobel 2007; Olsen et al. 2000), highly exposed residents (Emmett et al. 2006a; Wang et al. 2012), and the general population (Fisher et al. 2013) have not found statistically significant associations. The epidemiology database lacks studies in which actual exposure concentration or doses were measured; however, most studies provided serum perfluoroalkyl levels, which is a biomarker of exposure. Exposures likely occurred via multiple routes of exposure. It is assumed that workers were primarily exposed via inhalation;*

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however, oral exposure may have also contributed to the total perfluoroalkyl body burden. Similarly, it has been determined that drinking water was the primary source of perfluoroalkyls in residents living near a PFOA facility; it is likely that they were also exposed to airborne perfluoroalkyls. However, a study of residents living near industrial facilities where PFOA is used found little difference in serum PFOA levels between residents with minimal expected exposure to airborne PFOA (mean serum PFOA level of 418 ng/mL) and those with higher than expected exposure to airborne PFOA (mean serum PFOA level of 418 ng/mL) (Emmett et al. 2006a). It should also be noted that most, if not all, subjects were exposed to a number of perfluoroalkyl compounds. Studies of highly exposed residents and the general population have often reported significant associations for both PFOA and PFOS, and the possible interaction of the various perfluoroalkyl compounds with the health end point of concern is not known. Lastly, the mechanisms of toxicity of the observed health effects have not been established and these effects have not been reported in laboratory animals. Serum cholesterol and other lipid levels are also affected by PFOA and PFOS exposure in rats and mice; however, in rodents, exposure to perfluoroalkyls resulted in significant decreases in serum lipid levels. These uncertainties preclude the use of currently available epidemiology studies as the basis for developing an MRL for PFOA or PFOS."

Summaries of studies reviewed

Occupational exposure studies- inhalation exposure

The ATSDR reported on 10 studies in the 'Inhalation exposure – systemic/hepatic effects' section.

Of the study by Costa (2004), the ATSDR reported: "*A small study of 35 workers at a manufacturing facility in Italy found higher total cholesterol and nonhigh-density lipoprotein (HDL)-cholesterol levels in the PFOA-exposed workers, as compared to levels in 94 workers who were not exposed to PFOA (Costa 2004).*"

Costa et al. (2009) completed a second study at the same Italian facility. The ATSDR reported: "*A second study at this facility also found significantly higher total cholesterol levels in 34 currently employed workers (mean serum PFOA level of 12,930 ng/mL), as compared to unexposed workers (Costa et al. 2009). No significant differences in HDL-cholesterol or triglyceride levels were found between the exposed and unexposed workers.*"

The ATSDR reported on a study by Olsen et al. (1999): "*Two studies measuring serum PFOS levels found a positive association with total cholesterol (Olsen et al. 1999, 2003a).*"

The ATSDR reviewed a study by Olsen et al. (2000), and reported that: "*Workers at a PFOA production facility were examined in 1993 (111 subjects), 1995 (80 subjects), and 1997 (74 subjects) (Olsen et al. 2000). Only 17 subjects were examined at all 3 time periods; 21 subjects were examined in 1995 and 1997 and 68 subjects were examined in 1993 and 1995. The study did not adjust for the use of cholesterol-lowering medication. When workers were categorized by blood PFOA levels (0–<1,000, 1,000–<10,000, and >10,000 ng/mL), no significant differences in serum cholesterol, HDL-cholesterol, LDL-cholesterol, or triglyceride levels were found at any of the monitoring periods.*"

Of the study by Olsen and Zobel (2007), the ATSDR reported: "*A study of 506 male workers at 3M facilities in Cottage Grove, Minnesota, Decatur, Alabama, and Antwerp, Belgium (mean serum PFOA level of 2,210 ng/mL) not taking cholesterol-lowering medications did not find associations between serum PFOA levels and total cholesterol or low-density lipoprotein (LDL) cholesterol levels; however, serum PFOA levels were positively associated with triglyceride levels and there was an increased risk of having high triglyceride levels (≥150 mg/dL) in workers with serum PFOA levels in the three highest deciles (odds ratios [ORs] of 2.7 [95% CI 1.2–6.5], 2.4 [95% CI 1.0–5.9], and 2.4 [95% CI 1.0–5.8], respectively) (Olsen and Zobel 2007). Additionally, there was a negative association between serum*

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PFOA levels and HDL-cholesterol levels and an increased risk of low HDL cholesterol levels (≤40 mg/dL) in workers with the highest serum PFOA levels (OR 2.6, 95% CI 1.0–6.8)."

Of the study by Sakr et al. (2007a), the ATSDR reported: "*Sakr et al. (2007a) used medical records for 454 male and female current and former workers (74% male) at the DuPont Washington Works facility (mean serum PFOA level of 1,130 ng/mL) and found a positive association between serum PFOA and total cholesterol levels; no associations with triglycerides, LDL-cholesterol, or HDL-cholesterol were found."*

Sakr et al. (2007b) completed a larger-scale study at the same facility. The ATSDR reviewed the study: "*A larger-scale study of this facility (1,025 current workers, 76% males) (mean serum PFOA level 428 ng/mL) found significant associations between serum PFOA levels and total cholesterol, LDL-cholesterol, and very-low-density lipoprotein (VLDL) cholesterol levels in all subjects and in a subset of subjects not taking cholesterol-lowering medication (Sakr et al. The study did not find any association between serum PFOA and HDL-cholesterol or triglyceride levels."*

Of the studies by Olsen et al. (1999), and Olsen et al. (2003a), the ATSDR reported: "*Workers at 3M facilities in Decauter, Alabama and Antwerp, Belgium were examined in 1995 and 1997 (178 male workers) (Olsen et al. 1999) and in 1995, 1997, and 2000 (421 male and 97 female workers) (Olsen et al. 2003a); neither study notes whether workers taking cholesterol-lowering medication were excluded. In workers with serum PFOS levels between 3,000 and 6,000 ng/mL, total cholesterol and LDL cholesterol levels were significantly higher compared to workers with serum PFOS levels < 1,000 ng/mL (Olsen et al. 1999), but this was only found in workers examined in 1997. The latter study (Olsen et al. 2003a) found positive associations between serum PFOS levels and total cholesterol and triglycerides among male workers. The triglyceride levels of men with PFOS levels in the fourth quartile (mean PFOS level of 2,690 ng/mL) were significantly higher than men with PFOS in the first quartile (mean PFOS of 270 ng/mL). No differences in total cholesterol or HDL-cholesterol levels were seen across PFOS quartiles.*

Longitudinal analysis was conducted using data for 174 workers with medical surveillance data in 2000 and 1997 and/or 1995 (Olsen et al. 2003a). No significant differences in serum PFOS levels were observed across the three time periods and serum PFOS level was not a significant predictor of cholesterol or triglyceride levels. In contrast, there were significant differences in serum PFOA levels between 1997 and 2000; serum PFOA levels were increased in 69 workers with only 1997 and 2000 data and decreased in 41 workers with 1995, 1997, and 2000 data. Serum PFOA was a significant predictor of cholesterol and triglyceride levels, which was primarily due to 21 workers at the Antwerp facility (mean serum level 8,400 ng/mL) whose serum PFOA levels increased and serum PFOS levels decreased over time."

Olsen et al. (2012) examined 179 workers. The ATSDR reviewed the study and stated: "*Olsen et al. (2012) examined 179 workers (none of the subjects reported using cholesterol-lowering medication) involved in the demolition of 3M perfluoroalkyl manufacturing facilities; serum PFOA and lipid levels were measured prior to the demolition and after demolition (mean time interval of 164 days). The mean baseline serum PFOA levels were 881 ng/mL in 14 3M workers with prior PFOA or PFOS exposure and 28.9 ng/mL in the remaining 165 workers. A decline in serum PFOA and PFOS levels were observed among the 3M workers. Among the workers with increased serum PFOA/PFOS levels (mean increase 50.9 ng/mL), there was a significant increase in HDL-cholesterol levels, but no change in total cholesterol or non HDL-cholesterol levels. No significant alterations in serum lipid levels were observed in the workers with decreased serum PFOA/PFOS levels. In workers whose baseline levels of PFOA and PFOS were <15 and <50 ng/mL, respectively, there were no significant differences between pre- and post-exposure serum lipid levels."*

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The ATSDR reported about the Mundt et al. (2007) study: "*Mundt et al. (2007) measured serum lipid levels in 592 workers at a polymer production facility using PFNA; blood samples were collected in 1976, 1989, 1995, 1998, and 2001. Significantly higher total cholesterol levels were observed in workers with high potential exposure to PFNA (based on job titles), as compared to the low exposure group, in 1976 and 1989; no differences were observed at other time points and no differences were found between the high-exposure and no-exposure groups. No significant alterations were observed for serum triglyceride, HDL-cholesterol, LDL-cholesterol, or VLDL cholesterol at any of the time points. Longitudinal analysis did not find significant differences in serum lipid levels over time.*"

ATSDR conclusion about occupational studies

The ATSDR provided the following summary and conclusion about the studies they reviewed on occupational exposure to PFAS and serum cholesterol in occupationally exposed workers: "*Seven occupational studies examined the possible associations between serum PFOA levels and serum lipid levels. Five of the studies found positive associations between serum PFOA and total cholesterol levels (Costa 2004; Costa et al. 2009; Olsen et al. 2003a; Sakr et al. 2007a, 2007b); however, studies by Olsen et al. (2000) and Olsen and Zobel (2007) did not find statistically significant associations. Two studies measuring serum PFOS levels found a positive association with total cholesterol (Olsen et al. 1999, 2003a). In general, significant association were not found between serum PFOA levels and LDL-cholesterol (Olsen and Zobel 2007; Olsen et al. 2000; Sakr et al. 2007a) or triglycerides (Costa et al. 2009; Olsen et al. 2000; Sakr et al. 2007a, 2007b), although some studies did report a positive association with LDL-cholesterol (Sakr et al. 2007a) and triglycerides (Olsen and Zobel 2007; Olsen et al. 2003a). With the exception of one study that reported a negative association (Olsen and Zobel 2007), no association was found between HDL-cholesterol and serum PFOA levels (Costa et al. 2009; Olsen et al. 2000, 2003a; Sakr et al. 2007a, 2007b). One limitation of cross-sectional studies is that they do not establish causality. Longitudinal assessments provide additional insight since they can examine serum lipid levels in response to changes in serum PFOA or PFOS. Olsen et al. (2012) did not find any changes in total cholesterol in works with increasing or decreasing serum PFOA levels; the mean interval between measurements was approximately 5 months. In contrast, Olsen et al. (2003a) found that serum PFOA was a significant predictor of cholesterol and triglyceride levels in workers whose serum PFOA levels increased over a 3–5-year period. Serum PFOS levels did not predict serum lipid levels.*"

Populations living near manufacturing facilities and the general population

The ATSDR reported on nine studies in the 'Oral exposure – systemic effects' section.

High-exposure community studies

Of the study by Emmett et al. (2006), the ATSDR reported the following:

"*A study of 328 adults and 43 children living in a community serviced by the Little Hocking Water Authority with a median serum PFOA level of 354 ng/mL did not find a significant association between serum PFOA levels and total cholesterol levels.*"

Of the study by Wang et al. (2012), the ATSDR stated: "*Similarly, Wang et al. (2012) found no associations between serum PFOA levels and total cholesterol, HDL-cholesterol, LDL-cholesterol, or triglycerides in a study of 132 adults living near a PFOA manufacturing facility in China; the mean serum PFOA level was 378.30 ng/mL.*"

The ATSDR also noted that neither Emmett et al. (2006) nor Wang et al. (2012) "*included an adjustment for the use of cholesterol-lowering medication*".

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The ATSDR noted that three larger-scale studies of participants in the C8 Science Panel studies found significant associations between serum PFOA and PFOS levels and serum lipid levels (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009).

Of the study by Frisbee et al. (2010), the ATSDR reported the following: "*Positive associations between serum PFOA and PFOS levels and total cholesterol and LDL-cholesterol were found in a study of over 12,000 children and adolescents; the respective mean serum PFOA and PFOS levels were 32.6 and 20.7 ng/mL in children (aged 1.0– 11.9 years) and 26.3 and 19.3 ng/mL in adolescents (12.0–17.9 years) (Frisbee et al. 2010). Serum PFOA was also positively associated with triglyceride levels and serum PFOS was positively associated with HDL-cholesterol. Additionally, there was an increased risk of high cholesterol (≥170 mg/dL) in subjects with serum PFOA levels in the fourth or fifth quintiles; ORs of 1.2 (95% CI 1.1–1.4) and 1.2 (95% CI 1.1–1.4), respectively, and with serum PFOS levels in the second through fifth quintiles (ORs of 1.3 [95% CI 1.1–1.4], 1.3 [95% CI 1.2–1.5], 1.3 [95% CI 1.2–1.6], and 1.6 [95%CI 1.4–1.9], respectively). Increased odds of high LDL-cholesterol (≥110 mg/dL) were also observed for the fifth PFOA quintile (OR 1.4, 95% CI 1.2–1.7) and fourth and fifth PFOS quintiles (ORs of 1.3 (95% CI 1.1–1.6) and 1.6 (95% CI 1.3–1.9). The investigators noted that the dose-response relationship between serum PFOA and serum lipids was nonlinear, with greater increases in lipids observed at the lower serum PFOA levels.*"

Of the longitudinal study by Fitz-Simon et al. (2013), the ATSDR reported: "*560 adults participating in the C8 Health Project and not taking cholesterol-lowering medication were examined twice, with an average of 4.4 years between examinations. Mean serum PFOA levels were 74.8 ng/mL at the first examination and 30.8 ng/mL at the second examination and serum PFOS levels were 18.5 and 8.2 ng/mL in the first and second examinations, respectively. In subjects whose serum PFOA levels halved between examinations, there was a 3.6% decrease in LDL-cholesterol levels and 1.7% decrease in total cholesterol levels. However, there were very small changes in LDL-cholesterol and total cholesterol levels in subjects whose serum PFOA levels decreased by >64% and there were slight increases in LDL-cholesterol and total cholesterol levels in subjects whose serum PFOA levels fell by <50%. For PFOS, halving the serum levels resulted in a 5.0% decrease in LDL-cholesterol and a 3.2% decrease in total cholesterol levels. Changes in PFOA or PFOS levels were not associated with changes in HDL-cholesterol or triglyceride levels.*"

The ATSDR reported on a second large-scale study of participants in the C8 Science Panel by Steenland et al. (2009): "*Similar findings were reported in a study of >46,000 adults with a median serum PFOA level of 26.6 ng/mL and a median serum PFOS level of 19.6 ng/mL; the study excluded subjects who reported taking cholesterol-lowering medication (Steenland et al. 2009). Positive associations were found between serum PFOA levels and total cholesterol, LDL-cholesterol, and non-HDL cholesterol; a positive association between serum PFOA and triglycerides was also found. No significant associations between serum PFOA and PFOS levels and HDL-cholesterol levels were found. Increased risks of having high cholesterol (≥240 mg/dL) were found in subjects with serum PFOA levels in the second, third, and fourth quartiles (ORs 1.21 [95% CI 1.12–1.31], 1.33 [95% CI 1.23–1.43], and 1.38 [95% CI 1.25–1.50], respectively). Subjects with serum PFOS levels in the second, third, and fourth quartiles also had elevated risks of high cholesterol (ORs 1.14 [95% CI 1.05–1.23], 1.28 [95% CI 1.19–1.39], 1.51 [95% CI 1.40– 1.64], respectively). The investigators noted that the odds of high cholesterol from the first quartile to the fifth quartile were approximately 40% for PFOA and 50% for PFOS, which may be important given that the Framingham study found that the risk of coronary heart disease was about 1.8 times higher in subjects with total cholesterol levels >240 mg/dL as compared to subjects with levels <200 mg/dL. Steenland et al. (2009) also found a significant association between serum PFOA levels and total cholesterol levels in a study of 10,746 adults taking cholesterol-lowering medication; no*

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consistent findings were identified for PFOS. Using both groups of subjects (taking or not taking cholesterol-lowering medication), the investigators analyzed whether taking cholesterol medication was associated with lower serum PFOA or PFOS levels, which may be indicative of reverse causality. Although serum PFOA levels were significantly lower in subjects taking cholesterol-lowering medication, the difference between the groups was low (4%); no differences in serum PFOS levels were found between the two groups."

General population studies

Of the study by Nelson et al. (2010), the ATSDR reported: "*Using NHANES data for 860 adults not taking cholesterol-lowering medication (mean serum PFOA level of 4.6 ng/mL), there was a significant positive association between serum PFOA levels and non-HDL-cholesterol levels across PFOA quartiles; total cholesterol levels also increased with serum PFOA levels, but were not statistically associated (Nelson et al. 2010). Serum PFOS and PFNA levels (mean levels of 25.3 and 1.3 ng/mL, respectively) were also positively associated with total cholesterol and non-HDL-cholesterol levels. No associations were found for HDL-cholesterol or LDL-cholesterol levels with serum PFOA, PFOS, or PFNA levels. Serum PFHxS levels (mean level of 2.6 ng/mL) were negatively associated with a change in non-HDL-cholesterol levels across PFHxS quartiles and were not significantly associated with other serum lipid levels. No significant associations were found between HDL-cholesterol levels and serum PFOA, PFOS, PFHxS, or PFNA levels.*"

The ATSDR reported on the following study by Eriksen et al. (2013): "*Positive associations between serum PFOA and PFOS levels and total cholesterol levels were also found in a study of 753 Danish adults not taking cholesterol-lowering medication (mean serum PFOA and PFOS levels of 7.1 and 36.1 ng/mL, respectively) (Eriksen et al. 2013).*"

Of the study by Fisher et al. (2013), the ATSDR stated: "*No significant associations between serum PFOA or PFOS levels and total cholesterol, LDL-cholesterol, or non-HDL-cholesterol levels were found in 2,368 Canadian adults not taking cholesterol medication with a geometric mean serum PFOA level of 2.46 ng/mL and a PFOS level of 8.40 ng/mL (Fisher et al. 2013). The study did find positive associations between serum PFHxS levels (geometric mean level of 2.16 ng/mL) and total cholesterol, LDL-cholesterol, and non-HDL-cholesterol. Increased odds of having a high cholesterol level were also found for increasing PFHxS levels (OR 1.27, 95% CI 1.11–1.45).*"

The ATSDR reported the study by Château-Degat et al. (2010) as: "*A study of 723 Inuit adults living in Nunavik, Canada with a high dietary exposure to n-3 polyunsaturated fatty acids found in traditional food items and not taking cholesterol lowering medication found linear trends for total cholesterol and HDL-cholesterol levels across serum PFOS quartiles (Château-Degat et al. 2010). Regression analysis showed a significant positive association between serum PFOS and HDL-cholesterol; serum PFOS level was also significantly associated with triglyceride levels, but only in females.*"

Summary and conclusions of ATSDR on high-exposure communities and general population exposure studies on PFAS exposure and serum cholesterol

The ATSDR reported the following about the studies they reviewed:

"*In summary, the available epidemiology data provide strong support for a positive association between serum PFOA and serum PFOS levels and total cholesterol and non-HDL-cholesterol, particularly LDL-cholesterol, in populations living near PFOA/PFOS facilities or the general population with mean or median serum PFOA levels >7 ng/mL and PFOS levels >20 ng/mL (Eriksen et al. 2010; Frisbee et al. 2010; Nelson et al. 2010; Steenland et al. 2009). However, in two studies of highly exposed populations (mean or median serum PFOA level >350 ng/mL), no association was found between serum PFOA and*

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total cholesterol (Emmett et al. 2006b; Wang et al. 2012). It is not known if the conflicting result is to a nonlinear relationship between serum PFOA and total cholesterol levels, the low statistical power of the studies (328 and 132 adults), or the lack of adjustment for the use of cholesterol-lowering medication. Studies of the general population with lower serum PFOA levels (<5ng/mL) or PFOS (<8 ng/mL) did not find significant alterations in serum lipid levels (Fisher et al. 2013; Nelson et al. 2010). Two studies of the C8 Health Project participants also found increased risk of high cholesterol levels in adults (Steenland et al. 2009) or children and adolescents (Frisbee et al. 2010). The small number of studies examining possible associations between serum lipid levels and PFHxS (Fisher et al. 2013; Nelson et al. 2010) or PFNA (Nelson et al. 2010) levels preclude drawing conclusions about possible associations. Inconsistent results have been found for HDL-cholesterol. A positive association between serum PFOS and HDL-cholesterol was found in children and adolescents participating in the C8 Health Project studies (mean serum PFOS level of approximately 20 ng/mL) (Frisbee et al. 2010) and in a Canadian population with a mean serum PFOS level of 26 ng/mL (Château-Degat et al. 2010), but was not found in adult C8 participants with a mean serum PFOS level of 20 ng/mL (Steenland et al. 2009). No studies have found associations between serum PFOA and HDL-cholesterol (Frisbee et al. 2010; Nelson et al. 2010; Steenland et al. 2009)."

United States Environmental Protection Agency (US EPA, 2016a and 2016b)

In 2016, the US EPA (2016a and 2016b) in their health effects support documents for PFOA and PFOS reviewed evidence of the link between PFOA/PFOS and cholesterol.

Studies reviewed

The US EPA (2016a and 2016b) in their health effects support documents for PFOA and PFOS, reviewed human studies on cholesterol and serum lipids.

PFOA:

Seven occupational exposure studies of PFOA and serum lipids (Costa et al. 2009, Olsen et al. 2000, Olsen et al. 2003a; Olsen and Zobel 2007, Sakr et al. 2007a, Sakr et al. 2007b, Steenland et al. 2015);

Six high-exposure community studies (Emmett et al. 2006, Fitz-Simon et al. 2013, Winquist and Steenland, 2014, Steenland et al. 2009; Frisbee et al. 2010; Fletcher et al. 2013);

Five general population epidemiology studies of PFOA and serum lipids (Eriksen et al. 2013, Fisher et al. 2013, Geiger et al. 2014a, Nelson et al. 2010 and Starling et al. 2014b);

PFOS:

- Three occupational exposure studies of PFOS and serum lipids (Olsen et al. 2001a, 2001b, 2003a);
- Four studies on high-exposure communities (Fitz-Simon et al. 2013, Steenland et al. 2009; Frisbee et al. 2010; Fletcher et al. 2013);
- Six studies on PFOS and serum lipids in the general population (Nelson et al. 2010; Lin et al. 2009; Château-Degat et al. 2010; Eriksen et al. 2013; Starling et al. 2014b; Fisher et al. 2013);
- Five studies of children and adolescents and PFOS and serum lipids (Frisbee et al. 2010, Geiger et al. 2014a, Lin et al. 2009, Maisonet at al. 2015b, and Timmermann et al. 2014); and
- One study of pregnant women and PFOS and serum lipids (Starling et al. 2014b).

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Findings for the five studies on children were reported in Table 3-1 but not discussed further in the text under serum lipids.

Multiple studies reviewed by the US EPA were also reviewed by the ATSDR. However, the US EPA (2016a and 2016b) also included more detail when reporting on many of the studies. In this case, the additional information has been provided.

Considerations and conclusions

In the 'Executive Summary', the US EPA 2016a reported on PFOA: "*These epidemiology studies have generally found positive associations between serum PFOA concentration and total cholesterol (TC) in the PFOA-exposed workers and the high-exposure community (i.e., increasing lipid level with increasing PFOA); similar patterns are seen with low-density lipoproteins (LDLs) but not with high-density lipoproteins (HDLs). These associations were seen in most of the general population studies, but similar results also were seen with PFOS, and the studies did not always adjust for these correlations.*"

In the 'Summary and conclusions' from the 'Human epidemiology studies' section, the US EPA (2016a) reported: "*Cross-sectional and longitudinal studies in occupational settings (Costa et al. 2009; Olsen et al. 2000, 2003a; Olsen and Zobel 2007; Sakr et al. 2007a, 2007b; Steenland et al. 2015) and in the high-exposure community (the C8 Health Project study population) (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009; Winquist and Steenland 2014a) generally observed positive associations between serum PFOA and TC in adults and children (aged 1–< 18 yrs); most of these effect estimates were statistically significant. Although exceptions to this pattern are present (e.g., some of the analyses examining incidence of self-reported high cholesterol based on medication use [Steenland et al. 2015; Winquist and Steenland 2014a]), the results are relatively consistent and robust. Similar associations were seen in analyses of LDL, but were not seen with HDL. The range of exposure in occupational studies is large (with means varying between 400 and > 12000 ng/mL), and the mean serum levels in the C8 population studies were around 80 ng/mL. Positive associations between serum PFOA and TC (i.e., increasing lipid level with increasing PFOA) were observed in most of the general population studies at mean exposure levels of 2–7 ng/mL (Eriksen et al. 2013; Fisher et al. 2013; Geiger et al. 2014a; Nelson et al. 2010; Starling et al. 2014b). The interpretation of results for these general population studies is limited, however, by the moderately strong correlations (Spearman r > 0.6) and similarity in results seen for PFOS and PFOA. Additionally, many of the C8 studies do not appear to have controlled for the impact of diet on serum lipids.*"

The US EPA (2016b) reported, in the 'Executive Summary' of the 'Health Effects' support document for PFOS: "*Numerous epidemiological studies have examined occupational populations at large-scale PFOS production plants in the United States and a residential population living near a PFOA production facility in an attempt to determine the relationship between serum PFOS concentration and various health outcomes. Epidemiology data report associations between PFOS exposure and high cholesterol. The strongest associations are related to serum lipids with increased total cholesterol and high-density lipoproteins (HDLS).*"

In the 'Summary and conclusions' from the Human epidemiology studies' section, the US EPA (2016b) stated: "*Hypercholesterolemia, which is clinically defined as cholesterol > 240 mg/dL, was associated with PFOS exposure in a Canadian cohort (Fisher et al. 2013) and in the C8 cohort (Steenland et al. 2009). Cross-sectional occupational studies demonstrated an association between PFOS and total cholesterol (Olsen et al. 2001a, 2001b, 2003a). Evidence for associations between other serum lipids and PFOS is mixed, including HDL cholesterol, LDL, VLDL, and non-HDL cholesterol, as well as triglycerides. The studies on serum lipids in association with PFOS serum concentrations are largely cross-sectional in nature and were largely conducted in adults, but some studies exist on children*

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and pregnant females. The location of these cohorts varied from the U.S. population including NHANES volunteers, to the Avon cohort in the UK, to Scandinavian countries. Limitations to these studies include the frequently high correlation between PFOA and PFOS exposure; not all studies control for PFOA in study design. Also studied were populations with known elevated exposure to other environmental chemicals including PFOA in the C8 population and PBDEs and other persistent organic chemicals in the Inuit population."

Summaries of studies reviewed

Occupational exposure studies, PFOA and serum lipids

The US EPA (2016a) reviewed seven occupational exposure studies that explored the link between PFOA and serum lipids. These studies came from the 'Noncancer – Serum lipids and cardiovascular diseases' section. There is one study that was not reviewed by the ATSDR (Steenland et al. 2015).

Of the study by Steenland et al. (2015), the US EPA (2016a) reported:

"*Steenland et al. (2015) conducted an analysis of the incidence of several conditions, including high cholesterol (based on prescription medication use) among 3,713 workers at the Washington Works plant in West Virginia who participated in the C8 Health Project. Yearly serum estimates were modeled from work history information and job-specific concentrations. Cox proportional hazard models, stratified by birth year, were used to assess self-reported incidence of high cholesterol in relation to time-varying cumulative estimated PFOA serum concentration, controlling for gender, race, education, smoking, and alcohol consumption. No association was seen when analyzed without a lag (HRs by quartile 1.0, 1.11, 1.06, 1.05; trend p = 0.56 for log cumulative exposure), or when using a 10-year lag (HRs by quartile 1.0, 0.93, 1.01, 0.96; trend p = 0.62)."*

The US EPA (2016a) provided additional information on the following studies (compared to the ATSDR) for Costa et al. (2009), Olsen et al. (2000), Olsen and Zobel (2007), Sakr et al. (2007a), and Sakr et al. (2007b). This supplementary information is noted below.

For Costa et al. (2009), the US EPA (2016a) reported: "*Costa et al. (2009) examined serum lipid data using 30 years of medical surveillance data from workers of a PFOA production plant in Italy. The workers (n = 53 males, 20–63 years of age) participated in the medical surveillance program yearly from 1978 to 2007. The length of work exposure was 0.5–32.5 years. In 2007, 37 males were active workers and 16 males were retired or had transferred to other departments and were no longer being exposed. Unexposed male workers (n = 107, 12 executives and 95 blue collar workers) from different departments also participated in the medical surveillance program and served as controls. Beginning in 2000, serum PFOA was monitored yearly except in 2005. Serum PFOA concentrations in the workers decreased after plant renovations partially automated the PFOA production process and procedures for the use of protective devices were instituted in 2002. In 2007, the geometric mean serum PFOA was 4020 and 3760 ng/mL, respectively, in currently exposed and retired workers. Three analyses were conducted: a t-test comparing 34 exposed workers matched to 34 unexposed workers by age, work seniority, day/shift work, and living conditions; linear regression with 34 exposed workers and 107 unexposed workers adjusting for age, work seniority, BMI, smoking, and alcohol consumption; and a repeated measures analysis with a total of 56 individuals with more than one measure, adjusting for age, work seniority, BMI, smoking, alcohol consumption, and year of observation. TC and uric acid were significantly increased (p<0.05) in relation to PFOA exposure in each of these analyses. No correlations were observed between serum PFOA concentration and Apo-A (HDL-associated) or Apo-B (LDL-associated) proteins, HDL, or triglycerides in any of the analyses. PFOS was not included in this study."*

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For Olsen et al. (2000), the US EPA (2016a) reported: "*Cholesterol, LDL, HDL, and triglycerides were measured in male workers (n = 111 in 1993, n = 80 in 1995, and n = 74 in 1997). Multivariable regression analyses, conducted separately by year (cross-sectional), were adjusted for age, BMI, alcohol consumption, and cigarette use. Employees' serum PFOA levels were stratified into three categories—<1000, 1000-<10000, and ≥10000 ng/mL. The sample size in the highest category ranged from 11 to 15 in the three examination years. There was little variation by exposure category in mean or median TC, LDL, HDL, or triglycerides across the workers in 1993, 1995, or 1997.*"

For the study by Olsen and Zobel (2007), the US EPA (2016a) reported: "*Olsen and Zobel (2007) examined data from the 2000 medical surveillance program at the three 3M plants, which is an expanded and refined analysis of the data reported in Olsen et al. (2003a). The fluorochemical workers consisted of males (age 21–67) from the Antwerp, Belgium (n = 196); Cottage Grove, Minnesota (n = 122); and Decatur, Alabama (n = 188) production facilities who volunteered to participate in the medical surveillance program and did not take cholesterol-lowering medication. Blood was collected for fluorochemical concentration determination and serum lipid parameters including cholesterol, LDL, HDL, and triglycerides. Analysis of variance (ANOVA), analysis of covariance, logistic regression, and multiple regression models were used to analyze the data with age, BMI, and alcohol consumption as covariates. Potential associations with PFOS levels were not evaluated because a previous analysis had shown no association between PFOS and the selected outcomes. Serum PFOA concentrations ranged from 10 to 92030 ng/mL for the male workers (all sites combined), with a mean serum PFOA concentration of 2210, 1020, 4630, and 1890 ng/mL for all sites combined, and the Antwerp, Cottage Grove, and Decatur sites, respectively. Serum PFOA (all sites combined) was not associated with TC or LDL. A negative association was observed between serum PFOA concentration (all sites combined) and HDL. Serum triglyceride was positively associated with serum PFOA at all sites combined and independently at the Antwerp site. Nonadherence to the fasting requirement for blood collection, especially for night-shift workers, and potential binding of PFOA to albumin and LDL, were identified by the authors as possible factors that influenced the triglyceride results.*"

Of the study by Sakr et al. (2007b), the US EPA (2016a) provided the following details: "*The employees who volunteered to participate in the study (n = 1025, 782 males, 243 females) each had a physical examination, provided a fasting blood sample, and answered a medical and occupation history questionnaire in 2004. The association between PFOA and lipid levels was evaluated by ANOVA, $\chi 2$ test, student's t-test, and linear regression models. Confounders including age, BMI, gender, alcohol consumption, and parental heart attack were considered in the models. Mean serum PFOA concentration in the workers was 428 ± 189 ng/mL (interquartile range 0.099–0.381). For those with current occupational exposure to PFOA, the range was 7.4–9550 ng/mL and for workers with intermittent occupational exposure, the range was 8.1–2070 ng/mL. The range was 8.6–2590 ng/mL for workers with past occupational exposure and the 4.6–963 ng/mL for workers with no occupational exposure. Serum PFOA was positively associated with cholesterol, very low-density lipoprotein (VLDL), and LDL (p< 0.03) in the participating workers, whether or not they were taking lipid-lowering medication. No association was observed between serum PFOA and HDL or triglycerides. PFOS was not included in the study.*"

For Sakr et al. (2007a), the US EPA (2016a) reported: "*Sakr et al. (2007a) conducted a longitudinal analysis among the workers at the DuPont Washington Works plant in West Virginia using data from 1979 to 2004... Serum PFOA concentration was measured every 1–2 years in PFOA-exposed workers and every 3–5 years in non-PFOA-exposed workers on a volunteer basis. This study included 454 workers who had two or more serum PFOA measurements. The study population included 334 males and 120 females ranging in age from 24 to 66 years who had worked at the plant for at least 1 year*

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since 1979. A linear mixed effects regression model was used to analyze the data and accounted for age (and age-squared), gender, BMI, and decade of hire as potential confounders. Serum PFOA concentrations ranged from 0 to 22660 ng/mL, with a mean of 1130 ng/mL over the 23-year monitoring period in the study population. For employees with two or more PFOA measurements, the mean of the first and last sample was 1040 ng/mL and 1160 ng/mL, respectively, with an average of 10.8 years between samples. Serum PFOA concentration was positively associated with TC after age, BMI, gender, and decade of hire adjustment in the model (Beta = 1.06, 95% CI 0.24, 1.88) per ppm increase in PFOA. Information on lipid-lowering medications and alcohol intake by the participants was not available. PFOS was not included in this study."

High-exposure community studies, PFOA and serum lipids

The US EPA reviewed six high-exposure community studies (Emmett et al. 2006, Fitz-Simon et al. 2013, Winquist and Steenland 2014, Steenland et al. 2009; Frisbee et al. 2010; Fletcher et al. 2013).

The ATSDR reviewed four of the same studies (Emmett et al. 2006, Fitz-Simon et al. 2013, Steenland et al. 2009; Frisbee et al. 2010). The US EPA reviewed the studies by Winquist and Steenland (2014) and Fletcher et al. (2013) which the ATSDR did not review. The ATSDR reviewed the study by Wang et al. 2012, which the US EPA did not review.

The US EPA noted at the start of the section where they reviewed the high-exposure community studies: "*Several studies examined serum lipids in populations serviced by water districts contaminated by the Washington Works PFOA production plant in Ohio and West Virginia (Table 3-2). Emmett et al. (2006) is a small study (n = 371) with limited analysis (t-tests comparing PFOA levels in people with abnormal versus normal TC); the larger studies were conducted as part of the C8 Health Project. This collection of studies includes analyses of current serum PFOA levels in relation to serum lipids in adults (Steenland et al. 2009) and children (Frisbee et al. 2010), longitudinal analysis of the change in lipids seen in relation to a change in serum PFOA (Fitz-Simon et al. 2013), and analyses of the incidence of hypercholesteremia in relation to modeled exposure (Winquist and Steenland 2014a). With the exception of one set of analyses within the Winquist and Steenland study (2014a), these data provide consistent evidence of positive associations between PFOA exposure (measured directly in blood or modeled based on environmental and drinking water data) and TC.*"

Of the study by Winquist and Steenland (2014), that was not reviewed by the ATSDR, the US EPA (2016a) reported: "*More recently, participants in the C8 Health Project were examined for an association between PFOA levels and incidence of several conditions, including high cholesterol (based on prescription medication use) (Winquist and Steenland 2014a). The cohort included 28,541 community members and 3,713 workers who had completed study questionnaires during 2008– 2011. The median serum PFOA level at enrollment in 2005–2006 was 26.1 ng/mL for the combined cohort, 24.2 ng/mL for the community members, and 112.7 ng/mL for the workers. Retrospective serum levels for the community cohort were estimated from air and water concentrations, residential history, and water consumption rates. For the workers, yearly serum estimates were modeled from work history information and job-specific concentrations. Cox proportional hazard models, stratified by birth year, were used to assess self-reported adult heart disease hazard in relation to time-varying yearly or cumulative (sum of yearly estimates) estimated PFOA serum concentration, controlling for gender, race, education, smoking, and alcohol consumption. Using the cumulative exposure metric, the HRs for hypercholesterolemia for quintiles 2–5 versus quintile 1 were 1.24, 1.17, 1.19, and 1.19 (Ptrend = 0.005). Using the yearly exposure metric, the HRs for high cholesterol for quintiles 2–5 versus quintile 1 were 1.07, 1.11, 1.05, and 1.20 (Ptrend = 0.001). The strongest association was in males aged 40–59. No associations were found between PFOA level and hypertension or coronary artery disease incidence.*"

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The US EPA reported the following about the study by Fletcher et al. (2013): "*A subset of 290 individuals in the C8 Health Project was evaluated for evidence that PFOA exposure can influence the transcript expression of genes involved in cholesterol metabolism, mobilization, or transport (Fletcher et al. 2013). RNA was extracted from whole blood samples taken from 144 males and 146 females aged 20–60 years; serum collected at the same time was used to measure PFOA concentration. The association between candidate gene expression levels and PFOA levels was assessed by multivariable linear regression with adjustments for confounders. Inverse associations were found between PFOA levels and expressions of transcripts involved in cholesterol transport (NR1H2, NPC1, and ABCG1; p = 0.002, 0.026, and 0.014, respectively). When genders were analyzed separately, PFOA was negatively associated with expression of genes involved in cholesterol transport in males (NPC1, ABCG1, PPARα) and females (NCEH1). Similar associations were found with PFOS.*"

Additional details of the studies that were reported under the ATSDR section for high-exposure communities are included below.

The US EPA (2016a) reported for the study by Emmet et al. (2006): "*Emmett et al. (2006) is a small study (n = 371) with limited analysis (t-tests comparing PFOA levels in people with abnormal versus normal TC)... Emmett et al. (2006) examined the association of serum PFOA concentration with serum TC in residents of the Little Hocking water district in Ohio. The study population (n = 371, 2–>60 years of age) was a random sample of the population served by LHWA*[16]*. The subjects completed questionnaires (e.g., demographic, occupational, health conditions, and so forth) and provided blood samples. PFOA concentration was determined by HPLC/MS/MS; no other PFASs were measured. Regression models were used to analyze the data. The median serum PFOA concentration was 354 ng/mL. No association was observed between serum PFOA and TC.*"

Of the study by Fitz-Simon et al. (2013), the US EPA noted: "*A cohort of 521 members of the C8 Health Project was evaluated for an association between changes in serum PFOA levels and changes in serum LDL-cholesterol, HDL-cholesterol, TC, and triglycerides over a 4.4-year period (Fitz-Simon et al. 2013). Linear regression models were fit to the logarithm (base 10) of ratio change in each serum lipid measurement in relation to the logarithm of ratio change in PFOA. Mean serum PFOA concentration decreased by approximately one-half between baseline (140 ± 209 ng/mL) and follow-up (68 ± 144 ng/mL). No corresponding changes in serum lipids were found. However, those individuals with the greatest declines in serum PFOA had a larger decrease in LDL cholesterol.*"

Of the study by Frisbee et al. (2010), the US EPA (2016a) reported: "*The mean serum PFOA concentration was 77.7 ng/mL and 61.8 ng/mL, respectively, for children and adolescents. TC, LDL, and triglycerides were positively associated (p≤0.02) with serum PFOA concentration, adjusting for age, gender, BMI, exercise, and length of fast. Assessment of the quintile trends showed significant differences (p≤0.02) between the first and fifth quintile for TC and LDL for children and adolescents of both genders combined and separated. A significant difference (p = 0.04) was observed for fasting triglycerides in female children only. An increased risk of abnormal TC and LDL were positively associated with serum PFOA. The ORs were 1.0 first (reference), 1.1 (95% CI: 1.0–1.3, second), 1.2 (95% CI: 1.0–1.4, third), and 1.2 (95% CI: 1.1–1.4, fourth and fifth) for TC, and 1.0 (reference, first), 1.2 (95% CI: 1.0–1.5, second), 1.2 (95% CI: 1.0–1.4, third and fourth), and 1.4 (95% CI: 1.2–1.7, fifth) for LDL. An increased risk of abnormal fasting triglyceride and HDL was not associated with serum PFOA. PFOS also was positively associated with TC, LDL cholesterol, and HDL cholesterol.*"

Of the study by Steenland et al. (2009), the US EPA noted: "*No association was observed between mean level of serum PFOA and HDL cholesterol. PFOS also was positively associated with TC, LDL*

[16] Little Hocking Water Association, Inc. *Lipid outcomes Perfluorooctane*

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cholesterol, and triglycerides. The results of the study were consistent with occupational studies that found a positive association between PFOA exposure and serum lipids."

General population epidemiology studies, PFOA and serum lipids

The US EPA (2016a) reviewed the following general population epidemiology studies regarding PFOA and serum lipids (Eriksen et al. 2013; Fisher et al. 2013; Geiger et al. 2014; Nelson et al. 2010; Starling et al. 2014b). The studies were found in the 'Hazard identification – human studies – noncancer – serum lipids and cardiovascular diseases' section.

The ATSDR also reviewed the studies by Eriksen et al. (2013), Fisher et al. (2013), and Nelson et al. (2010). The studies by Geiger et al. (2014) and Starling et al. (2014b) were not reviewed by the ATSDR.

Additional information about those studies reviewed by the ATSDR along with the two studies reviewed by the US EPA, but not by the ATSDR is provided below.

Of the study by Starling et al. (2014b), that was not reviewed by the ATSDR, the US EPA (2016a) reported: "*Starling et al. (2014b) examined the association between PFOA (and six other PFASs) and serum lipids in pregnant females in the Norwegian Mother and Child Cohort Study. Most of the blood samples were drawn during weeks 14–26 of gestation. Weighted multiple linear regression was used to estimate the association between PFOA level and each lipid level. Covariates considered included age, prepregnancy BMI, nulliparous or interpregnancy interval, breastfeeding duration, education, current smoking, gestation week at blood draw, oily fish consumption, and weight gain during pregnancy. The median plasma PFOA level was 2.25 ng/mL. No association was observed between PFOA and triglycerides, TC, or LDLcholesterol. PFOA was positively associated with HDL-cholesterol, although the CI was large for the association. With HDL-cholesterol, each interquartile range- (IQR-) unit increase in lnPFOA was associated with an increase of 1.28 mg/dL (95% CI: -0.15, 2.71). Five of the seven PFASs studied were positively associated with HDL cholesterol and all seven had elevated HDL associated with the highest quartile."*

For the study by Geiger et al. (2014) the US EPA reported: "*A similar analysis [to Nelson et al. 2010], using 1999–2008 NHANES data for 815 adolescents (aged 12–18 years) by Geiger et al. (2014a) found an association between serum PFOA and TC (Beta 4.55, 95% CI 0.90, 8.20, per ln-unit increase in PFOA) and LDL (Beta 5.75, 95% CI 2.16, 9.33, per ln-unit increase in PFOA)."*

Of the study by Nelson et al. (2010), and the US EPA (2016a) reported: "*Nelson et al. (2010) examined the relationship between polyfluoroalkyl chemical serum concentration, including PFOA, and lipid and weight outcomes in the general population of the United States by analyzing data from the 2003–2004 NHANES. The population (n = 860) included persons aged 20–80 years with no missing covariate information who were not pregnant, breast-feeding, taking insulin or cholesterol medicine, or undergoing dialysis. Cholesterol (TC, HDL, LDL) was measured from serum samples. Data for covariates predicting cholesterol and body weight including age, gender, race/ethnicity, socioeconomic status, saturated fat intake, exercise, alcohol consumption at ≥ 20 years of age, smoking, and parity were obtained from the questionnaires. Regression analyses were performed for gender and the age groups 12–19 years, 20–59 years, and 60–80 years. The mean PFOA concentration was 4.6 ± 3 ng/mL. A positive association was found between TC and non-HDL (TC-HDL, ~70–80% TC) cholesterol and serum PFOA (effect estimate 9.8; 95% CI, -0.2–19.7). No association was found between serum PFOA concentration and HDL, or LDL. No association was found between serum PFOA concentration and body weight. Similar results were found with PFOS."*

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For the study by Eriksen et al. (2013), the US EPA (2016a), reported the following information: "*Eriksen et al. (2013) examined the association between plasma PFOA (and PFOS) levels and TC levels in a middle-aged Danish population. This cross-sectional study included 663 males and 90 females aged 50–65 years who were enrolled in the Danish Diet, Cancer and Health cohort. Generalized linear models were used to analyze the association between PFOA and TC levels, adjusted for age, gender, education, BMI, smoking, alcohol consumption, egg intake, animal fat intake, and physical activity. The mean plasma PFOA level was 7.1 mg/mL. A significant, positive association was found between PFOA (and PFOS) and TC such that, in the fully adjusted model, a 4.4-mg/dL (95% CI 0.8, 8.5) higher concentration of TC was found per interquartile range of plasma PFOA (quartile cut-points were not reported).*"

For the study by Fisher et al. (2013), the US EPA (2016a) noted: "*Fisher et al. (2013) examined the association of plasma PFAS levels, including PFOA, with metabolic function and plasma lipid levels. This population-based sample included 2,700 participants aged 18–74 years (~50% male) in the Canadian Health Measures Survey. The geometric mean PFOA concentration was 2.5 ± 1.8 ng/mL. In analyses that included sampling weights, no associations were found between PFOA (or PFOS) and TC, HDL- and LDL-cholesterol, and metabolic syndrome and glucose homeostasis parameters. Covariates considered included age, gender, marital status, income adequacy, race, education, BMI, physical activity, smoking, and alcohol consumption.*"

US EPA Summary of all studies reviewed on PFOA and cholesterol and serum lipids

At the end of the section where the studies were reviewed, the US EPA made the following summary statement: "*The association between PFOA and serum lipids has been examined in several studies in different populations. Cross-sectional and longitudinal studies in occupational settings (Costa et al. 2009; Olsen et al. 2000, 2003a; Olsen and Zobel 2007; Sakr et al. 2007a, 2007b; Steenland et al. 2015) and in the high-exposure community (the C8 Health Project study population) (FitzSimon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009; Winquist and Steenland 2014a) generally observed positive associations between serum PFOA and TC in adults and children (aged 1–< 18 yrs); most of the effect estimates were statistically significant. Although exceptions to this pattern are present (i.e., some of the analyses examining incidence of self-reported high cholesterol based on medication use in Winquist and Steenland [2014a] and in Steenland et al. [2015]), the results are relatively consistent and robust. Similar associations were seen in analyses of LDL, but were not seen with HDL. The range of exposure in occupational studies is large (with means varying between 400 and > 12000 ng/mL), and the mean serum levels in the C8 population studies were around 80 ng/mL. Positive associations between serum PFOA and TC (i.e., increasing lipid level with increasing PFOA) were observed in most of the general population studies at mean exposure levels of 2–7 ng/mL (Eriksen et al. 2013; Fisher et al. 2013; Geiger et al. 2014a; Nelson et al. 2010; Starling et al. 2014b). The interpretation of these general population results is limited, however, by the moderately strong correlations (Spearman r > 0.6) and similarity in results seen for PFOS and PFOA.*"

Occupational exposure studies, PFOS and serum lipids

The US EPA reviewed three occupational exposure studies on PFOS and serum lipids (Olsen et al. 2001a, 2001b, 2003a).

The following data can be found in the 'Human effects – long-term noncancer epidemiological studies – serum lipids and cardiovascular diseases' section.

The US EPA noted: "*Cross-sectional, as well as a longitudinal analyses of medical surveillance data from the 3M Decatur, Alabama and Antwerp, Belgium plants were conducted to evaluate possible associations between PFOS levels and hematology, clinical chemistry, and hormonal parameters (Olsen et. al 2001a, 2001b, 2003a).*"

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The ATSDR also reviewed the study by Olsen et al. (2003a) and a summary can be found in the above section.

Of Olsen et al. (2001a,), the US EPA (2016b) reported: "*In the cross-sectional study, male (n = 215) and female (n = 48) volunteers working at the Decatur plant and male (n = 206) and female (n = 49) volunteers working at the Antwerp plant underwent clinical chemistry tests to evaluate hepatic enzyme activity, renal function, thyroid activity, and cholesterol levels. Data on employees from both plants appeared to be combined for the regression analyses; however, it was not clear whether females were included or whether the analyses only included males. The mean PFOS level in all employees from the Decatur and Antwerp plants was 1400 ng/mL (range: 110– 10060 ng/mL) and 960 ng/mL (range: 40–6240 ng/mL), respectively. Positive significant associations were reported between serum PFOS and cholesterol (probability [p] = 0.04) and between serum PFOS and triglycerides (p = 0.01); similar results were found for PFOA. Age was also significant in both analyses. Alcohol consumed per day was significant in the cholesterol model, while body mass index (BMI) and cigarettes smoked per day was significant for triglycerides. PFOS was positively associated with alkaline phosphatase (ALP). Hepatic enzymes and bilirubin were not associated with PFOA. However, there were many limitations to combining and comparing the data from the two plants.*"

Of the studies by Olsen et al. (2001b; 2003a), the US EPA reported: "*A longitudinal analysis of the above data was performed to determine whether occupational exposure to fluorochemicals over time was related to changes in clinical chemistry and lipids (Olsen et al. 2001b, 2003a). The medical surveillance data from 175 individuals who had participated in two or more medical exams in 1995, 1997, and 2000 were analyzed using multivariable regression. Mean PFOS levels at the beginning and end of the surveillance period were 2620 ng/mL and 1670 ng/mL, respectively, in Decatur employees and 1870 ng/mL and 1160 ng/mL, respectively, in Antwerp employees. When male employees from both plants were combined, no statistically-significant (p < 0.05) associations were observed over time between PFOS and serum cholesterol or triglycerides. There were no significant associations between PFOS and changes over time in HDL, ALP, gamma-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), or alanine transaminase (ALT) activities, total bilirubin, or direct bilirubin. PFOA was positively associated with cholesterol and triglycerides in the Antwerp employees.*"

High-exposure community studies – PFOS and serum lipids

The US EPA reviewed four studies on the C8 Health Project communities (Steenland et al. 2009; Frisbee et al. 2010; Fitz-Simon et al. 2013; Fletcher et al. 2013). Additional information on these studies is provided below. The ATSDR did not review the study by Fletcher et al. (2013).

The US EPA provided the following context about the C8 Health Project studies it reviewed: "*The C8 Health Project conducted in 2005–2006 on approximately 69,000 residents in Ohio and West Virginia evaluated general population exposures to PFOS and other perfluorochemicals. Public drinking water was contaminated in six water districts surrounding the plant (≥ 0.05 ng/mL of PFOA). Residents were eligible to participate in the study if they had consumed water from any of the 6 water districts for at least one year prior to the study. Blood samples were collected from the participants to determine PFOA and PFOS serum levels and clinical chemistry was performed. Extensive questionnaires were administered as well. The levels of PFOA were elevated, however, levels of PFOS in this population were similar to those reported in the general U.S. population (median 20 ng/mL).*"

Of the study by Fletcher et al. (2013), the US EPA provided the following summary: "*A subset of 290 individuals in the C8 Health Project was evaluated for evidence that PFOS exposure can influence the transcript expression of genes involved in cholesterol metabolism, mobilization, or transport (Fletcher et al. 2013). Ribonucleic acid (RNA) was extracted from whole blood samples taken from 144 males and*

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146 females aged 20–60 years; serum collected at the same time was used to measure PFOS concentration. The association between candidate gene expression levels and PFOS levels was assessed by multivariable linear regression with adjustments for confounders. A positive association was seen between PFOS and a transcript involved in cholesterol mobilization (Neutral Cholesterol Ester Hydrolase 1 [NCEH1]; p = 0.018), and a negative relationship with a transcript involved in cholesterol transport (Nuclear Receptor Subfamily 1, Group H, Member 3 [NR1H3]; p = 0.044). When sexes were analyzed separately, PFOS was positively associated with expression of genes involved in cholesterol mobilization and transport in females (NCEH1 and Peroxisome Proliferator Activated Receptor alpha [PPARα]; p = 0.003 and 0.039, respectively), but no effects were evident in males. Similar associations were also found for PFOA."

The study by Steenland et al. (2009) was reported above under PFOA. For PFOS, the US EPA reported: "*The mean serum PFOS level among participants was 22 ng/mL, with a range of 0.25–75902 ng/mL. Lipid outcomes (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides) were examined in relation to PFOS and PFOA serum levels. All lipid outcomes, except for HDL, showed significant increasing trends with increasing PFOS levels (similar for PFOA). The predicted increase in cholesterol from lowest to highest PFOS decile was 11–12 mg/deciliter (dL). Logistic regression analyses indicate statistically-significant incidence of hypercholesterolemia (≥ 240 mg/dL) with increasing PFOS serum levels. Cholesterol levels ≥ 240 mg/dL are characterized as high, and medical intercession is recommended. The odds ratios (ORs) across quartiles for cholesterol ≥ 240 mg/dL were 1.00, 1.14 (95% CI: 1.05–1.23), 1.28 (95% CI: 1.19– 1.39) and 1.51 (95% CI: 1.40–1.64).*"

The US EPA made the following comment about this study: "*The cross-sectional design of this study, as well as the lack of cumulative exposure measurements, are limitations in the study design.*"

For the study by Frisbee et al. (2010), the US EPA reported the following regarding PFOS: "*The mean level of PFOS was 23 ng/mL. PFOS was significantly associated with increased total cholesterol, HDL-cholesterol, and LDL- cholesterol in a linear regression analysis after adjustment for covariables. A statistically-significant increased risk of high total cholesterol [OR 1.6 (1.4–1.9)] and LDL-cholesterol [OR 1.6 (1.3–1.9)] was also observed between the first and fifth quintiles of PFOS serum levels. No trends were observed with triglycerides. Total cholesterol, LDL, and triglycerides were also positively associated with serum PFOA concentration.*"

The US EPA commented about this study: "*As with the other C8 project data, the authors acknowledge that the cross-sectional nature of this study limits causal inference.*"

For the study by Fitz-Simon et al. (2013), the US EPA reported for PFOS: "*Linear regression models were fit to the logarithm (base 10) of ratio change in each serum lipid measurement in relation to the logarithm of ratio change in PFOS. Mean serum PFOS concentration decreased by approximately one-half between baseline (23 ± 14 ng/mL) and follow-up (11 ± 7 ng/mL). No corresponding changes in serum lipids were found. However, those individuals with the greatest declines in serum PFOS had a tendency for a slight decrease in LDL-cholesterol. Similar results were found with PFOA.*"

General population studies

The US EPA reviewed six studies on PFOS and serum lipids in the general population (Nelson et al. 2010; Lin et al. 2009; Château-Degat et al. 2010; Eriksen et al. 2013; Starling et al. 2014b; Fisher et al. 2013). The ATSDR did not review the studies by Lin et al. (2009) and Starling et al. (2014b).

The US EPA (2016b) also noted the studies by Geiger et al. (2014a), Maisonet et al. (2015b) and Timmermann et al. (2014) in summary tables but did not provide any further comment in the text.

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Of the study by Lin et al. (2009), the US EPA reported: "*Lin et al. (2009) explored associations of serum lipid levels with NHANES PFOA data from 1999–2000 and 2003–2004. Serum HDL was inversely associated with serum PFOS concentration OR ((95% CI): 1.61 (1.15–2.26), p < 0.05). Triglycerides did not show an association with PFASs.*"

For the study by Starling et al. (2014b), the US EPA reported the following summary for this study: "*A cross-sectional study of 891 pregnant females evaluated the association between plasma PFOS levels and plasma lipids (Starling et al. 2014b). Six other perfluoroalkyl substances were also quantified and evaluated. The females were a cohort of the Norwegian Mother and Child Cohort Study, and the majority of blood samples were drawn during weeks 14–26 of gestation. Weighted multiple linear regression was used to estimate the association between PFOS level and each lipid level. The median plasma PFOS level was 13 ng/mL. No association was observed between PFOS and triglycerides. PFOS was positively associated with total cholesterol, HDL-cholesterol, and LDL-cholesterol, although confidence intervals were broad for all associations. Each ln-unit increase in PFOS was associated with an increase of 8.96 mg/dL (95% CI: 1.70–16.22) in total cholesterol and for each interquartile range (IQR)-unit increase in the ln-PFOS concentration, total cholesterol increased by 4.25 mg/dL (95% CI: 0.81–7.69). With HDL-cholesterol, each IQR-unit increase in ln-PFOS was associated with an increase of 2.08 mg/dL (95% CI: 1.12–3.04). For LDL-cholesterol, each IQR-unit shift in ln-PFOS was associated with a change of 3.07 mg/dL LDL (95% CI: −0.03–6.18). Five of the seven PFASs studied were positively associated with HDL cholesterol, and all seven had elevated HDL associated with the highest quartile.*"

The ATSDR reviewed Château-Degat et al. (2010); the US EPA (2016b) also reported: "*Effects of PFOS on plasma lipid levels in the Inuit population of Northern Quebec were examined in a cross-sectional epidemiology study (Château-Degat et al. 2010). The relationship between consumption of PFOS-contaminated fish and wild game with blood lipids was assessed in 723 Inuit adults (326 man and 397 females). This traditional diet is also rich in n-3polyunsaturated fatty acids (n-3 PUFAs) which are known to have hypolipidemic effects; therefore, the n-3 PUFAs were considered as a confounder in the analyses. Multivariate linear regression modeling was used to evaluate the relationship of PFOS levels and blood lipids, including total cholesterol (TC), HDL cholesterol, LDL cholesterol, and triacylglycerols. Plasma levels of HDL cholesterol were positively associated with PFOS levels, even after adjustment for circulating levels of n-3 PUFAs, but the other blood lipids were not associated with PFOS levels. The geometric mean level of PFOS in plasma for females and males was 19 ng/mL.*"

The ATSDR reviewed Eriksen et al. (2013); the US EPA (2016b) added: "*Eriksen et al. (2013) examined the association between plasma PFOS levels and total cholesterol levels in a middle-aged Danish population. This cross-sectional study included 663 males and 90 females aged 50–65 years who were enrolled in the Danish Diet, Cancer and Health cohort. Generalized linear models were used to analyze the association between PFOS and total cholesterol levels and adjusted regression analyses were performed. The mean plasma PFOS level was 36.1 ng/mL. A significant, positive association was found between PFOS (and PFOA) and total cholesterol such that in the fully adjusted model, a 4.6 mg/dL (95% CI: 0.8–8.5) higher concentration of total cholesterol was found per interquartile range of plasma PFOS. The quartiles of PFOS used in the analyses were not defined and no comparison was made for cholesterol levels between the highest and lowest PFOS quartile.*"

The ATSDR reviewed Fisher et al. (2013); the US EPA (2016b) also commented: "*Fisher et al. (2013) examined the association of plasma PFAS levels, including PFOS, with metabolic function and plasma lipid levels. This cross-sectional study included 2,700 participants, aged 18–74 years (approximately 50% male), in the Canadian Health Measures Survey. Multivariate linear and logistic regression models were used for analyses of associations between PFOS levels and cholesterol outcomes, metabolic syndrome, and glucose homeostasis. The geometric mean PFOS concentration was 8.4 ± 2 ng/mL. In*

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weighted analyses, no association was found between PFOS (or PFOA) and total cholesterol, HDL- and LDLcholesterol, and metabolic syndrome and glucose homeostasis parameters. Hypercholesterolemia (cholesterol greater than 240 mg/dL), was associated with PFOS exposure in unadjusted analyses of this cohort."

Nelson et al. (2010) was reviewed by the ATSDR; the US EPA (2016b) provided supplementary information: "*Homeostatic model assessment (HOMA) was used to assess insulin resistance (calculated from fasting insulin and fasting glucose measurements collected in NHANES). BMI and waist circumference were used to measure body size. Exclusion criteria included current use of cholesterol-lowering medications, participants over the age of 80, pregnant/breastfeeding females or insulin use. After exclusion criteria, approximately 860 participants were included in the analyses. The mean PFOS serum concentration for participants 20–80 years old was 25 ng/mL (range: 1.4–392 ng/mL). A positive association was identified between total serum cholesterol and serum PFOS concentrations. When analyzed by PFOS serum quartiles, adults in the highest PFOS quartile had total cholesterol levels of 13.4 mg/dL (95% CI: 3.8–23.0), higher than those in the lowest quartile. As expected, non-HDL cholesterol accounted for most of the total cholesterol. Consistent trends were not observed for HDL or LDL. Adjusting the cholesterol models for serum albumin produced similar results. Body weight and insulin resistance were not consistently associated with serum PFOS levels. Similar results were found for PFOA."*

The study by Frisbee et al. (2010) was reported by the ATSDR; the US EPA (2016b) added: "*PFOS was significantly associated with increased total cholesterol, HDL-cholesterol, and LDL- cholesterol in a linear regression analysis after adjustment for covariables. A statistically-significant increased risk of high total cholesterol [OR 1.6 (1.4–1.9)] and LDL-cholesterol [OR 1.6 (1.3–1.9)] was also observed between the first and fifth quintiles of PFOS serum levels. No trends were observed with triglycerides. Total cholesterol, LDL, and triglycerides were also positively associated with serum PFOA concentration. As with the other C8 project data, the authors acknowledge that the cross-sectional nature of this study limits causal inference."*

US EPA Summary of all studies reviewed on PFOS and cholesterol and serum lipids

At the end of the section on 'Serum lipids and cardiovascular diseases', the US EPA noted that: "*Multiple epidemiologic studies have evaluated serum lipid status in association with PFOS concentration (Table 3-1). These studies provide support for an association between PFOS and small increases in total cholesterol in the general population at mean serum levels of 22.4– 36.1 ng/mL (Frisbee et al. 2010; Nelson et al. 2010; Eriksen et al. 2013). Hypercholesterolemia, (clinically defined as cholesterol greater than 240 mg/dL), was associated with PFOS exposure in a Canadian cohort (Fisher et al. 2013) and in the C8 cohort (Steenland et al. 2009). Cross-sectional occupational studies demonstrated an association between PFOS and total cholesterol (Olsen et al. 2001a, 2001b, 2003b). Evidence for associations between other serum lipids and PFOS is mixed, including HDL cholesterol, LDL, very low density lipoprotein (VLDL), non-HDL cholesterol, and triglycerides. The studies on serum lipids in association with PFOS serum concentrations are largely cross-sectional in nature and were largely conducted in adults, but some studies exist on children and pregnant females. The location of these cohorts varied from the U.S. population including NHANES volunteers, to the Avon cohort in the United Kingdom (UK), to Scandinavian countries. Limitations to these studies include the frequently high correlation between PFOA and PFOS exposure; not all studies control for PFOA in study design. Studies also included populations with known elevated exposure to other environmental chemicals including PFOA in the C8 population or polybrominated diphenyl ethers (PBDEs) and other persistent organic compounds among the Inuit population. Overall, the epidemiologic evidence supports an association between PFOS and increased total cholesterol.*

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Some of the studies that examined serum LDL and HDL cholesterol also found significant increases these measures. Neither of these lipoprotein complexes is a stand-alone indicator for cardiovascular decrease risk. Rather, it is the relationship across the lipoprotein complexes within the same individuals that is important with HDLs considered as protective and LDLs a biomarker for potential atherosclerosis. Relatively few studies of triglycerides noted a significant increase with the serum PFOS levels."

New Jersey Drinking Water Quality Institute (DWQI, Public Review Draft 2016)

The DWQI reported in its health-based maximum contaminant level support document for PFOA on the evidence of PFOA on cholesterol in humans.

Studies reviewed

The DWQI reported: *"Associations of serum lipids and PFOA were evaluated in 24 studies, each of which included one or more of the following end points: total cholesterol, high density lipid cholesterol (HDL), non-HDL, ratio of total cholesterol to HDL, low-density lipid cholesterol (LDL), very low-density lipid cholesterol (VLDL), ratio of HDL to LDL, and triglycerides. There is also one additional study which only evaluated expression of genes related to cholesterol transport in humans (Fletcher et al. 2013)."*

For total cholesterol, the DWQI reviewed a total of twenty studies that: *"evaluated serum total cholesterol and two self-reported clinically defined high cholesterol"*. The two studies that examined self-reported clinically defined high cholesterol were Steenland et al. (2015) and Winquist and Steenland (2014); the DWQI did not further discuss these studies regarding PFAS exposure and cholesterol.

The DWQI also noted Fletcher et al. (2013), an additional study on the expression of genes related to cholesterol.

The DWQI reported the results by study type and noted that among the 20 serum total cholesterol studies:

- Fifteen studies were cross-sectional (Emmett et al. 2006; Eriksen et al. 2013; Fisher et al. 2013; Frisbee et al. 2010; Fu et al. 2014; Geiger et al. 2014a; Gilliland and Mandel 1996; Nelson et al. 2010; Olsen et al. 2000; Olsen and Zobel 2007; Sakr et al. 2007b; Starling et al. 2014b; Steenland et al. 2009; Wang et al. 2012; and Zeng et al. 2015), including:
  - Seven studies of the general population or individuals with low level exposures (Eriksen et al. 2013; Fisher et al. 2013; Fu et al. 2014; Geiger et al. 2014a; Nelson et al. 2010; Starling et al. 2014b; and Zeng et al. 2015);
  - Four studies of residents of highly exposed communities (Emmett et al. 2006; Frisbee et al. 2010; Steenland et al. 2009; Wang et al. 2012); and
  - Five studies of occupationally exposed individuals (Gilliland and Mandel 1996; Olsen et al. 2000; Olsen and Zobel 2007; Sakr et al. 2007b; and Wang et al. 2012).
- Two studies included cross-sectional and other analyses (Costa et al. 2009; and Olsen et al. 2003a).
- Five studies were occupational exposure studies evaluating serum total cholesterol and PFOA, including one case control study (Costa et al. 2009), and four cohort studies, including one study of residents of a highly-exposed community (Fitz-Simon et al. 2013) and three studies of occupationally exposed individuals (Olsen et al. 2003a; Olsen et al. 2012; and Sakr et al. 2007a).

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The studies reviewed regarding HDL, Non-HDL, Ratio of HDL to Total cholesterol and LDL are reported under the respective sections in 'Summaries of studies reviewed'.

There were 10 studies on PFOA and serum lipids that were not reported on by the ATSDR (Fletcher et al. 2013; Fu et al. 2014; Geiger et al. 2014a; Gilliland and Mandel 1996; Lin et al. 2011; Lin et al. 2013; Starling et al. 2014b; Steenland et al. 2015; Winquist and Steenland 2014; and Zeng et al. 2015).

The US EPA did not review six of the studies reviewed by the DWQI (Gilland et al. 1996; Wang et al. 2012; Fu et al. 2014; Lin et al. 2011; Lin et al. 2013; Zeng et al. 2015).

Considerations and conclusions

In the 'Executive Summary', the DWQI made the following statements: "*Of the end points that were evaluated comprehensively, the evidence for associations with PFOA was strongest for increases in serum levels of cholesterol. PFOA was associated with clinically defined hypercholesterolemia in a community exposed through drinking water. The epidemiological evidence supports multiple criteria for a causal relationship between PFOA and both serum cholesterol and ALT. Notably, the steepest dose response for associations with these end points was within the range of serum PFOA concentrations found in the general population and communities with drinking water exposures, with a much flatter curve at higher serum concentrations.*

For some other end points that were comprehensively reviewed, limited evidence of an association with PFOA was found. Other end points with limited evidence of an association include LDL. There was ...no evidence for association with HDL."

At the end of the section 'Serum lipids', the DWQI stated: "*In summary, the epidemiologic database for serum cholesterol and PFOA, which included twenty studies, provides evidence of consistency, strength and dose-response, including some evidence of temporality. Associations with clinically defined hypercholesterolemia were reported in some studies. These findings provide evidence supporting a causal relationship between PFOA and serum cholesterol. Overall, the epidemiologic evidence suggests no evidence of an association with HDL and PFOA. There were a limited number of epidemiologic studies evaluating an association with non-HDL or the ratio of total cholesterol to HDL and PFOA. The epidemiologic database for PFOA and LDL appears inconsistent. Although there is some evidence of an association with LDL, it remains limited due to the interpretation of other studies which found no evidence of an association. There is limited epidemiologic evidence evaluating associations of VLDL, the ratio of HDL to LDL, and triglycerides with PFOA.*"

Summaries of studies reviewed

Occupational exposure studies – PFOA and total cholesterol

The DWQI stated the following about the occupational studies they reviewed on total cholesterol: "*Of the five occupational cross-sectional studies, only one U. S. occupational study (n=840) with a median serum PFOA concentration of 189 ng/ml found a positive statistically significant association with serum cholesterol (Sakr et al. 2007b). The remaining four occupational cross-sectional studies which did not find evidence of an association include two U.S. male only worker studies, one with a mean serum PFOA concentration of 3,300 ng/ml and a sample size of 115 (Gilliland and Mandel 1996), and one with a mean serum PFOA concentration of 1,190 ng/ml with a sample size of 265 (Olsen et al. 2000). The third study took place in both the U.S. and Belgium with a median PFOA concentration of 2210 ng/ml and a sample size of 506 (Olsen and Zobel 2007) and the fourth cross-sectional study included 55 workers in China with a median PFOA concentration of 1,636 ng/ml (Wang et al. 2012). Five of the 20 studies had study designs other than cross-sectional. A longitudinal analysis of workers from*

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Belgium and U.S. with a range of PFOA means of 1,220 to 1,900 ng/ml (Olsen et al. 2003a), and another longitudinal worker cohort analysis from the U.S. with a range of PFOA exposure from 1,010 to 1,160 ng/ml (Sakr et al. 2007a), both found evidence of an association with PFOA and serum cholesterol. A third occupational cohort study utilizing matched-pair analysis of 98 to 179 workers (highly exposed of 881 ng/ml PFOA mean v. lower exposed of 28.9 ng/ml PFOA mean) did not find a statistically significant association (Olsen et al. 2012). None of these studies found evidence of a statistically significant inverse association with serum cholesterol and PFOA. An Italian male occupational case-control study with PFOA median concentration 4,400 among formerly exposed workers and a median of 5,700 ng/ml among currently exposed workers, with cross-sectional analysis, found evidence of a positive association (Costa et al. 2009). Among the cohort studies, a longitudinal study of individuals in highly-exposed mid-Ohio Valley communities, with geometric mean PFOA concentrations of 74.8 ng/ml at baseline and 30.8 ng/ml at follow-up, found evidence of a positive association (Fitz-Simon et al. 2013)."

The DWQI continued: "*Several of the studies mentioned above showed statistically significant trends for increased serum cholesterol with increasing serum PFOA. A decile analysis of PFOA with total cholesterol among a large study of residents of a highly exposed community showed an increasing effect of PFOA on cholesterol and additionally the odds of clinically defined hypercholesterolemia (≥240 mg/dL) increased 40-50% from the lowest to the highest quartile of PFOA (Steenland et al. 2009). A statistically significant trend of increasing serum cholesterol with increasing PFOA was also reported in at least five other studies (Frisbee et al. 2010, Fu et al. 2014, Geiger et al. 2014a; and Zeng et al. 2015)."*

High-exposure communities – PFOA and total cholesterol

The DWQI reported: "*Two large cross-sectional studies evaluated individuals residing in communities located in the mid-Ohio Valley with drinking water contaminated with PFOA. One study included 12,476 children aged 1 to 17.9 years with a mean serum PFOA concentration of 69.2 ng/ml (Frisbee et al. 2010) and the other included 46,294 individuals aged 18 years or older with a median serum 65 PFOA concentration of 27 ng/ml (Steenland et al. 2009). Both studies found a positive, statistically significant association of serum PFOA and cholesterol. A third smaller (n=371) cross-sectional study from the water district in the mid-Ohio Valley with the highest PFOA levels in its drinking water, with a much higher median serum PFOA concentration, 354 ng/ml, did not find a statistically significant association (Emmett et al. 2006). A fourth study from China, which in addition to a study of 132 residents located near a plant utilizing PFOA with a median PFOA concentration of 284 ng/ml also included a worker study, did not find an association with serum cholesterol in either group (Wang et al. 2012)."*

General population or low exposure communities – PFOA and total cholesterol

The DWQI reported: "*Six of seven cross-sectional studies of the general population or populations with low-level exposures found evidence of statistically significant positive associations with serum cholesterol and PFOA. These studies of general population level exposures include a study nested in a larger cohort in Denmark of adults, aged 50 to 65 years, with mean serum PFOA concentration of 7.1 ng/ml (Eriksen et al. 2012); a general population study in Canada with a PFOA geometric mean of 2.5 ng/ml (Fisher et al. 2013); a small study of individuals randomly selected from attendees at a health check-up clinic with a median serum PFOA concentration of 1.4 ng/ml (Fu et al. 2014); a study of children in the U.S. general population with a serum PFOA mean concentration of 4.3 ng/ml (Geiger et al. 2014a); a study of the general U.S. population aged 12 years older with a median PFOA concentration of 3.8 ng/ml (Nelson et al. 2010); and a study of subjects recruited from the control group of another study in Taiwan with median PFOA exposures of 1.1 ng/ml in boys and 0.9 ng/ml in girls (Zeng et al. 2015). A study of pregnant women recruited from a larger cohort in Norway, with a median*

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serum PFOA concentration of 2.3 ng/ml, did not find a statistically significant positive association with PFOA and serum cholesterol; however, results showed a positive and increasing association of cholesterol with increasing quartiles of PFOA (Starling et al. 2014b)."

DWQI Summary on PFOA and total cholesterol studies reviewed

The DWQI made the following comments and conclusion about total cholesterol: "*In summary, general population level exposure studies (seven), found consistent evidence of a positive association between PFOA and serum cholesterol. Additionally, three very large studies (two cross-sectional and a cohort study) of highly exposed community populations found evidence of a positive association between PFOA and serum cholesterol. Two longitudinal occupational studies also found a positive association, along with one case-control occupational study. In contrast, results from two much smaller cross-sectional studies of highly exposed community populations (with higher median population exposures than the three larger studies) and a matched-pairs occupational study did not find an association. Although findings from the occupational cross-sectional studies in general (four out of five) found no evidence of an association, they may be biased toward the null by a healthy worker effect. This is suggested by a similar pattern of inconsistency among these study's findings as compared to the findings from the corresponding database were also noted for other serum lipid end points (HDL and LDL – discussed below). In general, studies of the general population, as well as large, mid-exposure range community studies and occupational studies with longitudinal designs, found consistent evidence of an association, while a few smaller, higher exposure range community and occupational studies found no evidence. None of the 20 studies evaluated found evidence of an inverse association.*"

"*A review by Steenland et al. (2010a) summarized and evaluated the epidemiologic literature on PFOA and cholesterol available at that time. The authors noted that the lower the range of PFOA that was studied, the greater the change in cholesterol per unit change in PFOA. They suggest that, as discussed in Occupational Studies (above), an exposure-response relationship that is steep at low PFOA concentrations and then flattens out (i.e. approaches a plateau) at higher serum PFOA concentrations is a possible explanation for the observed differences in effect magnitudes. Therefore, studies of populations with high serum PFOA concentrations may not detect an association of PFOA with serum cholesterol if there is a steep dose-response curve for the association in the lower exposure ranges. For dose-response curves of this type, associations may not be evident in populations with higher exposures since even the least exposed individuals in the comparison group may have exposures that fall on the much flatter (approaching a plateau) portion of the exposure/response curve.*"

HDL

The DWQI reviewed 19 studies on PFOA exposure and HDL. They noted that: "*An increase in HDL is considered to be beneficial, as compared to increases in total cholesterol, LDL, and non-HDL, which are considered to be undesirable.*"

The DWQI reported on these studies that: "*None of these studies found an association with increased HDL, while four of the 19 studies found evidence of statistically significant decreased association with HDL (Gilliland and Mandel 1996; Olsen et al. 2000; Olsen and Zobel 2007; and Wang et al. 2012). Interestingly, these four studies are all occupational cross-sectional studies which also did not find evidence of an association with PFOA and increased serum cholesterol (described above), whereas the only other additional occupational cross-sectional study found no evidence of an association with HDL but did find a statistically significant positive association between PFOA and cholesterol (Sakr et al. 2007b). These differences in findings suggest that these occupational cross-sectional studies may be biased from a healthy worker effect. There was no evidence of statistically significant associations with HDL in any of the other 15 studies (Costa et al. 2009; Fisher et al. 2013; Fitz-Simon et al. 2013; Frisbee et*

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al. 2010; Fu et al. 2014; Geiger et al. 2014a; Lin et al. 2011; Nelson et al. 2010; Olsen et al. 2003a; Olsen et al. 2012; Sakr et al. 2007a; Sakr et al. 2007b; Starling et al. 2014b; Steenland et al. 2009; Wang et al. 2012 [resident study]; and Zeng et al. 2015)."

Non-HDL cholesterol (i.e. total cholesterol – HDL cholesterol)

The DWQI reported "*Non-HDL was evaluated in four studies: two general population cross-sectional studies (Fisher et al. 2013; and Nelson et al. 2010), a U.S. occupational longitudinal study (Olsen et al. 2012), and a large cross-sectional study of residents in highly exposed communities (Steenland et al. 2009). Three of the studies found statistically significant positive associations with non-HDL and PFOA (Fisher et al. 2013; Nelson et al. 2010; and Olsen et al. 2012), while the occupational longitudinal study had a negative association with non-HDL which was not statistically significant (Olsen et al. 2012)."*

Ratio of HDL to total cholesterol

The DWQI reported the following of the literature they reviewed: "*The ratio of total cholesterol to HDL was evaluated in three studies with inconsistent findings. A general population study in Canada did not find evidence of a statistically significant association (Fisher et al. 2013), U.S. occupational longitudinal study found a statistically significant negative association (Olsen et al. 2012), and a large study of residents from a highly exposed community found a statistically significant positive association (Steenland et al. 2009)."*

LDL cholesterol

The DWQI reported the following on the studies they reviewed that investigated PFOA and serum LDL: "*Associations of LDL and PFOA were evaluated in 16 studies. Fourteen of the studies are cross-sectional, which includes seven low level exposure populations (Fisher et al. 2013; Fu et al. 2014; Geiger et al. 2014a; Lin et al. 2013; Nelson et al. 2010; Starling et al. 2014b; and Zeng et al. 2015), three studies of residents from a highly exposed community (Frisbee et al. 2010; Steenland et al. 2009; Wang et al. 2012), and five studies of occupationally exposed individuals (Gilliland and Mandel 1996; Olsen et al. 2000; Olsen and Zobel 2007; Sakr et al.; 2007b ; and Wang et al. 2012). The other two studies of LDL and PFOA include an occupational longitudinal study (Sakr et al. 2007a) and a cohort study of residents from the highly exposed community, mid-Ohio Valley (Fitz-Simon et al. 2013). Among the cross-sectional studies of populations with low level exposure, three found evidence of statistically significant positive associations with LDL (Fu et al. 2014; Geiger et al. 2014a; and Zeng et al. 2015) and four found no statistically significant evidence of an association (Fisher et al. 2013; Lin et al. 2013; Nelson et al. 2013; and Starling et al. 2014b). Of the three cross-sectional studies of residents from a highly exposed community; the two large studies in the mid-Ohio Valley, one which included children and the other of adults, found evidence of statistically significant positive association (Frisbee et al. 2010, and Steenland et al. 2009); while the third smaller study of 132 residents in China found no evidence of an association (Wang et al. 2012).*

Four of the five occupational cross-sectional studies found no association (Gilliland and Mandel 1996; Olsen et al. 2000; Olsen and Zobel, 2007; and Wang et al. 2012) while only one of the studies found evidence of a statistically significant association with both LDL and VLDL (Sakr et al. with LDL (Sakr et al. 2007a) while a cohort study of residents from a highly exposed 2007b). Additionally, an occupational longitudinal study found a positive, non-statistically significant association community found a statistically significant positive association (Fitz-Simon et al. 2013). Finally, the ratio of HDL to LDL was evaluated in a cross-sectional study which assessed both occupational and highly exposed residential populations and found a negative association with the worker population and no evidence of a statistically significant association with the residential population (Wang et al. 2012)."

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DWQI Summary on PFOA and LDL

The DWQI made the following comments about the studies they reviewed: "*In summary, positive associations with PFOA and LDL were inconsistent among low level exposure populations, and largely unassociated in occupational studies, but there is consistent evidence of an association with PFOA and LDL among larger studies of the highly exposed mid Ohio Valley communities: two cross-sectional studies one among children and another among adults, and a longitudinal study.*"

Triglycerides

The DWQI reported of the studies they reviewed: "*Sixteen studies evaluated triglycerides with inconsistent findings. Four of the studies found evidence of positive statistically significant association (Frisbee et al. 2010; Olsen et al. 2003a; Olsen and Zobel, 2007; and Zeng et al. 2015), one found evidence of a negative statistically significant association (Lin et al. 2013), and 11 studies found no evidence of a statistically significant association (Costa et al. 2009; Fisher et al. 2013; Fitz-Simon et al. 2013; Fu et al. 2014; Geiger et al. 2014a; Lin et al. 2011; Olsen et al. 2000; Sakr et al. 2007a; Sakr et al. 2007b; Starling et al. 2014b; and Wang et al. 2012).*"

Issues with studies

The DWQI raised the following concern about the study by Fu et al. (2014), stating: "*Selection bias may be an issue in Fu et al. (2014) since the study included only individuals attending a health clinic check-up such that individuals concerned with existing health issues may be more likely to be included. Selection bias may also be an issue in Lin et al. (2013), which included individuals with an abnormal urinalysis from a population-based screening program in which the final study population was made up of 246 (37%) individuals with elevated blood pressure. Information bias is unlikely to have an impact in the general population studies which relied on serum concentrations and clinical biomarkers. In contrast, some occupational studies relied on medical record abstraction of clinical parameters. Other limitations of occupational studies include small sample size that may limit power to detect associations, possibility of healthy worker effect, inclusion of few or no women, and the possibility that exposure in the least exposed groups may be well above the population exposure range in occupationally exposed individuals.*"

Biological plausibility

The DWQI also considered a paper by Fletcher et al. (2013) and commented: "*The biological plausibility of the association of PFOA and serum cholesterol was investigated in a study of associations of serum PFOA and changes in expression of genes involved in cholesterol metabolism. In this cross-sectional study, expression of 13 genes involved in cholesterol metabolism (cholesterol biogenesis, peroxisome proliferation, cholesterol transport, downstream transcriptional activation of PPAR-alpha, and mobilization of cholesterol) was evaluated in whole blood from 290 subjects from a highly exposed community (geometric mean serum PFOA, 32.2 ng/ml). Statistically significant associations between genes involved in cholesterol transport and mobilization and PFOA were found, and the affected genes differed in men and women. The authors state that these change in gene expression "appear consistent with PFOA promoting a hypercholesterolemic environment" (Fletcher et al. 2013).*"

Dutch National Institute for Public Health and the Environment (RIVM, 2017).

The RIVM reviewed international reports and epidemiological studies that had reported on PFAS exposure and blood lipid concentrations.

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Studies reviewed

The RIVM considered the findings of five international reviews: (C8Science Panel (2012); ATSDR (2015); ECHA-RAC (2015); DWQI (2016); US EPA (2016a).

The RIVM also reviewed 23 studies on blood lipids and exposure to PFOA. The studies included:

- nine occupational exposure studies with workers from PFOA production plants (Costa et al. 2009; Gilliland and Mandel 1996; Olsen et al. 2000; Olsen et al. 2003a; Olsen et al. 2012; Olsen and Zobel 2007; Sakr et al. 2007a; Sakr et al. 2007b; Steenland et al. 2015);
- six studies of high-exposure communities (Emmett et al. 2006, Fitz-Simon et al. 2013, Frisbee et al. 2010, Steenland et al. 2009), including two studies in which workers were also examined (Wang et al. 2012; Winquist and Steenland, 2014a); and
- seven studies in the general population (Eriksen et al. 2013; Fisher et al. 2013; Fu et al. 2014; Geiger et al. 2014a; Lin et al. 2011; Lin et al. 2013a; Nelson et al. 2010; Starling et al. 2014b; and Zeng et al. 2015).

All of the studies were mentioned by previous reports (ATSDR, DWQI, US EPA 2106a, and US EPA 2016b). The RIVM reviewed all of the studies in succinct paragraphs and these are added below to complement the information provided in the above sections of each report.

Considerations and conclusions

The RIVM concluded in the 'Synopsis': "*The strength of evidence for the existence of a possible association differs between the observed effects. The clearest evidence has been found for a relationship between exposure to PFOA and higher total cholesterol concentrations in blood... For all other examined associations, the evidence is less clear. There are indications of an association with higher blood concentrations of LDL-cholesterol.*"

Summaries of studies reviewed

Occupational exposure studies

The RIVM cited nine studies that focused on occupational exposure to PFAS and cholesterol with workers from PFOA production plants (Costa et al. 2009; Gilliland and Mandel 1996; Olsen et al. 2000; Olsen et al. 2003a; Olsen et al. 2012; Olsen and Zobel 2007; Sakr et al. 2007a; Sakr et al. 2007b; Steenland et al. 2015). The RIVM noted the study by Wang et al. (2012) included both an occupational study population as well as a high exposure community.

All 10 of the studies have been reviewed by one or more of the previous international reports (ATSDR, DWQI, or US EPA 2016a and 2016b). The ATSDR reviewed Costa et al. (2009); Olsen et al. (2000); Olsen et al. (2003a); Olsen et al. (2012); Olsen and Zobel (2007); Sakr et al. (2007a); Sakr et al. (2007b); and Wang et al. (2012). The DWQI reported on all 10 of the studies but did not expand upon the results of Steenland et al. (2015). The US EPA (2016a) reviewed Costa et al. (2009); Olsen et al. (2000); Olsen and Zobel (2007); Sakr et al. (2007a); Sakr et al. (2007b); and Steenland et al. (2015). The US EPA (2016b) reviewed Olsen et al. (2003a).

The RIVM reviewed the following results from the nine studies: "*Five studies (Costa et al. 2009; Olsen et al. 2003a; Olsen and Zobel, 2007; Sakr et al. 2007a; Sakr et al. 2007b) found positive and statistically significant associations between blood PFOA and total cholesterol concentrations. Positive non-significant associations were reported in three studies (Gilliland and Mandel, 1996; Olsen et al. 2000; Wang J. et al. 2012). One study reported a negative non-significant association (Olsen et al. 2012) and one study (Steenland et al. 2015) did not include total cholesterol concentrations (but self-reported elevated cholesterol with medication).*"

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Of the six studies that examined LDL-cholesterol, the RIVM found: "*Five studies (Gilliland and Mandel, 1996; Olsen and Zobel, 2007; Sakr et al. 2007a; Sakr et al. 2007b; Wang J. et al. 2012) showed positive associations with PFOA concentrations, one of which (Sakr et al. 2007b) was statistically significant. One study reported cholesterol concentrations per tertile of PFOA, with no apparent positive or negative association (Olsen et al. 2000).*"

Of the nine studies that examined HDL-cholesterol, the RIVM stated: "*Five studies (Gilliland and Mandel, 1996; Olsen et al. 2000; Olsen and Zobel, 2007; Sakr et al. 2007b; Wang J. et al. 2012) found negative associations with PFOA, two of which (Olsen and Zobel, 2007; Wang J. et al. 2012) were statistically significant. One study (Sakr et al. 2007a) found a nonsignificant positive association. Two studies (Costa et al. 2009; Olsen et al. 2012) found non-significant associations that were either positive or negative, depending on the statistical model, and in one study (Olsen et al. 2003a) the association was not quantified.*"

High-exposure communities

The RIVM reviewed six studies of high-exposure communities. Four were from the C8 Health Project (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009; and Winquist and Steenland 2014a). One study was conducted in the C8 Health Project area (Emmett et al. 2006). One study took place in China (Wang et al. 2012).

All six of the studies of high-exposure communities have been cited by earlier reports (ATSDR, DWQI, US EPA 2016a and 2016b). The ATSDR cited Emmett et al. (2006); Fitz-Simon et al. (2013); Frisbee et al. (2010); Steenland et al. (2009); and Wang et al. (2012). The DWQI reviewed all six studies, but did not explore any details regarding the study by Winquist and Steenland (2014). The US EPA (2106a) reported on Emmett et al. (2006); Fitz-Simon et al. (2013); Frisbee et al. (2010); Steenland et al. (2009); and Winquist and Steenland (2014). The US EPA (2016b) reported on Fitz-Simon et al. (2013); Frisbee et al. (2010); and Steenland et al. (2009).

The RIVM reported on the six studies of high-exposure communities, stating: "*Three studies from the C8 Health Project found positive and statistically significant associations between serum PFOA concentrations and total and LDL-cholesterol concentrations. These studies included cross-sectional studies in 46,294 adults (Steenland et al. 2009) and 12,476 children (Frisbee et al. 2010) and one longitudinal study in 560 adults (Fitz-Simon et al. 2013). Two studies also found an association between PFOA and elevated total cholesterol concentrations (Frisbee et al. 2010; Steenland et al. 2009), and one study between PFOA and elevated LDL-cholesterol levels (Frisbee et al. 2010) (not studied in Steenland et al. (2009)). A longitudinal study from the C8 Health Project included both workers and members of the high-exposure community and used modelled serum PFOA concentrations (Winquist and Steenland, 2014a). They also found a higher incidence of medically validated diagnosis of hypercholesterolemia with medication in those with higher cumulative, modelled serum PFOA concentrations, i.e. hazard ratios were significantly higher in quintiles 2 (>142 ng/mL per year) through 5 (≥3,579 ng/mL per year) (hazard ratios in quintiles 2 through 5: 1.24, 1.17, 1.19, 1.19, see table 11) (Winquist and Steenland, 2014a). The study conducted by Emmett et al. (2006) in the C8 Project area studied total cholesterol levels and found a positive non-significant association with PFOA, but this study was based on a much smaller data set (n=371) and did not adjust for potential confounders in the statistical analysis. The study from China (Wang J. et al. 2012) included 132 residents and did not find associations with total, LDL or HDL cholesterol or triglyceride concentrations.*"

"*None of the community studies found an association with HDL-cholesterol. An association with triglycerides was found in the two large cross-sectional studies from the C8 Health Project (Frisbee et al.*

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2010; Steenland et al. 2009), but not in the longitudinal study (Fitz-Simon et al. 2013) or the study from China (Wang J. et al. 2012)."

General population studies

The RIVM cited seven studies that focused on total blood cholesterol concentration in the general population (Eriksen et al. 2013; Fisher et al. 2013; Fu et al. 2014; Geiger et al. 2014a; Nelson et al. 2010; Starling et al. 2014b; and Zeng et al. 2015).

All seven of the studies have been considered by earlier reports (ATSDR, DWQI, US EPA (2016a and 2016b)). The ATSDR reviewed Eriksen et al. (2013); Fisher et al. (2013); and Nelson et al. (2010). The DWQI reviewed all seven of the general population studies. The US EPA (2016a and 2016b) reviewed Eriksen et al. (2013); Fisher et al. (2013); Geiger et al. (2014a); Nelson et al. (2010); and Starling et al. (2014b).

The RIVM reported on the results of the seven general population studies: *"All seven studies that measured total blood cholesterol concentration in the general population found a positive association between serum or plasma PFOA and total cholesterol... In five (Eriksen et al. 2013; Fu et al. 2014; Geiger et al. 2014a; Nelson et al. 2010; Zeng et al. 2015) of the seven studies, the association was statistically significant. LDL was also measured in seven studies. Three studies (Fu et al. 2014; Geiger et al. 2014a; Zeng et al. 2015) observed a positive statistically significant association, two studies (Fisher et al. 2013; Starling et al. 2014b) a non-significant positive association and two studies (Lin et al. 2013a; Nelson et al. 2010) non-significant negative associations. Nelson et al. (2010), however, although they did not find an association with LDL-cholesterol, did find a positive statistically significant association between PFOA and non-HDL (i.e. LDL + VLDL) cholesterol. HDL-cholesterol was measured in seven studies. In one study positive and negative, statistically significant associations were found in adolescent girls and elderly men, respectively. The other six studies found statistically non-significant positive and negative associations."*

Food Standards Australia New Zealand (FSANZ, 2017)

In 2017, FSANZ made a number of statements about the evidence on PFAS and cholesterol in the 'Hazard assessment report for PFOA, PFOS and PFHxS'.

Studies reviewed

FSANZ used the US EPA (2016a and 2016b) and EFSA (2008) (not used for this report) reports to decide which studies were included in their analysis, along with additional studies identified by FSANZ. This information can be found in 'Appendix Two'. The following studies were included by FSANZ:

- two studies of pregnant women (Skuladottir et al. 2015; Starling et al. 2014b);
- five studies of children (Frisbee et al. 2010; Geiger et al. 2014a; Lin et al. 2013a; Maisonet et al. 2015b; Zeng et al. 2015);
- six studies of adults (Chateau-Degat et al. 2010; Olsen and Zobel 2007 (PFOA); Olsen et al. 2003a (PFOS); Steenland et al. 2009; Nelson et al. 2010; Eriksen et al. 2013); and
- five studies of adults (included in the qualitative analysis) (Christensen et al. 2016[17]; Costa et al. 2009; Fisher et al. 2013; Fu et al. 2014; Wang et al. 2012).

[17] FSANZ cites this reference as Christiansen et al. 2016 in the body of their report.

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Considerations and conclusions

In their 2017 'Hazard assessment report for PFOS, PFOA and PFHxS', FSANZ reported in the 'Executive Summary': "*The US EPA (2016) concluded that associations that appear to be reasonably consistent and repeatable are those with increased serum cholesterol... FSANZ has reviewed the available human epidemiological information and concluded that while there is evidence of these associations, it is not possible to determine whether PFOS or PFOA causes the changes, or whether other factors are involved. As these are observational studies, FSANZ considers that the meaning and clinical significance of the associations for PFOS and PFOA for... increased cholesterol in humans are uncertain and should be treated with caution.*"

PFOS

In the 'Serum lipids' sections for PFOS, FSANZ stated: "*FSANZ reviewed the available epidemiological data relating to PFOS and PFOA exposure and serum cholesterol (Appendix 2). A number of studies that were not referred to in the EFSA and US EPA reviews were identified and included in the analysis. The FSANZ review noted that overall the cross-sectional studies show a fairly consistent finding of a positive association between total and LDL cholesterol and low serum concentrations of PFOS, with the association plateauing at higher PFOS levels. At around 40 ng/mL serum PFOS concentration, total cholesterol was around 0.3 mmol/L higher than the lowest PFOS exposure groups. The lack of association in some occupational groups might be explained because there were not enough low concentrations in the study group to detect the effect at low PFOS concentrations. The FSANZ review observed that a number of studies note a correlation between concentrations of PFOS and PFOA but do not adjust the results for each other. Similarly, populations with high exposure to PFAS may also be exposed to other contaminants but these have not been considered in most studies. Another limitation is that most studies do not adjust for diet. In addition, kidney function does not seem to have been examined together with cholesterol concentrations. This may be important as PFAS concentrations increase as glomerular filtration rate (GFR) decreases, and it is also known that there is an inverse correlation between serum LDL cholesterol and GFR (Morita et al. 2010).*"

In the 'Discussion and conclusions' PFOS section, FSANZ stated that: "*A number of studies that were not referred to in the EFSA [European Food Safety Authority]*[18] *and US EPA reviews were identified and included in the analysis. The FSANZ review noted that overall the cross-sectional studies show a fairly consistent finding of a positive association between total and LDL cholesterol at low serum concentrations of PFOS, with the association plateauing at higher PFOS levels. However, a number of limitations were observed including that some studies note a correlation between concentrations of PFOS and PFOA but do not adjust the results for each other. Similarly, populations with high exposure to PFAS may also be exposed to other contaminants but these have not been considered in the studies, and most studies do not adjust for diet or consider the impact of GFR [glomerular filtration rate].*"

PFOA

In the 'PFOA – Serum lipids' section, FSANZ stated that it: "*considered that studies in both adults and children suggest a positive association between total and LDL cholesterol and PFOA concentration at very low concentrations of PFOA but not at higher concentrations (Appendix 2). At around 25 ng/mL, total cholesterol is about 0.2-0.3 mmol/ higher than the lowest groups in the studies and then the*

[18] EFSA is a European agency funded by the European Union that operates independently of the European legislative and executive institutions (Commission, Council, Parliament) and EU Member States. EFSA's scientific advice helps to protect consumers, animals and the environment from food-related risks. EFSA provide independent scientific advice to the decision makers who *regulate* food safety in Europe. Source: https://www.efsa.europa.eu/_The EFSA report referred to was published in 2008 and is outside the Panel's inclusion dates.

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association plateaus. The quantitative results from pregnant women are more inconsistent, but this may be related to haemostatic changes during pregnancy. There appears to be little or no association with HDL cholesterol, and not all studies have adverse findings. The few longitudinal data that are available do not contradict the findings in the cross-sectional studies. However, the results in humans do contradict the findings in animals because increased PFAS concentrations in animals decrease total cholesterol."

In the 'Discussion and conclusions' section for PFOA, FSANZ reported: "*PFOA is highly persistent in human beings, with an elimination half-life measured in years. This persistence gives rise to some concern, although PFOA appears to have few adverse effects. Toxic mechanism(s) in humans are unclear, but epidemiological evidence suggests that PFOA may be positively associated with serum levels of cholesterol, LDL, and serum triglycerides.*

The positive association of PFOA with elevated levels of cholesterol and triglycerides in the circulation in human beings are inconsistent with findings in experimental animals, and are also the reverse of those that would generally be expected of a PPARα agonist. Fibrates including gemfibrozil, bezafibrate and fenofibrate are PPARα agonists that are prescribed to lower cholesterol and decrease plasma triglycerides, and experimental evidence links these therapeutic effects with their PPARα agonism (Yu et al. 2015). It is noteworthy that there is an inverse correlation between serum LDL cholesterol and GFR, and that it has been suggested that LDL cholesterol reduces GFR by impairing the function of renal arterioles and capillaries (Morita et al. 2010)."

Summary of studies reviewed

In the 'Executive Summary' of Appendix 2: 'Observational studies of PFAS and Cholesterol Concentrations', FSANZ stated that: "*In summary, the cross-sectional studies overall present a fairly consistent picture. Studies in both adults and children suggest a positive association between between total cholesterol (total-C) and low density lipoprotein cholesterol (LDL-C) and PFOA concentration at very low concentrations of PFOA but not at higher concentrations. At around 25 ng/mL blood concentration, total-C is about 0.2 – 0.3 mmol/ higher than total-C in the lowest PFOA blood concentration groups in the studies, above this the association plateaus. The quantitative results from pregnant women are more inconsistent, but this may be related to changes in blood volume during pregnancy. There appears to be little or no association with high density lipoprotein cholesterol (HDL-C), and not all studies have adverse findings. Similar results were seen for PFOS with a plateau of 0.3 mmol/L total-C which is reached at around 40 ng/ mL blood concentration. The lack of association reported in some occupational groups might be due to the lack of sufficient subjects with low concentrations of PDAS to detect the effect. The few longitudinal data that are available do not contradict the findings in the cross-sectional studies. However, the results in humans do contradict the findings in animals because increased PFAS concentrations in animals decrease total-C."*

FSANZ also stated that: "*It is not possible to determine whether the inconsistent information presented across the studies occurs because the samples were not tested for certain cholesterol fractions or whether the authors have failed to report non-significant results. Therefore the question of whether there is publication bias affecting this body of literature must be raised. Studies have been included regardless of whether or not they have reported their results in a common format because failure to do this may have introduced a bias into the body of evidence. As far as it is possible to tell, the results of studies which could not be graphed do not contradict the results of studies which could be graphed in a qualitative sense although it is not possible to make a quantitative comparison."*

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Non-pregnant adults – PFOA

FSANZ reviewed the 10 studies that investigated exposure to PFOA and cholesterol concentrations in non-pregnant adults, and commented that: "*Overall, studies examining the lower ranges of exposure are consistent in reporting an increase in total-C with increasing blood PFOA concentrations which then plateaus at higher PFOA concentrations. The largest study reports that the association attenuates, which might reflect a plateau or an ongoing but much slower increase, from about 25 ng/mL. Other studies either do not cover this range or do not have enough sample size to examine where the change in slope might occur. Two of the studies reporting results for total-C did not report whether they had analysed their samples for LDL-C (Costa et al. 2009; Christensen et al. 2016) although one of these did analyse for HDL-C (Costa et al. 2009). Most report a similar pattern but a smaller effect on LDL-C than total-C (Figure A2.2). For example, Steenland et al. (2009) report an increase of 0.1 mmol/L at a PFOA concentration of about 25 ng/dL. An exception is the worker group of Wang et al. (2012) in whom the effect on LDL-C was larger than the effect on total-C.*

The results across studies are much more variable for HDL-C (Figure A2.3). At low PFOA concentrations, both the graphical and tabulated results show little or no effect on HDL-C. At concentrations greater than 1000 ng/dL, Wang et al. (2012) reports an inverse effect whereas Costa et al. (2009) reports a positive effect. FSANZ concludes that there is no association between PFOA and HDL-C concentration."

Non-pregnant adults – PFOS

FSANZ reported that: "*Four studies with low concentrations (Figure A2.6B) reported that total-C concentration was positively associated with PFOS concentration with a possible maximal increase of 0.3 mmol/L at a concentration of about 40 ng/ mL. Olsen et al. (2003a) examined a population with much higher blood concentrations and reported that, in women, total-C declined and then returned to the starting point as PFOS concentration increased above 70 ng/ mL. In men, however, there was no association in the range of 270 ng/mL to 1190 ng/mL followed by an increase. These variations may reflect random variation around a null effect or plateau at higher concentrations. Three of the four studies measuring total-C also reported LDL-C data. Two of these found that LDL-C increased in parallel and to much the same extent as total-C whereas Chateau-Degat et al. (2010) found a much lower increase in LDL-C (Figure A2.7B). Chateau-Degat et al. (2010) examined an Inuit population who had high consumption of fish; however, long-chain omega-3 fatty acids are generally thought to affect triglyceride concentration rather than cholesterol concentration (Nestel et al. 2015) and so this would not seem to explain the relatively small difference in LDL-C. Olsen et al. (2003) did not report measuring LDL-C. Studies examining HDL-C reported results varying around a null effect (Figure A2.8). The studies which could not be graphed are generally consistent with the graphed results in showing that total-C and LDL-C are positively associated with PFOS in the low concentration range and that HDL-C has little or no association.*"

Pregnant women – PFOA

FSANZ reported on the two studies they reviewed: "*One study described its results in quartiles of PFAS concentration (Starling et al. 2014b) and the other in quintiles (Skuladottir et al. 2015). Hence 25% of the population lie below the bottom point and above the top point plotted for the first study and 20% for the second. Consequently, more than half of the population studied by Starling et al. (2014b) had PFOA concentrations less than 80% of the population studied by Skuladottir et al. (2015). Both studies found that total-C increased as PFOA increased, and this was larger than was seen in the non-pregnant group and occurred across a smaller increment in PFOA (Figure A2.1). Only one of these studies reported on cholesterol subfractions. The one study reporting the association for subfractions*

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shows that LDL-C and HDL-C both increased by a small amount. The study reporting larger increases in total-C did not analyse their samples for LDL-C and HDL-C."

Pregnant women – PFOS

For the two studies FSANZ noted: "*Two studies examined the association between total-C and PFOS concentration in the range of 10-30 ng/mL PFOS in pregnant women and found a positive association. The range of PFOS concentrations covered by the studies was more similar to the general population (See Figure A2.6B) than was the case for PFOA. The greatest increase was 0.44 mmol/L for the quintile with PFOS concentration of 27.7 ng/mL or greater. This was paralleled by LDL-C and there was as an increase in HDL-C (good cholesterol) of the same magnitude (Figure A2.9)."*

Children and young people – PFOA

For the five studies on children included in FSANZ review, FSANZ reported: "*All except Maisonet et al. (2015b) are cross-sectional studies. Unlike the studies in adults and pregnant women, two studies only described their results as regression coefficients and thus have been shown on the graph as lines without points. It is difficult to know how to represent this fairly compared to the studies which report results in PFOA quantiles. Frisbee et al. (2010) analysed 12,000 children which was a much larger sample than the other studies. It should be noted that this is the only age group in which papers did not report results as quantiles, and so it is difficult to know how to zero the presentation of the regression lines of Frisbee et al. (2010) and Maisonet et al. (2015b) relative to the other studies. Frisbee et al. (2010) describe the relationship between PFOA and cholesterol in 12,000 children from the C8 study and show the same effect that was seen in the adults of the same study in Figure A2.1 (Steenland et al. 2009). Geiger et al. [2014a] analysed data from the NHANES as did Nelson et al. (Figure A2.1), albeit from a slightly different range of years. They found essentially the same pattern in children that was shown for adults (Figure A2.1). The smaller studies of Zeng et al. (2015) and Lin et al. (2013a) from Taiwan examined low concentrations of PFOA in this age group and had opposite results for LDL-C. Maisonet et al. (2015b) examined 88 girls and used different methods from the other studies. Firstly, their study is longitudinal and compared PFOA concentrations from prenatal maternal blood to the girls' cholesterol concentrations at age 7 and age 15 years. The plot shows the unadjusted data at age 15, but this was similar to the adjusted data at the same age and at 7 years old."*

FSANZ then commented on the findings by Maisonet et al. (2015b): "*The pattern shown in the study of Maisonet et al. (2015b) was very different from that of the other studies. The apparent size of effect may be related to the zeroing problem mentioned above when graphing the results from the various studies. It is difficult to explain how maternal PFOA concentrations during pregnancy would have an effect at only one concentration in children aged 15 years. As noted, this shows the unadjusted results which were not very different from the adjusted results. However, the only adjustment factors considered were maternal age at delivery, maternal education and previous live births. 25% of maternal blood samples were drawn after 28 weeks and so there could be differences in PFAS concentration due to haemodilution which occurs in the later weeks of pregnancy. No other factors related to cholesterol seem to have been considered, such as weight or diet, and there could be correlations between these and maternal PFAS owing to similarity in the familial environment. However, with only 88 subjects, it is not appropriate to include a large number of covariates in a regression model. The authors comment that they are conducting a similar analysis on blood from boys who are members of the same cohort study but these data were not available."*

Children and young people – PFOS

FSANZ noted: "*The results in children are similar to the results in adults (Figure A2.10). As noted above, Maisonet et al. (2015b) reported on a longitudinal study which compared the concentration of PFAS in*

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maternal blood during pregnancy to the cholesterol concentration of daughters at age 15 and the apparent size of the effect relative to other studies may be related to a zeroing problem. The association between LDL-C and PFOS parallels that for total-C and is consistent in direction across the studies. By contrast, the studies have variable findings relating to HDL-C which suggests that there is no association overall."

Summary of FSANZ literature review on PFAS and cholesterol concentrations

FSANZ reported the following summary: "*In summary, the cross-sectional studies overall present a fairly consistent picture. Studies in both adults and children suggest a positive association between total-C and LDL-C and PFOA concentration at very low concentrations of PFOA but not at higher concentrations. At around 25 ng/mL, total cholesterol is about 0.2-0.3 mmol/L higher than at the lowest concentrations measured; after this point the association plateaus. The peak may be reached at lower concentrations or be a little higher. The quantitative results from pregnant women are more inconsistent, but this may be related to haemodilution changes during pregnancy. There appears to be little or no effect on HDL-C, and not all studies have adverse findings. Similar results were seen for PFOS with a maximum increment in total-C of 0.3 mmol/L which is reached at around 40 ng/mL. The lack of association reported in some occupational groups might be due to the lack of sufficient subjects with low concentrations of PFAS to detect the effect. The few longitudinal data that are available do not contradict the findings in the cross-sectional studies."*

6.2.3. Systematic reviews

Saikat et al. (2013)

In their literature review on the impact of PFOS exposure on the health in the general population, Saikat et al. (2013) reviewed three studies that investigated the impact of PFOS exposure on cholesterol in the general population.

Studies reviewed

Saikat et al. reviewed Frisbee et al. (2010); Nelson et al. (2010[19]); and Steenland et al. (2009). Frisbee et al. (2010) and Steenland et al. (2009) are both high-exposure general population studies, while Nelson et al. (2009) used a general population sample.

All three studies have been explored by the international reports (ATSDR, DWQI, US EPA (2016a and 2016b), RIVM and FSANZ). All the information on these studies can be found in the above sections.

Considerations and conclusions

Saikat et al. in their literature review on the impact of PFOS exposure on the health in the general population stated in the Abstract: "*Small but statistically significant associations have been reported with PFOS and total cholesterol [and several other health related outcomes]. The true significance of these findings is uncertain due to the inconsistencies in some of the study results and the limitations in the literature. The majority of studies were cross-sectional and considered surrogate markers of health (e.g. cholesterol levels)."*

In the 'Coherence with evidence' section, Saikat et al. noted: "*Steenland et al[20] and Nelson et al[21] both demonstrated a similar small but significant positive association between PFOS and*

[19] Saikat et al. report that this study was published in 2009; however, it is the study published in 2010 that is referred to throughout this section.

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cholesterol but neither study demonstrated a convincing association between PFOS and HDL cholesterol. Frisbee et al.[22] *also indicated a significant association between PFOS and increased Total-C, HDL-C and LDL-C. These studies used a cross-sectional design; therefore it is not possible to conclude a cause-effect relationship between PFOS and cholesterol and the association might have been confounded by selection bias from underlying demographic risk factors because sorting cohort by dose may disproportionately increase more younger female and low-BMI individuals in the lowest dose quartile used as the referent population*[33]*."*

"*These studies used a cross-sectional design; therefore it is not possible to conclude a cause-effect relationship between PFOS and cholesterol and the association might have been confounded by selection bias from underlying demographic risk factors....*"

Priestly (2016)

Priestly (2016) reviewed studies that examined the link between PFAS exposure and cholesterol.

Studies reviewed

Priestly reviewed:

- six general population or low exposure studies (Château-Degat et al. 2010; Fisher et al. 2013; Fu et al. 2014, Geiger et al. 2014a; Starling et al. 2014; and Zeng et al. 2015);
- five high-exposure general population studies (Fitz-Simon et al. 2013; Frisbee et al. 2010; Kerger et al. 2011; Steenland et al. 2009; and Kerger et al. 2011);
- three studies of infants, children and adolescents (Maisonet et al. 2015; Goudarzi et al. 2016a; Itoh et al. 2016);
- one study of pregnant women and newborns (Kishi et al. 2015);
- one study on mode of action (Fletcher et al. 2013); and
- an editorial summary of the Nelson et al. (2010) paper (Tillett, 2010).

Priestly also cited an *in vitro* study on mouse and human cells (Xu et al. 2016), which has not been reported further here.

The four studies that were not previously reviewed by the international documents and/or systematic reviews in the section above are Kerger et al. (2011); Kishi et al. (2015); Goudarzi et al. (2016a); and Itoh et al. (2016). Information on the other studies can be found in the sections above.

Considerations and conclusions

Priestly (2016) stated: "*The epidemiological studies are suggesting, but not yet proving, a possible link between PFOS/ PFOA and blood lipid disorders.*"

Under the section 'Altered serum lipids', Priestly commented that: "*Selective interference with gene expression could explain the promotion of hypercholesterolemia by PFAS, but it does not allow for making a distinction between different PFAS. However, Heuval (2013), in a critique of the Fletcher et al. (2013) study, pointed out that a hypercholesterolemic state did not exist in the subjects, because geometric means of cholesterol and LDL were in the normal range, nor did other studies on this population indicate any excess risk to coronary artery disease.*"

Summaries of studies reviewed

Of the following studies, Priestly provided the following details in a Table (Table 4, pages 32-33).

- Kerger et al. (2011): "*Total cholesterol Subjects taking lipid-lowering drugs excluded from analysis. Cross-sectional; C8 Health project; West Virginian residents living near Dupont*

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facility; 46,294 adults >18y in 2005-06. Median PFOA serum levels 26.6 ng/mL; odds ratios for increased serum cholesterol (>240mg/dL) across the top three quartiles of serum PFOA, compared to the lowest quartile (1.21, 1.33, 1.4); cholesterol levels overall lower than in the general US population, with age, sex and BMI found to be stronger correlates with hypercholesterolemia; separate analysis of 19% of subjects taking cholesterol-lowering drugs showed similar, but attenuated trends, on a lower serum cholesterol base (mean 173 vs 206 mg/dL)."

- Kishi et al. (2015): "*Prenatal maternal serum PFOS & PFOA; 9 fatty acids & triglycerides; infant birth size. Subjects from Hokkaido Study on Environment & Children's Health; n=306 mother-child pairs; 2002-05 enrolment. PFOS (but not PFOA) blood levels negatively associated with levels of palmitic, palmitoleic, linoleic, α-linolenic and arachidonic acids (but not stearic, oleic EPA, DHA or triglycerides); some of these effects may have been confounded by dietary fish intake or the time of blood sampling duration gestation; pregnancy effect discussed in Table 5.*"

Of the editorial summary by Tillett (201), on the study by Nelson et al. (2010), Priestly reported: "*An editorial summary of the Nelson et al. (2010) paper discussed the potential implications of the findings, but also cautioned about drawing conclusions pending further confirmatory studies and also pointed out that the findings could indicate reverse causality, with higher cholesterol levels and dyslipidemia resulting in a tendency to accumulate PFOS in blood (Tillett 2010). Since then, there have several more epidemiological studies that examined serum lipid and lipid transport/metabolism and metabolic gene expression in various populations. Findings in most of the studies were only partially consistent with those reported by Nelson et al. (2010) for an adult population, although there was a degree of consistency across different regional groups.*"

Of the studies by Gourdazi et al. (2016a) and Itoh et al. (2016), Priestly reported: "On the basis that serum cholesterol is the precursor of androgenic and glucocorticoid steroid hormone synthesis, the effects of prenatal PFOS/PFOA exposures on cord blood levels of various hormones was assessed in 185 infants from the Hokkaido Study. A dose-related reduction of cortisol and cortisone levels (-24 ng/mL 95% CI -0.47, -12.11; -63.21 ng/mL 95% CI -26.72, -132.56) was associated with PFOS levels in the highest-lowest quartiles, and a similar negative association with PFOA for DHEA levels (-1.23 95% CI -0.25, -1.72). No effect was found on androstenedione levels (Goudarzi et al. 2016a). Also from the Hokkaido Study, variable, but small, changes were observed in cord blood levels of some reproductive hormones, with different hormones and different change directions seen in girls and boys (Itoh et al. 2016)."

Rappazzo et al. (2017)

Rappazzo et al. (2017) reviewed five studies on the relationships between prenatal and/or childhood exposure to PFAS and health outcomes in children. The study also provided a risk of bias analysis of the literature.

Studies reviewed

The authors reviewed five studies that examined dyslipidemia (Frisbee et al. 2010; Geiger et al. 2014a; Lin et al. 2009; Maisonet et al. 2015b; Zeng et al. 2015).

All five of the studies have been discussed by the international reports and/or systematic reviews. Information on each study can be found in the above sections.

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Considerations and conclusions

The authors concluded in the Abstract that: "*While there are a limited number of studies for any particular health outcome there is evidence of a positive association between PFAS and dyslipidemia... while PFASs are mixtures of multiple compounds few studies examine them as such, therefore the role of these compounds as complex mixtures remains largely unknown.*"

The authors stated the measures of dyslipidemia as being abnormal levels of serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), or triglycerides.

In the 'Cardiometabolic' section where the studies on dyslipidemia were reviewed, Rappazzo et al. commented that: "*Cardio-metabolic effects in children were reported in multiple studies. Analyses reported generally higher or abnormal levels of total cholesterol and LDL-C in association with PFAS serum concentration. Some mechanistic analyses have also been performed by Fletcher et al. (2013), who found changes in the expression of genes involved in cholesterol metabolism (transport and mobilization) to be associated with serum PFAS in the C8 population.*"

In their 'Discussion', they stated: "*Blood samples may also be taken from either fasting or non-fasting participants, which may make between study comparison of cardiovascular and lipid-related markers difficult.*"

Summaries of studies reviewed

Rappazzo et al. reported the following about the five studies they reviewed: "*Five cross-sectional studies examined dyslipidemia. In adolescents from NHANES, increases in PFOA, PFOS, or total PFAS serum concentrations were positively associated with high total cholesterol (>170 mg/dL) and high LDL-C; PFAS were not associated with clinically abnormal HDL-C and triglyceride levels [Geiger et al. (2014a)]. Linear associations were similar, with increases in PFAS associated with increases in total cholesterol and LDL-C, and no associations with triglycerides or HDL-C, though HDL-C levels did show small decreases with increases in plasma PFOA levels [Geiger et al. (2014a)]. Also in NHANES, Lin et al. [2009] found no associations between PFASs and HDL-C or triglyceride levels examined as components of metabolic syndrome in NHANES adolescents. Children and adolescents from the Ohio Valley C8 population had PFOS and PFOA serum concentration associated with increased odds of abnormal total cholesterol and LDL-C; PFOS was also associated with decreased odds of abnormal HDL-C [Frisbee et al. (2010)]; Linear changes in lipids were also examined, with [Frisbee et al. (2010)] reporting positive though non-linear trends between PFOS and total cholesterol, LDL-C, and HDL-C....A study in Taiwan found log increases in serum PFOA, PFOS, and PFNA associated with increases in total cholesterol, LDL-C, and triglyceride concentrations [Zeng et al. (2015)]. In the ALSPAC cohort examination of lipids in association with maternal serum PFAS, Maisonet, et al. [2015b] observed positive, though non-linear and non-monotonic, associations with increases in maternal serum PFOA and PFOS and total and LDL-cholesterol.*"

Kirk et al. (2018)

Studies reviewed

Kirk et al. in their systematic review of the literature on PFAS and health effects, evaluated 29 papers investigating PFAS exposure on serum concentrations of cholesterol and triglycerides in children and adults (Château-Degat et al. 2010; Costa et al. 2009; Eriksen et al. 2013; Fisher et al. 2013; Fitz-Simon et al. 2013; Fleisch et al. 2016; Frisbee et al. 2010; Fu et al. 2014; Geiger et al. 2014a; Lin et al. 2009; Lin et al. 2011; Maisonet et al. 2015a; Mundt et al. 2007; Nelson et al. 2007; Olsen et al. 1999; Olsen et al. 2000; Olsen et al. 2003a; Olsen et al. 2012; Olsen and Zobel 2007; Rotander et

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al. 2015; Sakr et al. 2007a; Sakr et al. 2007b; Skuladottir et al. 2015; Starling et al. 2014b; Steenland et al. 2009; Timmermann et al. 2014; Wang et al. 2012; Winquist and Steenland 2014a; Zeng et al. 2015).

The studies included[20]:

- twenty-five studies reviewing total cholesterol (Château-Degat et al. 2010; Costa et al. 2009; Eriksen et al. 2013; Fisher et al. 2013; Fitz-Simon et al. 2013; Frisbee et al. 2010; Fu et al. 2014; Geiger et al. 2014a; Maisonet et al. 2015a; Mundt et al. 2007; Nelson et al. 2010; Olsen et al. 1999; Olsen et al. 2000; Olsen et al. 2003a; Olsen et al. 2012; Olsen and Zobel 2007; Rotander et al. 2015; Sakr et al. 2007a; Sakr et al. 2007b; Skuladottir et al. 2015; Starling et al. 2014b; Steenland et al. 2009; Winquist and Steenland 2014a; Wang et al. 2012; Zeng et al. 2015);
- twenty-two studies reviewing HDL (Château-Degat et al. 2010; Costa et al. 2009; Fisher et al. 2013; Fitz-Simon et al. 2013; Frisbee et al. 2010; Fu et al. 2014; Geiger et al. 2014a; Maisonet et al. 2015a; Mundt et al. 2007; Nelson et al. 2010; Olsen et al. 1999; Olsen et al. 2000; Olsen et al. 2003a; Olsen et al. 2012; Olsen and Zobel 2007; Rotander et al. 2015; Starling et al. 2014b; Sakr et al. 2007a; Sakr et al. 2007b; Steenland et al. 2009; Wang et al. 2012; Zeng et al. 2015);
- three studies examining total cholesterol to HDL ratio (Fisher et al. 2013; Olsen et al. 2012; Steenland et al. 2009);
- eighteen studies reviewing LDL levels (Château-Degat et al. 2010; Geiger et al. 2014a; Fisher et al. 2013; Fitz-Simon et al. 2013; Frisbee et al. 2010; Fu et al. 2014; Maisonet et al. 2015a; Mundt et al. 2007; Nelson et al. 2010; Olsen et al. 2000; Olsen and Zobel 2007; Rotander et al. 2015; Sakr et al. 2007a; Sakr et al. 2007b; Starling et al. 2014b; Steenland et al. 2009; Wang et al. 2012; Zeng et al. 2015);
- two studies examining very low density lipoprotein (VLDL) levels (Mundt et al. 2007; Sakr et al. 2007b);
- two studies reporting on HDL to LDL ratio (Steenland et al. 2009; Wang et al. 2012);
- four studies investigaing non-HDL levels (Château-Degat et al. 2010; Fisher et al. 2013; Nelson et al. 2010; Olsen et al. 2012);
- nineteen studies examining triglyceride (TG) levels (Château-Degat et al. 2010; Costa et al. 2009; Fisher et al. 2013; Frisbee et al. 2010; Fitz-Simon et al. 2013; Fu et al. 2014; Geiger et al. 2014a; Maisonet et al. 2015a; Olsen et al. 1999; Olsen et al. 2003a; Olsen et al. 2007; Rotander et al. 2015; Sakr et al. 2007a; Starling et al. 2014b; Steenland et al. 2009; Wang et al. 2012; Zeng et al. 2015).

Of these studies, all have been outlined under previous reviews by the ATSDR, DWQI and the US EPA, except for Rotander et al. (2015) and Skuladottir et al. (2015).

Kirk et al. noted: "*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 studies, 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.*"

[20] Note that studies may appear in more than one sub-section.

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Considerations and conclusions

In the 'Plain Language Summary', Kirk et al. stated that: "*We found sufficient*[21] *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.*"

In the 'Executive Summary', Kirk et al. made two statements about PFAS exposure and cholesterol levels in the blood:

- "*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*", and
- "*PFOA and PFOS were associated with higher blood total cholesterol levels (hypercholesterolaemia). We found evidence of a positive association between exposure to PFOA and blood total cholesterol levels in 12 of 19 relevant studies. Further, eight of 18 studies that reported on PFOS exposure and total cholesterol levels also found a positive association. Due to a lack of consistency in the way these studies were conducted, we were unable to conduct a meta-analysis. The findings for both chemicals applied to total cholesterol in children and in adults and suggest that elevated PFOA and PFOS concentrations in blood increase total blood cholesterol levels.*"

In the 'Discussion', Kirk et al. made the following statements and comments: "*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.

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[21] *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. (Source Kirk et al. page 24)

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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. [2017] 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. [2017] referred to, which may explain the difference between our findings and theirs."

In the 'Conclusion of the Systematic Review,' Kirk et al. made the following comment: "*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.*"

The table below shows the evaluation of the evidence determined by Kirk et al. for all cholesterol and triglycerides evaluated, by PFAS.

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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

Kirk et al. 2018, pp. 73.

Total cholesterol

Kirk et al. reviewed 25 studies that investigated the association between PFAS and total cholesterol level. Kirk et al. made the following comments about the studies: "*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.*"

Kirk et al. then made an overall statement about PFAS and cholesterol, before reporting specifically on PFOA and PFOS: "*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.*"

PFOA and total cholesterol

Kirk et al. noted that: "*Of 22*[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. [2009]; Eriksen et al. [2013]; Sakr et al. [2007a, 2007b]; Skuladottir et al. [2015]; Steenland et al. [2009] and Winquist & Steenland [2014a]) identified this relationship in adults, three papers (Frisbee et al. [2010]; Geiger et al. [2014a] and Zeng et al. [2015]) in children and two papers (Fu et al. [2014] and Nelson et al. [2010]) related to participants aged 12 to 80-years old and 0 to*

[22] Note the Executive Summary and Discussion reported in Considerations and Conclusions state that 12 out of 19 studies on PFOA and cholesterol but here it is stated that 22 studies were evaluated, of which 10 showed no effect. This is because four of the papers were by Olsen et al. and can be considered follow-up analyses of the same cohort, making 19 studies, as Olsen is included as one study.

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88-years old, respectively. In contrast, the remaining 10[23] *papers reported no association between PFOA and total cholesterol level in children and adults [Fisher et al. 2013; Fitz-Simon et al. 2013; Maisonet et al. 2015a; Olsen et al. 2000; Olsen et al. 2003a; Olsen et al. 2012; Olsen and Zobel 2007; Rotander et al. 2015; Starling et al. 2014b; Wang et al. 2012]."*

Kirk et al. discussed the evidence on the association between PFOA and total cholesterol level, noting that it: *"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. [Olsen et al. 1999; Olsen et al. 2003a; Olsen and Zobel 2007; Olsen et al. 2012] 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 studies 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 studies 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."*

PFOS and total cholesterol

With regards to the evidence of PFOS and total cholesterol, Kirk et al. reported that: *"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 [Eriksen et al. 2013; Skuladottir et al. 2015; Steenland et al. 2009; Frisbee et al. 2010; Geiger et al. 2014a; Zeng et al. 2015; Nelson et al. 2010; Starling et al. 2014b]. PFOS was concluded to have no effect on total cholesterol levels in 10 additional papers [Fu et al. 2014; Fisher et al. 2013; Fitz-Simon et al. 2013; Maisonet et al. 2015a; Olsen et al. 1999; Olsen et al. 2003a; Olsen et al. 2012; Olsen and Zobel 2007; Rotander et al. 2015; Château-Degat et al. 2010]."*

Of the papers they reviewed on PFOS, Kirk et al. reported that: *"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. [1999; 2003a; 2012] 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."*

Other PFAS and total cholesterol

Kirk et al. reviewed eight studies on exposure to other PFAS, including PFNA and PFHxS, and concluded that: *"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."*

[23] Refer to footnote 14 for differences in numbers from the Executive Summary.

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High-density lipoprotein (HDL) level

Kirk et al. reported on 22 studies that analysed the relationship between PFAS exposure and serum HDL concentration, and concluded that "*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.*"

PFOA and HDL

In providing more detail about the studies, Kirk et al. reported that: "*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 [Costa et al. 1999; Sakr et al. 2007a; Sakr et al. 2007b; Steenland et al. 2009; Frisbee et al. 2010; Geiger et al. 2014a; Zeng et al. 2015; Fu et al. 2014; Nelson et al. 2010; Fisher et al. 2013; Fitz-Simon et al. 2013; Maisonet et al. 2015a; Olsen et al. 2000; Olsen et al. 2003a; Olsen et al. 2012; Olsen and Zobel 2007; Rotander et al. 2015; Starling et al. 2014b]. Contrary to these results, Wang et al. [2012] 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.*"

PFOS and HDL

Kirk et al. reported the following for the papers they reviewed on PFOS and HDL: "*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 studies showing no significant effect of elevated PFOS exposure levels on HDL measurements [Geiger et al. 2014a; Fisher et al. 2013; Fitz-Simon et al. 2013; Fu et al. 2014; Maisonet et al. 2015a; Nelson et al. 2010; Olsen et al. 1999; Olsen et al. 2003a; Olsen et al. 2007; Olsen et al. 2012; Rotander et al. 2015; Steenland et al. 2009; Zeng et al. 2015]. In contrast, three studies supported a significant association between PFOS and HDL levels.*"

Other PFAS and HDL

Kirk et al. reported that: "*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 [Zeng et al. 2015; Fu et al. 2014; Nelson et al. 2010; Fisher et al. 2013; Mundt et al. 2007]. However, Starling et al. [2014b] 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*", and then commented "*In summary, the evidence largely supports that these additional PFAS exposures have no association with HDL level.*"

Total cholesterol to HDL ratio

Kirk et al. reported on three studies that investigated the effect of elevated PFAS exposure on the ratio of total cholesterol and HDL measurements (total cholesterol: HDL), and concluded that: "*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.*"

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Low-density lipoprotein (LDL) level

Kirk et al. reported on 18 studies that investigated the association between PFAS exposure and serum LDL concentrations, and summarised: "*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.*"

PFOA and LDL

Kirk et al. provided further detail about the studies on PFOA: "*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 discussing the effects of other PFAS exposures, Kirk et al. noted that: "*...results are also inconsistent... Largely, the results show that PFAS exposures are not associated with LDL level; however, there are discrepancies with some studies reporting significant results.*"

Very low density lipoprotein (VLDL) level

Kirk et al. reported on two studies which investigated the effect of PFAS exposure on VLDL levels, and provided the following detail: "*Sakr et al. [2007b] reported no significant relationship between PFOA and VLDL measurements and Mundt et al. [2007] found no association between PFNA and VLDL level.*"

HDL to LDL ratio

Kirk et al. reported on two studies which investigated the relationship between elevated PFAS levels and HDL to LDL ratio measurements (HDL: LDL) and concluded that: "*Results are conflicting for this health outcome.*"

Non-HDL level (total HDL cholesterol)

Kirk et al. reported on four studies that investigated the association between PFAS levels and non-HDL measurements, and summarised: "*...these findings suggest there is inconsistent evidence to support a significant association between PFAS exposures and non-HDL measurements.*"

Triglyceride (TG) level

Kirk et al. reported on 19 studies on the association of PFAS exposure on serum TG concentration, and noted that: "*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.*"

PFOA and TG level

In considering the results from the 17 studies on the relationship with PFOA exposure, Kirk et al. concluded that: "*Non-significant results related to the effects of PFOA on TG levels were reported by 14 papers [Costa et al. 2009; Sakr et al. 2007a; Frisbee et al. 2010; Geiger et al. 2014a; Fu et al. 2014; Fisher et al. 2013; Fitz-Simon et al. 2013; Maisonet et al. 2015a; Olsen et al. 2000; Olsen et al. 2003a;*

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Olsen and Zobel 2007; Rotander et al. 2015; Starling et al. 2014b; Wang et al. 2012]. Therefore, there is more evidence in the literature to suggest PFOA does not affect changes in TG levels in adults and children."

PFOS and TG level

Kirk et al. noted the following about the 13 studies that investigated PFOS exposure on TG measurements: "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 [Frisbee et al. 2010; Geiger et al. 2014a; Fu et al. 2014; Fisher et al. 2013; Fitz-Simon et al. 2013; Maisonet et al. 2015a; Olsen et al. 2003a; Rotander et al. 2015; Starling et al. 2014b; Olsen et al. 1999]."

Kirk et al. noted that three of the 19 studies on TG levels stated the effects of additional PFAS exposures, and summarised that: "*there is also insufficient evidence to state a positive association between PFNA and other PFAS exposures and TG concentrations in serum.*"

6.2.4. Summary of key national and international reports and systematic reviews

Recent key national and international reports:

- ATSDR reports that the most consistently found alteration in serum lipid levels was increased serum total cholesterol levels, while the evidence of associations between serum perfluoroalkyl levels and other serum lipids is not so strong. ATSDR reports the mechanisms for the increased serum cholesterol in individuals with high serum PFOA and/or PFOS levels have not been identified.
- The US EPA reported associations were found between PFOA/PFOS exposure and high cholesterol.
- DWQI concluded that PFOA was associated with clinically defined hypercholesterolemia in a community exposed through drinking water and epidemiological evidence supports multiple criteria for a causal relationship between PFOA and cholesterol.
- RIVM concluded evidence has been found between exposure to PFOA and higher total cholesterol concentrations in the blood.
- FSANZ considers that the meaning and clinical significance of the associations for PFOS and PFOA for increased cholesterol in observational studies are uncertain and should be treated with caution.

Systematic reviews:

- Saikat et al. concluded that there is an association between PFOS and elevated cholesterol levels, but the studies have limitations and the current evidence for health outcomes among the general population is inconclusive.
- Priestly concluded the epidemiological studies are suggesting but not yet proving a possible link between PFOS/PFOA and blood lipid disorders.
- Rappazzo et al. concluded there was evidence of a positive association between PFAS and dyslipidaemia in children.
- Kirk et al. reported 'sufficient evidence' that PFOA and PFOS were associated with higher blood total cholesterol levels (hypercholesterolaemia).

The systematic reviews and key reports highlighted:

- It is not possible to determine whether PFOS or PFOA causes the changes in cholesterol levels, or whether other factors are involved.
- Many of the studies were considered to have high risk of bias.

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6.2.5. Expert Health Panel synthesis to support advice to the Minister

- Many studies highlighted that although there was a small statistical association between PFOA and total cholesterol levels, this is unlikely to represent important differences for individual people; these might still have some relevance for PFAS risk assessment for regulating general population exposures.
- The association of PFAS with total cholesterol does not have an established causal mechanism. One point to note is that PFAS do interact with PPAR receptors and these are involved in lipid regulation. Drugs that are PPAR-α agonists (e.g. fibrates) generally lower total cholesterol; PPAR-γ agonists (-glitazones) increase total cholesterol.
- The current evidence is largely from cross-sectional studies, which is generally a weak study design, and stronger evidence would come from future cohort studies.
- Animal studies, including some primate studies, have found decreases in serum cholesterol levels which is the opposite effect observed in humans.

6.2.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and cholesterol and triglycerides:

- An association of PFAS with cholesterol, but not other lipids, is generally observed but it is of small magnitude, although there is an exposure-response relationship. Evidence to date does not establish whether or not PFAS causes higher cholesterol, due to weak studies, inconsistencies with animal studies, limited adjustment for confounders, the possibility of reverse causation and the lack of any clear causative mechanism.
- Due to the small association found and the other limitations noted above, the existing scientific evidence does not warrant any change to peoples' medical management or risk assessment for heart disease.
- In the clinic, established risk factors for high cholesterol and/or heart disease such as alcohol, diabetes, diet, smoking, blood pressure and kidney disease are usually of a much greater magnitude than these small differences.

To further investigate the association between PFAS exposure and cholesterol and triglycerides in an Australian setting, the Panel proposes the following research priorities:

- Studies that provide causal evidence are the key research need. Further cross-sectional studies are unlikely to provide this information, but well-designed longitudinal studies may provide stronger epidemiological evidence. Relevant studies would (for example) investigate direct evidence for activation of causal biochemical mechanism(s) in humans, or determine whether reducing PFAS concentrations in individuals alters cholesterol measurements.

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6.3. Liver function and PFAS exposure

Blood concentrations of liver enzymes can be measured as an indication of liver health. An increase in these enzymes may be indicative of liver problems; however, they normally vary so it is difficult to determine when health is affected. Liver enzymes can be affected by medical disorders (e.g. hepatitis, hyperthyroidism); temporary conditions (e.g. dehydration, infection, muscle trauma, burns, pregnancy); medications (e.g. antibiotics, anticonvulsants, statins, paracetamol); herbal supplements (e.g. black cohosh, comfrey tea, kava kava, noni juice; Chinese herbal medicines such as Ba Jiao Lian; alcohol intake; and obesity. In animal studies on PFAS exposure, the liver has been a principal target organ. Several international authority reports have evaluated the human evidence on PFAS exposure and liver function, along with two recent systematic reviews.

6.3.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of five published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and two systematic reviews since 2016 that analysed the human epidemiological evidence regarding exposure to PFAS and liver function.

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR, 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **United States Environmental Protection Agency (US EPA, 2016a).** Health effects support document for Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA, 2016b).** Health effects support document for Perfluorooctane Sulphonate (PFOS);
- **New Jersey Drinking Water Quality Institute (DWQI, Public Review draft 2016).** Health-based maximum contaminant level support document: Perfluorooctanoic Acid (PFOA);
- **Dutch National Institute for Public Health and the Environment (RIVM, 2017)**. PFOA exposure and health: A review of scientific literature.

Systematic reviews and reviews

- **Priestly (2016).** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS). Monash University;
- **Kirk et al. (2018).** The PFAS Health Study: systematic literature review. Australian National University.

The Panel acknowledges that FSANZ commented on PFAS exposure and liver function in its 'Hazard assessment report for PFOA, PFOS and PFHxS'; FSANZ did not undertake a review of the epidemiological literature. FSANZ cited several studies (not named), and noted these studies had been reviewed by other international authority reports, notably EFSA (2008), ATSDR (2015), US EPA (2016a, b). For this reason, the FSANZ (2017) report is not considered further in this section. No other systematic reviews or key national and international reports covered liver function.

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6.3.2. Key national and international reports

Agency for Toxic Substances and Disease Registry (ATSDR, 2015).

Studies reviewed

The ATSDR reviewed the following studies:

- twelve studies under 'Hepatic Effects – Inhalation Exposure' (Alexander et al. 2003; Olsen et al. 2004; Grice et al. 2007; Gilliland and Mandel 1996; Olsen et al. 1999; Olsen et al. 2000; Olsen et al. 2003a; Olsen and Zobel 2007; Olsen et al. 2012; Sakr et al. 2007a; Costa et al. 2009; Mundt et al. 2007).
- three studies under 'Hepatic effects – Oral Exposure' (Emmett et al. 2006; Gallo et al. 2012; Lin et al. 2010).

Considerations and conclusions

ATSDR reported in the 'Public health statement' section that: "*There is also some evidence that PFOA and PFOS exposure may cause liver damage.*"

In the 'Relevance to Public Health – Summary of health effects' section, ATSDR reported that: "*Consistent findings were found for association of serum PFOA and PFOS with ... alterations in biomarkers of liver damage.*" Specifically, regarding liver effects, the ATSDR reported: "*A number of human studies have used serum liver enzymes as biomarkers of possible liver effects. In occupational exposure studies, no associations between serum liver enzymes (primarily, alanine aminotransferase [ALT], aspartate aminotransferase [AST], and γ-glutamyl transpeptidase [GGT]) and serum PFOA or PFOS levels were consistently found. A study of residents highly exposed to PFOA found significant associations between serum PFOA and serum PFOS levels and ALT and bilirubin levels. The study also found increased risk of high ALT levels in subjects with higher PFOA and PFOS levels. Although associations were found, the magnitude of the increased serum enzymes were not great, and were probably not biologically significant. Occupational exposure studies have not found increases in deaths from liver cirrhosis or increases in the occurrence of liver disorders or cirrhosis. Studies in rats, mice, and monkeys have identified the liver as one of the most sensitive targets of toxicity; the data in humans are not as convincing. However, serum PFOA and PFOS levels were much lower than those associated with effects in animals.*"

In the 'Minimal risk levels' section, the ATSDR stated that: "*A wide range of effects have been statistically associated with serum perfluoroalkyl levels; however, there is a lack of consistency of the findings across studies and across types of studies. Based on the weight of evidence, there is support for identifying several health effects in humans that appear to be related to perfluoroalkyl exposure [a number of other health effects] and possible changes in biomarkers of liver damage. The magnitude of the changes in... serum liver enzymes observed in the human studies are small and not likely biologically relevant.*"

Summaries of studies reviewed

Inhalation exposure

Under 'Hepatic Effects – Inhalation Exposure', the ATSDR reviewed 12 studies.

Liver diseases

The ATSDR examined three studies of the possible association between PFOA/PFOS on liver diseases and reported that: "*No alterations in the SMR for cirrhosis of the liver were found in workers*

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at the 3M facility in Decatur, Alabama (Alexander et al. 2003). Another study of workers at this facility found no significant alterations in the episodes of care for liver disorders or cirrhosis of the liver (Olsen et al. 2004). A third study of workers at a PFOS facility in Cottage Grove, Minnesota, did not find increases in self-reported liver disease (including cirrhosis and hepatitis) (Grice et al. 2007)."

Liver function

For PFOA/PFOS and liver function, the ATSDR reported that: "*A number of occupational exposure studies have evaluated liver function (as assessed by serum liver enzymes) in workers exposed to PFOA and/or PFOS, and for the most part, no significant associations have been found.*"

Of the study by Gilliland and Mandel (1996), the ATSDR reported: "*A cross-sectional study of 115 workers exposed to PFOA found no significant alterations in activities of alanine aminotransferase (ALT), asparate aminotransferase (AST) or γ-glutamyl transpeptidase (GGT) at the serum PFOA levels measured (<1,000–26,000 ng/mL, mean 3,300 ng/mL).*" The ATSDR made the following comment about this study: "*It should be noted that in obese workers only, AST and ALT activities increased with increasing PFOA, which the investigators thought had biological plausibility because obesity has been associated with elevation of transaminases through fatty infiltration.*"

Of the studies by Olsen et al. (1999, 2000), the ATSDR reported that: "*A similar study was conducted with PFOS in male workers at plants in Decatur, Alabama and Antwerp, Belgium (Olsen et al. 1999). In 1995, the mean serum PFOS for 178 workers was 2,190 ng/mL (range 0– 12,830 ng/mL); in 1997, the mean for 149 workers was 1,750 ng/mL (range 100–9930 ng/mL). For both years, 95% of the measured PFOS levels were <6,000 ng/mL. Because the employees from the two plants were dissimilar by age, body mass index (BMI), and self-reported alcohol use, the authors conducted combined analyses as well as separate analyses by plant location. There were no substantial changes in serum ALT, AST, or GGT enzymes at PFOS levels <6,000 ng/mL; a positive association with total bilirubin levels was found. No conclusions were drawn from the few workers with serum PFOS ≥6,000 ng/mL due to their small number (7 in 1995 and 5 in 1997 data). Similarly, no association of ALT, AST, or GGT and serum PFOA levels were observed in groups of workers at these facilities examined in 1993 (111 subjects), 1995 (80 subjects), and/or 1997 (74 subjects) (Olsen et al. 2000).*"

Of the study by Olsen et al. (2003a), the ATSDR reported the following summary: "*A subsequent evaluation of workers from the same plants, but that included women and a longitudinal analysis of the workers, reported that, after adjusting for potential confounding factors, there were no substantial changes in hepatic parameters; GGT levels were significantly higher in females with PFOS levels in the fourth quartile, as compared to the first quartile, but this was not observed in males (Olsen et al. 2003a). In this study, the mean serum concentrations of PFOS and PFOA for 263 Decatur employees were 1,320 and 1,780 ng/mL, respectively. Workers at the Antwerp plant (n=255) had mean PFOA and PFOS serum values approximately 50% lower than those at the Decatur plant.*"

The study by Olden and Zobel (2007) was reported as: "*A more recent assessment of 506 employees who did not take cholesterol-lowering medications at three fluorochemical production plants (Cottage Grove, Minnesota; Decatur, Alabama; Antwerp, Belgium) reported no statistically significant association between serum PFOA and ALT, AST, or total bilirubin levels for the three facilities combined, although some modest positive associations were observed between PFOA and hepatic enzymes (ALT and GGT) at one of the three facilities (Olsen and Zobel 2007). Serum PFOA levels in this study ranged from 7 to 92,030 ng/mL (arithmetic mean 2,210 ng/mL, 95% CI 1,660– 2,770 ng/mL).*"

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Liver enzymes and biomarkers

Olsen et al. (2012) was reported to be: "*A study of workers (n=179) involved in the demolition of 3M perfluoroalkyl manufacturing facilities examined the effect of a change in serum PFOA levels over a mean period of 164 days on hepatic biomarkers (Olsen et al. 2012). In workers with prior exposure to PFOA who had a decrease in serum PFOA levels during the study period, there was a significant increase in ALT levels. An increase in serum PFOA levels did not significantly alter AST or total bilirubin levels. The study also found a negative association between the change in serum PFOS levels and ALT levels.*"

The ATSDR reviewed two studies that investigated the possible association between PFOA exposure and hepatic enzymes of workers at a facility that manufactures fluoropolymers in West Virginia.

Of the study by Sakr et al. (2007a), the ATSDR reported that it: "*examined the relationship between serum PFOA and liver enzymes in a longitudinal study of 454 workers using a linear mixed effects model. The cohort was comprised of employees who had two or more measurements of serum PFOA from 1979 until the study was conducted. The average length of employment among workers with multiple PFOA measurements was 11 years, and, on average, 10.8 years elapsed between their first and last serum PFOA measurement. The means of the first and last PFOA measurement were 1,040 and 1,160 ng/mL, respectively. After adjustment for potential confounders, PFOA was negatively associated with total bilirubin and positively with serum AST activity, but not ALT or GGT.*"

Of the study by Sakr et al. (2007b), the ATSDR reported: "*The same groups of investigators conducted a cross-sectional study of 1,025 active workers (76% males) at the same plant with potential exposure to PFOA (Sakr et al. 2007b). Serum PFOA levels ranged from 5 to 9,550 ng/mL among the total participants. After adjustment for confounders, which included control for cholesterol-lowering medications, there was a modest but statistically significant positive association between PFOA and GGT activity. The increases in serum AST activity in the longitudinal study and serum GGT activity in the cross-sectional study were small and were not likely biologically relevant. No associations were found for bilirubin, or ALT and AST activities.*"

The ATSDR noted that Costa et al. (2009) had undertaken a small study of Italian perfluoroalkyl workers (n=34) and that it: "*did not find significant associations between serum PFOA and AST or ALT activities or total bilirubin levels*".

In a health evaluation by Mundt et al. (2007), of workers exposed to PFNA, the ATSDR reported: "*The cohort consisted of 630 employees at a U.S. polymer production facility using PFNA at any time between January 1, 1989 and July 1, 2003. Annual cross-sectional analyses and longitudinal analyses that accounted for multiple measurements per person were conducted over a 5-year period. After adjusting for age and BMI, some small but not clinically significant differences between groups were found. However, these observations were not consistent between men and women or over the five analysis windows. GGT, AST, ALT, and bilirubin examined in separate longitudinal models showed no significant increase or decrease by unit increase in exposure intensity score.*"

Oral exposure

The ATSDR reviewed three studies under the 'Hepatic effects – oral exposure' section.

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Liver function

Of the study by Emmett et al. (2006), the ATSDR reported: "*No evidence of adverse liver function (assessed via serum transaminases, alkaline phosphatase, or bilirubin) was observed among the 371 individuals with high levels of PFOA in the water supply and verified high serum PFOA levels evaluated by Emmett et al. (2006). In 13 individuals with liver disease (information provided by the individuals), the mean serum PFOA was higher than in individuals without liver disease (527 vs. 441 ng/mL), but the difference was not statistically significant.*"

The ATSDR reported the study by Gallo et al. (2012) as: "*A study of over 47,000 subjects enrolled in the C8 Health Project found a significant association between PFOA and PFOS and ALT levels and with direct bilirubin levels (direct bilirubin levels were not significantly related to PFOA levels after adjustment for smoking status, BMI, physical activity, and insulin resistance) (Gallo et al. 2012). Additionally, the odds of having an abnormally high ALT value (≥45 IU/L in men and 34 IU/L in women) were significantly higher in subjects with serum PFOA levels in the third or higher decile and PFOS levels in the fifth or higher decile. In the fully adjusted model, there was a significant association between GGT values and serum PFOA levels; however, there was no exposure-related trend when serum PFOA levels were categorized by deciles.*"

The study by Lin et al. (2010): "*found significant trends for increasing serum ALT and GGT levels with increasing serum PFOA and PFOS levels in a general population study using the NHANES data set. Exposure-related trends were also observed for total bilirubin levels with serum PFHxS and PFNA levels. After adjusting for potential confounders, the association between serum PFOA and ALT and GGT remained statistically significant.*"

In this study, the levels of the enzyme ALT were still within the normal reference range for the population (see Figure 2, page 1,361 in Lin et al. 2010).

United States Environmental Protection Agency (US EPA, 2016a, b).

The US EPA reviewed the effects of PFOA under the 'Liver enzymes and liver disease' section.

Studies included

Under 'Hazard identification – liver enzymes and liver diseases' (PFOA), the US EPA reviewed nine studies in total, including eight studies on liver enzymes. These included:

- five cross-sectional occupational exposure studies (Olsen et al. 2000; Olsen and Zobel 2007; Sakr et al. 2007b; Costa et al. 2009; Olsen et al. 2003a);
- two cross-sectional high exposure community studies (Emmett et al. 2006; Gallo et al. 2012);
- one cross-sectional general population study (Lin et al. 2010).

All of these studies were reviewed by the ATSDR (2015).

The US EPA also reviewed one study on liver disease from a high exposure community (Steenland et al. (2015). This study was not reviewed by the ATSDR (2015).

Under 'Hazard identification – liver enzymes and liver diseases' (PFOS), the US EPA reviewed two studies (Lin et al. 2010; Gallo et al. 2012).

Conclusions and considerations

For PFOA, the US EPA reported in the 'Executive Summary' that: "*Human epidemiology data report associations between PFOA exposure and... increased liver enzymes.*" The US EPA then went on to state that: "*Associations between serum PFOA concentrations and elevations in serum levels of alanine*

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aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) were consistently observed in occupational cohorts, the high-exposure community, and the U.S. general population. The associations are not large in magnitude, but indicate the potential for PFOA to affect liver function."

The US EPA did not make any statements about studies investigating PFOS and the potential effect on the human liver in the 'Executive Summary'.

Summaries of studies reviewed

PFOA – liver function

The eight studies reviewed by the US EPA were the same as those reviewed by the ATSDR.

The US EPA provided more detail, including the size of the effect found, about the general population study by Lin et al. (2010) than the ATSDR did. The US EPA reported that: "*Lin et al. (2010) investigated the association between serum PFOA (plus three other PFASs) and liver enzymes in the adult population of the United States by analyzing data from the 1999–2000 and 2003–2004 NHANES. The study population included 2,216 adults (1076 males, 1140 females) older than 20 years who were not pregnant or nursing; had fasted more than 6 hours at the time of examination; were negative for hepatitis B or C virus; had body weight, height, educational attainment, and smoking status data available; and had serum tests for PFAS, liver function, or the five physiological measures associated with metabolic syndrome. Regression models were used to analyze the data and adjust for confounders. Mean PFOA levels were 5.05 ng/mL and 4.06 ng/mL for males and females, respectively. Serum PFOA concentration was divided into quartiles (Q1 = ≤ 2.9; Q2 = ≤ 4.2; Q3 = ≤ 5.95; Q4 = > 5.95 ng/ml). In the univariate regression models, liver enzymes, serum ALT, and logGGT increased across quartiles of PFOA (p ≤ 0.012), but total bilirubin showed no trend. The linear regression models were adjusted for (1) age, gender, and race/ethnicity; (2) age, gender, race/ethnicity, and lifestyle (smoking status, drinking status, education level), and (3) additional data for BMI, metabolic syndrome biomarkers, iron saturation status, and insulin resistance. An association was found between serum log-PFOA concentration and increasing serum ALT and log GGT. One unit increase in serum log-PFOA was associated with an increase of 1.86 units in serum ALT measurements and a 0.08-unit increase in log-GGT measurements. Effect modification was seen: For example, stronger associations between serum PFOA concentration and serum ALT (or GGT) were found among non-Hispanic Caucasians. PFOS also was positively associated with ALT in the fully adjusted model."*

Of all of the eight studies reviewed under the section 'Liver function', the US EPA made the following conclusion: "*The results of the occupational studies provide evidence of an association with increases in serum AST, ALT, and GGT, with the most consistent results seen for ALT. The associations were not large and they might depend on the covariates in the models such as BMI, use of lipidlowering medications, and triglycerides (Costa et al. 2009; Olsen et al. 2000, 2003a; Olsen and Zobel 2007; Sakr et al. 2007a, 2007b). Two population-based studies of highly exposed residents in contaminated regions near a fluorochemical industry in West Virginia have evaluated associations with liver enzymes, and the larger of the two studies reported associations of increasing serum ln ALT and ln GGT levels with increasing serum PFOA concentrations (Emmett et al. 2006; Gallo et al. 2012). A cross-sectional analysis of data from NHANES, representative of the U.S. national population, also found associations with ln PFOA concentration with increasing serum ALT and ln GGT levels. Serum bilirubin was inversely associated with serum PFOA in the occupational studies. A U-shaped exposure-response pattern for serum bilirubin was observed among the participants in the C8 Health Project, which might explain the inverse associations reported for occupational cohorts. Overall, an association of serum PFOA concentration with elevations in serum levels of ALT and GGT has been consistently observed in*

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occupational and highly exposed residential communities, and the U.S. general population. The associations are not large in magnitude, but indicate the potential of PFOA to affect liver function."

PFOA – liver disease

The US EPA reported on the one high-exposure community study by Steenland et al. (2015): "*Few studies of the relationship between PFOA and liver disease are available, but the C8 Health Project did not observe associations with hepatitis, fatty liver disease, or other types of liver disease in their initial studies. The most recent update of disease incidence in the workers identified 35 cases of nonhepatitis liver disease (with medical validation) (Steenland et al. 2015); no association was seen with cumulative exposure when analyzed without a lag (HRs by quartile 1.0, 0.58, 1.43, 1.20; trend p = 0.86 for log cumulative exposure), but there was a possible trend in the analysis using a 10-year lag (HRs by quartile 1.0, 1.46, 2.13, and 2.02; trend p = 0.40)."*

PFOS- liver disease and liver enzymes

The US EPA reported on two studies in the section 'Liver enzymes and liver disease' (Gallo et al. 2012; and Lin et al. 2010).

The US EPA provided more detail on the study by Lin et al. (2010) regarding PFOS than the ATSDR, including: "*Mean PFOS levels were 27.4 and 22.2 ng/mL for males and females, respectively. Serum PFOS concentration was divided into quartiles. Unadjusted liver enzymes, serum ALT, and log-GGT increased across quartiles of PFOS (p ≤ 0.03), but total bilirubin showed no trend. The linear regression models were adjusted for:*

- *age, gender, and race/ethnicity;*
- *lifestyle (smoking status, drinking status, education level);*
- *biomarker data (BMI, metabolic syndrome, iron saturation status, insulin resistance).*

In the fully adjusted model, a slight positive association was found between serum PFOS concentration and serum ALT (p = 0.066). A positive association was also found between serum PFOA concentration and serum ALT and PFOA concentration and serum GGT. Data interpretation was limited by the cross-sectional study design, and the fact that other environmental chemicals (possible covariates or explanatory variables) and medication use were not included in the study."

Of the study by Gallo et al. (2012), the US EPA reported that the authors: "*investigated the correlation between serum PFOS levels and liver enzymes in a total of 47,092 samples collected from members enrolled in the C8 Health Project. The association of ALT, GGT, and direct bilirubin with PFOS was assessed using linear regression models adjusted for age, physical activity, body mass index, average household income, education level, race, alcohol consumption, and cigarette smoking. Median PFOS level was 23.3 ng/mL with an interquartile range of 13.7–29.4 ng/mL. The ln-transformed values of ALT were significantly associated with ln-transformed PFOS levels (and PFOA) and showed a steady increase in fitted levels of ALT per decile of PFOS, leveling off after approximately 30 ng/mL PFOS. Fitted values of GGT showed no overall association with ln-transformed PFOS levels. A positive association was seen with direct bilirubin and PFOS levels in linear regression models, but this was not evident with logistic regression models. Limitations of the study include the cross-sectional design and self-reported lifestyle characteristics. Only a small number of ALT values were outside the normal range, making the results difficult to interpret in terms of health."*

The US EPA concluded from these two studies that: "*The epidemiological data supporting liver damage based on serum ALT and GGT as reported by Gallo et al. (2012) are not strong enough to support an association of serum PFOS alone with liver damage in humans, because in most of the epidemiology studies the serum contains a mixture of PFASs and possibly other exogenous chemicals."*

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New Jersey Drinking Water Quality Institute (DWQI, Public Review Draft 2016).

Studies reviewed

In the section 'Health effects – human studies – liver enzymes/bilirubin', the DWQI reported that the Health Effects Subcommittee evaluated 18 studies that investigated associations between PFOA and clinical biomarkers used in the diagnosis and/or evaluation of treatment of liver function or metabolic disease or liver disease including:

- ten studies of occupationally exposed populations (Costa et al. 2009; Gilliland and Mandel 1996; Olsen et al. 2000; Olsen et al. 2003a; Olsen and Zobel 2007; Olsen et al. 2012; Sakr et al. 2007a; Sakr et al. 2007b; Steenland et al. 2015);
- four studies of highly exposed communities (Darrow et al. 2016; Emmett et al. 2006; Gallo et al. 2012);
- five studies of the general population (Gilliland and Mandel 1996; Gleason et al. 2015; Lin et al. 2010; Melzer et al. 2010; Yamaguchi et al. 2013);
- one study of pregnant women (Jiang et al. 2014); and
- one study that analysed occupationally exposed and high exposure communities (Wang et al. 2012).

All of the studies except Darrow et al. (2016); Gleason et al. (2015); Melzer et al. (2010); Yamaguchi et al. (2013); and Jiang et al. (2014) were reviewed by the ATSDR (2015) and US EPA (2016a).

Considerations and conclusions

The DWQI evaluated the literature on liver enzymes/bilirubin and liver disease, and stated in the 'Executive Summary' that: "*Of the end points that were evaluated comprehensively, the evidence for associations with PFOA was strongest for increases in serum levels of ... the liver enzyme ALT.*" The DWQI also reported that: "*Other end points with limited evidence of an association include the liver enzymes GGT and AST, bilirubin, liver disease*" and that: "*There was limited or no evidence of association of PFOA with the liver enzyme ALP.*"

In the section 'Health effects – human studies – liver enzymes/bilirubin', the DWQI provided the following summary of the evaluation: "*In summary, the evaluation of epidemiologic studies provides evidence of some inconsistencies among the group of studies evaluated. However, there was consistency among the larger nonoccupational studies, as well as evidence of specificity, exposure-response, strength, and biologic plausibility for PFOA and ALT. These findings provide evidence supporting a causal relationship between PFOA and ALT. The epidemiologic evidence of an association with PFOA and GGT, AST, and bilirubin is inconsistent, while there was no evidence of an association with PFOA and ALP. There is also limited epidemiologic evidence of a causative relationship with PFOA and liver disease, and the available studies did not find an association.*"

Summaries of studies reviewed

PFOA and ALT

Regarding PFOA and liver enzyme ALT, the DWQI reported: "*Two larger cross-sectional general population studies utilizing different survey cycles of the U.S. National Health and Nutrition Examination Survey (NHANES) both found evidence of statistically significant positive associations with PFOA and the liver enzyme ALT (Gleason et al. 2015 and Lin et al. 2010). Two other low cross-sectional studies of a population with low level exposure cross-sectional studies have also evaluated this association. A study that was based on a population recruited from a larger cohort in Taiwan (n=608) found a positive statistically significant correlation (Yamaguchi et al. 2013), while a small study*

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(n=141) of pregnant women in China did not find a significant correlation between PFOA and ALT (Jiang et al. 2014). Of the three cross-sectional studies of mid-Ohio Valley residents, the smaller study (n=371) with a higher median and narrower range of PFOA exposure found no evidence of an association (Emmett et al. 2006), while the two larger studies (n=47,092) with a wider range of exposures found a consistent positive statistically significant association with ALT and PFOA (Gallo et al. 2012; and Gallo et al. 2016). Nine additional occupational studies investigated associations of ALT and PFOA with inconsistent findings. Among these studies only one cross sectional study found evidence of a positive association (Olsen et al. 2007), one found evidence of a negative association (Gilliland et al. 1996) and four cross-sectional studies found no consistent evidence of an association (Olsen et al. 2000; Olsen et al. 2003a; Sakr et al. 2007a; and Wang et al. 2012). An occupational case-control study, with cross-sectional components, found some evidence of a positive association (Costa et al. 2009); one longitudinal occupational study found evidence of a negative association (Olsen et al. 2012), and a second longitudinal occupational study found no evidence of an association (Sakr et al. 2007b). Although results of occupational studies were inconsistent, both cross-sectional general population studies found evidence of an increasing trend (Gleason et al. 2015 and Lin et al. 2010). The much larger studies of a highly-exposed community also found increasing levels of ALT with increasing serum concentrations of PFOA (Darrow et al. 2016; Gallo et al. 2012). Further, the associations noted by Gallo et al. (2012) were consistent both between water districts and among individuals within the same district, which also increased the strength of evidence. Additionally, the modeled serum PFOA exposure assessment used by Darrow et al. (2016) complements evidence from previous studies because these estimates are not affected by reverse causation."

PFOA and GGT

The DWQI reported the following about the studies they reviewed on GGT: "*Thirteen studies evaluated associations of PFOA and GGT: six studies found evidence of a positive statistically significant association (Costa et al. 2009; Gallo et al. 2012; Gleason et al. 2015; Lin et al. 2010; Olsen and Zobel, 2007; and Sakr et al. 2007a) and the remaining seven studies found no statistically significant evidence of an association (Darrow et al. 2016; Emmett et al. 2006; Gilliland et al. 1996; Olsen et al. 2000; Olsen et al. 2003a; Sakr et al. 2007b; and 72 Yamaguchi et al. 2013). Twelve studies also evaluated the association of PFOA and AST; three found evidence of a positive statistically significant ... association (Gleason et al. 2015; Sakr et al. 2007b; Yamaguchi et al. 2013); two studies found some evidence of a negative association (Gilliland et al. 1996 and Wang et al. 2012) and seven other studies found no evidence (Emmett et al. 2006; Jiang et al. 2014; Olsen et al. 2000; Olsen et al. 2003a; Olsen and Zobel 2007; Olsen et al. 2012; and Sakr et al. 2007a)."*

PFOA and ALP

The DWQI reported that: "*Eight studies evaluated the association of PFOA and the liver enzyme ALP. Only one found some limited evidence of a positive statistically significant association (Costa et al. 2009), while the other seven studies found no evidence of an association (Emmett et al. 2006; Gleason et al. 2015; Olsen et al. 2000; Olsen et al. 2003a; Olsen and Zobel, 2007; Olsen et al. 2012; and Sakr et al. 2007b)."*

PFOA and bilirubin

The DWQI reported the following about the studies they reviewed: "*Thirteen studies evaluated the association of PFOA and either total or direct bilirubin. A component of total bilirubin is direct bilirubin, a product of hemoglobin metabolism for which increased serum concentrations reflect increases in liver and bile duct disease. Therefore, total bilirubin serves only as an inferential measure of liver function. Among studies of total bilirubin, three studies found evidence of a statistically significant association*

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(Costa et al. 2009; Olsen and Zobel, 2007; and Sakr et al. 2007b); one study found a positive statistically significant association (Gleason et al. 2015); and seven found no association with total bilirubin (Emmett et al. 2006; Jiang et al. 2014; Lin et al. 2010; Olsen et al. 2000; Olsen et al. 2003a; Olsen et al. 2012; and Sakr et al. 2007a). Two additional studies found no association with direct bilirubin (Gallo et al. 2012; and Darrow et al. 2016), and Olsen et al. (2000) also found no association with total or direct bilirubin."

Liver disease

The DWQI reviewed three studies that investigated the association with PFOA and clinical liver disease (Darrow et al.2016; Melzer et al. 2010; Steenland et al. 2015). The study by Steenland et al. (2015) was reviewed by the US EPA (2016a). The other two studies were not reviewed by the ATSDR (2015) or US EPA (2016a). The DWQI provided the following summaries on these two studies: "*Melzer et al. 2011*[24] *found no statistically significant association of PFOA and current liver disease in a crosssectional study of the U.S. general population (NHANES). Also Darrow et al. (2016) found no evidence of an association with modeled serum PFOA and medically-validated liver disease when categorized as either any liver disease or restricted to enlarged liver, fatty liver, or cirrhosis among the highly exposed C8 Health Study community."*

For further information about the summary of findings on PFOA and liver enzymes see Table 6B pg 94-96 in the DWQI report available at http://www.nj.gov/dep/watersupply/pdf/pfoa-hb--mcl-public-review-draftwithappendices.pdf.

The DWQI made the comment: "*As previously described, cross-sectional studies limit interpretation of temporality. Information bias is unlikely to have an impact in the general population studies which relied on serum concentrations and clinical biomarkers. Small sample sizes in some studies may have limited their power to detect associations (Emmett et al. 2006; Jiang et al. 2014; Wang et al. 2012; and Yamaguchi et al. 2013). In addition to small sample size, some occupational studies relied on abstraction of clinical parameters from medical records. Other limitations of occupational studies include the possibility of healthy worker effect, inclusion of few or no women, and the possibility that exposure in the least exposed groups may be well above the population exposure range in occupationally exposed individuals."*

Dutch National Institute for Public Health and the Environment (RIVM, 2017)

In their report, 'PFOA exposure and health', the RIVM reviewed the literature on liver enzymes and liver disease.

Studies reviewed

The RIVM reviewed four international reviews and reports (C8 Science Panel 2012; ATSDR 2015; DWQI, 2016; US EPA, 2016a) and 15 epidemiological studies:

- Three cross-sectional studies were performed in a general population (Gleason et al. 2015; Jiang et al. 2014; Lin et al. 2010).
- Three studies were performed in high-exposure communities (all part of the C8 Health Study population) (Emmett et al. 2006; Gallo et al. 2012; Darrow et al. 2016).
- Nine occupational studies (Olsen et al. 2000; Olsen and Zobel 2007; Sakr et al. 2007a; Costa et al. 2009; Olsen et al. 2003; Sakr et al. 2007b; Olsen et al. 2012; Steenland et al. 2015; Gilliland and Mandel 1996).

[24] The DWQI references Melzer et al. 2011 in the body of the report, but the correct source is Melzer et al. 2010.

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The RIVM reviewed the same studies as the ATSDR (2015) and US EPA (2016a). They also reviewed the studies by Darrow et al. (2016), Jiang et al. (2014) and Gleason et al. (2015), which were reviewed by the DWQI, but not the ATSDR and the US EPA. The RIVM did not review the studies by Melzer et al. (2011) and Yamaguchui et al. (2013), which were reviewed by the DWQI (2016).

Considerations and conclusions

The RIVM (2017) stated in the 'Synopsis' that: "*The strength of evidence for the existence of a possible association differs between the observed effects. The clearest evidence has been found for a relationship between exposure to PFOA and ... higher concentrations of the liver enzyme ALT in blood ...*", and that "*There are indications of an association with higher blood concentrations of other liver enzymes.*"

In the 'Discussion and conclusions' section, the RIVM did not make any comments or conclusions about the 15 studies they reviewed. Instead they focussed on the findings of the international authority reports in coming to their conclusion: "*In summary, all previous reviews that evaluated liver effects concluded that PFOA is associated with small changes in blood concentrations of liver enzymes, but not with liver disease.*"

Summaries of studies reviewed

The RIVM reported on the 15 studies they reviewed under the 'Liver enzymes and liver disease' section.

As mentioned above, many of the studies reviewed by RIVM were also reviewed by the ATSDR, US EPA and DWQI, with details provided in those respective sections above. Additional information, particularly on the ranges of blood concentrations within which the associations were observed and the magnitude of the associations, both objectives of the RIVM review, has been included below.

Of the studies by Gleason et al. (2015) and Jiang et al. (2014), both cross-sectional studies performed in a general population, the RIVM reported that: "*Gleason et al. (2015) observed an association between serum PFOA and higher AST, ALT, GGT and total bilirubin (also in the NHANES study; interquartile range: 2.5-5.2 ng/mL). Jiang et al. (2014) found no association between serum PFOA and blood levels of liver enzymes (i.e. AST, ALT and total bilirubin) in a general population in China (range: 1.82-33.2 ng/mL).*"

Of the two cross-sectional studies conducted in high-exposure communities (Emmett et al. 2006; Gallo et al. 2012) and the one longitudinal study (Darrow et al. 2016), the RIVM reported the following:

Of the study by Emmet et al. (2006): "*One of those cross-sectional studies had PFOA concentrations of 0-3,000 ng/mL and reported no significant association with blood levels of liver enzymes (Emmett et al. 2006). This is, however, a study that carried less weight because it is a relatively small study (n=371).*"

Of the study by Gallo et al. (2012), the RIVM reported the results and commented on the findings: "*In the other study (Gallo et al. 2012), a median PFOA concentration of 28.0 ng/mL (interquartile range: 13.5-70.8 ng/mL) was observed among 47,092 individuals. They found an association between PFOA and ALT. In addition, Gallo et al. (2012) discuss that a study previously performed by Steenland et al. (2009) observed an association between individual serum levels of PFOA and the water district of residence. Factors related to the water district of residence may therefore have affected the examined association between PFOA and ALT. For this reason, Gallo et al. (2012) examined the association within and between water districts, thereby adjusting for those factors related to the water district.*

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Associations were found both within and between water districts, which strengthens the notion that a relationship between PFOA exposure and the blood levels of liver enzymes exists."

Of the study by Darrow et al. (2016), the RIVM reported that the authors: "*performed a longitudinal study and observed an association between modelled PFOA concentrations and higher ALT and lower direct bilirubin (a median serum PFOA concentration of 16.5 ng/mL was observed and a full range of 2.6-3,559 ng/mL). No association was found between serum PFOA and liver disease (diagnosis of liver disease was validated by healthcare providers)."*

The RIVM reported the following about the nine occupational studies they reviewed on PFOA: "*Multiple studies were performed in which occupational study populations were examined. Similar to the results taken from studies conducted among the general population and a high-exposure community, associations were found, although not consistently. Cross-sectional occupational studies observed some associations with the blood levels of liver enzymes (although inconsistent, i.e. sometimes only in a certain factory or certain year of examination) (Olsen et al. 2000; Olsen and Zobel, 2007). Those studies examined individuals with serum PFOA concentrations ranging between 0.1-81,300 ng/mL (Olsen et al. 2000), 7-92,030 ng/mL (Olsen and Zobel, 2007), 5-9,550 ng/mL (Sakr et al. 2007a) and 200-47,040 ng/mL (Costa et al. 2009). One occupational study conducted among workers with 40-12,700 ng/mL of serum PFOA concentration found no association between PFOA and the blood levels of liver enzymes (Olsen et al. 2003). Gilliland and Mandel (1996) observed no association between serum fluorine (examined as a proxy for PFOA) and changes in GGT. Three longitudinal studies were performed. One longitudinal study found some association (serum concentration range: 0-2,266 ng/mL) (Sakr et al. 2007b). Another longitudinal study conducted among an occupational population with serum PFOA concentrations ranging from 0.1-10,000 ng/mL found no association with blood levels of liver enzymes (Olsen et al. 2012). No association of estimated cumulative PFOA concentrations with nonhepatitis liver disease was found in a longitudinal study among an occupational population with a median PFOA concentration of 113 ng/mL (full range not reported) (Steenland et al. 2015)."*

6.3.3. Systematic reviews

Priestly, 2016

Priestly (2016) reviewed studies on liver biomarkers published between 2012 and 2014 in the 'Miscellaneous end point' section.

Studies reviewed

Priestly reviewed three studies (Gallo et al. 2012; Yamaguchi et al. 2013; Jiang et al. 2014).

Only Priestly and the DWQI reviewed the study by Yamaguchi et al. (2013).

Considerations and conclusions

Priestly did not make any specific conclusions about these three studiess.

Summaries of studies reviewed

The studies by Gallo et al. (2012) and Jiang et al. (2014) are reported previously in this section. However, the DWQI (2016) and RIVM (2017) only reported on the findings for PFOA in the study by Jiang et al. (2014). Additional information about the findings on other PFAS was noted by Priestly and this has been included below. As Priestly provided different information to the DWQI on the study by Yamaguchi et al. (2013), this information is also included below.

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Of the studies by Yamaguchi et al. (2013) and Jiang et al. (2014), Priestly reported that: "*Yamaguchi et al. (2013) also found associations between increase liver biomarkers (SGOT, SGPT, but not γGGT with serum PFOS and PFOA levels associated with fish consumption patterns (median PFOS 5.8 ng/mL; PFOA 2.1 ng/mL), as well as an association of PFOA/S with increasing serum levels of ω-3 unsaturated fatty acids. In contrast, there were no PFAS effects on AST, ALT or total bilirubin among Chinese pregnant women, although there were inconsistent small changes in some blood cell counts and serum albumin associated with some PFAS isomers, but not others (Jiang et al. 2014).*"

Kirk et al. 2018

Kirk et al. evaluated the human evidence on the effect of PFAS on liver function and liver disease, reporting on 10 health outcomes, including Gilbert syndrome and lobular inflammation of the liver.

Studies reviewed

Kirk et al. (2018) evaluated seven papers that investigated PFAS exposure on liver function in humans (Darrow et al. 2016; Costa et al. 2009; Gallo et al. 2012; Rantakokko et al. 2015; Emmett et al. 2006; Lundin et al. 2009; Fan et al. 2014). These included:

- five studies on liver enzymes (Darrow et al. 2016; Costa et al. 2009; Gallo et al. 2012; Rantakokko et al. 2015; Emmett et al. 2006);
- three studies on liver disease end points (Emmett et al. 2006; Darrow et al. 2016; Lundin et al. 2009);
- one study on Gilbert syndrome in adults (Fan et al. 2014);
- one study on liver histology (Rantakokko et al. 2015).

Considerations and conclusions

Kirk et al. made the following comment regarding all of the studies they reviewed: "*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. [2015] 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.*"

- The table below has been reproduced from Kirk et al. (pg. 83) and provides Kirk et al.'s evaluation of the reported association between PFAS and 10 liver function outcomes, from their review of the literature.

Associations at a glance (Liver function outcomes)

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

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Health outcome	PFAS exposure	Evaluation of 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

Source: Kirk et al. 2018, pp. 83.

Summaries of studies reviewed

Liver enzymes

The studies by Darrow et al. (2016), Costa et al. (2009), Emmett et al. (2006) and Gallo et al. (2012) were reviewed by the ATSDR (2015), US EPA (2016), DWQI (2016) and RIVM (2017), with summaries provided above.

Of the study by Rantakokko et al. (2015), Kirk et al. reported that: "*In contrast to the results presented by Darrow et al. [2016], Costa et al. [2009] and Gallo et al. [2012], Rantakokko et al. [2015] 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.*"

Liver disease end points

Kirk et al. reported, of the three studies they reviewed: "*All papers investigating liver disease end points reported that there was no evidence that exposure to PFOA increases the risk of clinically diagnosed liver disease. [Emmettt et al. 2006; Darrow et al. 2016; Lundin et al. 2009].*"

Gilbert syndrome

Kirk et al. reviewed one study by Fan et al. (2014) and reported the following about the study: "*Fan et al. [2014] 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. [2014] reported non-significant associations for eight PFAS, including PFOA, PFOS, PFHxS, PFNA, PFUdA, PFDoA, PFPeA and PFHpA.*"

Liver histology

Of the study by Rantakokko et al. (2015), Kirk et al. reported *that: "The effect of PFOA exposure on liver histology was further assessed by Rantakokko et al. [2015]. 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.*"

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6.3.4. Summary of key national and international reports and systematic reviews

Recent key national and international reports:

- ATSDR concluded there is some evidence that PFOA and PFOS exposure may cause liver damage; however, the magnitude of the changes in serum liver enzymes in human studies is small and not likely biologically relevant. ATSDR report studies have not found increases in deaths from liver cirrhosis or increases in the occurrence of liver disorders or cirrhosis in occupationally exposed cohorts.
- The US EPA reports that associations exist between serum PFOA concentrations and increased serum levels of ALT and GGT in occupational cohorts, high-exposure communities and in the general population; while the associations are not large, they indicate a potential for PFOA to affect liver function. For PFOS, the US EPA consider the data are not strong enough to support an association between PFOS and liver damage in humans.
- DWQI concluded the evidence for an association with PFOA was strongest for increased liver enzyme ALT, with the evidence supporting a causal relationship between PFOA and ALT. Available studies did not find an association between PFOA and liver disease. They noted the issue of cross-sectional studies and temporality of exposure, and for occupational studies, the healthy worker effect and few or no women included in the studies.
- RIVM concluded that the clearest evidence is for a relationship between PFOA exposure and higher concentrations of the liver enzyme ALT; all previous reviews that evaluated liver effects have concluded PFOA is associated with small changes in blood concentrations of liver enzymes, but not with liver disease.

Systematic reviews:

- Priestly reviewed three studies but did not make a specific overall conclusion.
- Kirk et al. concluded while there are several reported positive associations between exposure to PFOA and markers of liver function, results are inconsistent and evidence is 'inadequate'; all of the studies investigating liver disease end points showed no evidence that PFOA exposure increases the risk of clinically diagnosed liver disease; and as most of the studies are cross-sectional, the temporality and causality of the exposure is unknown.

6.3.5. Expert Health Panel synthesis to support advice to the Minister

- An association of PFAS with elevated levels of the liver enzyme ALT was observed in many studies. This was generally of small magnitude, is not considered biologically significant and no link to clinically important liver disease was noted. Evidence to date does not establish whether or not PFAS causes a high ALT, and it is possible this reflects confounding by other factors.
- The scientific evidence does not support an association between PFAS and specific liver conditions, such as hepatitis, cirrhosis or fatty liver.
- The liver is a target organ for PFAS toxicity in high dose animal toxicity studies, where hepatic steatosis (fatty liver) is observed. It is also a key organ for metabolic regulation relevant to PPAR and other nuclear receptors. It is unclear if these are activated at concentrations relevant to Australian exposures.
- Liver issues were a concern of a significant number of those exposed to PFAS who responded in the public consultation.

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6.3.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and liver function and liver disease:

- There are small but inconsistent assoications of LFTS and PFAS in some studies. Current standard medical tests for liver damage and function in Australians frequently show minor abnormalities such as those associated with PFAS. These can be due to underlying disease (e.g. chronic hepatitis, alcoholic liver disease, viral diseases), medications, herbal supplements and obesity, or just be a transient and reversible abnormality.
- No routine medical monitoring of liver function for residents or others exposed to PFAS is required on the basis of current evidence.

To further investigate the association between PFAS exposure and liver function in an Australian setting, the Panel suggests the following research priorities:

- Studies that provide causal evidence are the key research need. Further cross-sectional studies are unlikely to provide useful information. Well-designed longitudinal studies which take into account confounders (chronic hepatitis, alcoholic liver disease, viral diseases, medications, herbal supplements and obesity) may provide stronger epidemiological evidence to indicate whether long-term alteration of metabolism occurs and increases the risk of clinically important liver disease (e.g. hepatic steatosis and subsequent fibrosis). Relevant studies would also explore measurement of activation of biochemical mechanisms that disturb liver metabolism, especially those pathways relevant to lipids and cholesterol.

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6.4. Kidney function effects and PFAS exposure

There is a growing body of evidence on PFAS exposure and kidney function, particularly uric acid levels, with several recent reports from international authorities and systematic reviews having undertaken analyses of the epidemiological literature on PFAS exposure, uric acid and kidney disease. Uric acid is a product of purine metabolism eliminated by the kidney, and elevated levels are a marker of kidney disease. Elevated uric acid is also the cause of gout, may also directly cause kidney disease (uric acid nephropathy) and is also associated with cardiovascular disease, diabetes, hypertension and a range of other diseases.

Most PFAS are eliminated by the kidney, possibly influenced by similar mechanisms to those altering uric acid elimination (filtration and then reabsorption of weak acids in the kidney tubules). Thus, reverse causation or confounding for any associations between PFAS and kidney disease/uric acid is difficult to exclude, especially in short term or cross-sectional studies.

6.4.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of five published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and four systematic reviews since 2013 that analysed the human epidemiological evidence regarding exposure to PFAS and kidney function, uric acid and kidney disease.

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR, 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **New Jersey Drinking Water Quality Institute (DWQI, Public Review draft 2016).** Health-based maximum contaminant level support document: Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA, 2016a).** Health effects support document for Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA, 2016b)**. Health effects support document for Perfluorooctane Sulphonate (PFOS);
- **Dutch National Institute for Public Health and the Environment (RIVM, 2017).** PFOA exposure and health: A review of scientific literature.

Systematic reviews and reviews

- **Saikat et al. (2013).** The impact of PFOS on health in the general population: a review;
- **Priestly (2016).** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS), Monash University;
- **Rappazzo et al. (2017).** Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature. Int J Environ Res Public Health 14:691;
- **Kirk et al. (2018).** The PFAS Health Study: systematic literature review. Australian National University.

While the Panel acknowledges that FSANZ (2017) did comment on PFOS and human data on kidney function, FSANZ did not review kidney epidemiological studies in detail. Instead, they reported on conclusions by other authoritative groups, notably the ATSDR (2015) and US EPA. Under PFOA, FSANZ reported that: "*Findings on kidney disease are conflicting (based on data presented on human epidemiology data detailed in Bull et al. 2014) and might reflect reverse causation (i.e. declining kidney*

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function may result in increased PFOA levels)." FSANZ also noted the ATSDR conclusion about PFOA and uric acid levels. No other systematic reviews or key national and international reports covered kidney function and uric acid.

6.4.2. Key national and international reports

Agency for Toxic Substances and Disease Registry (ATSDR, 2015)

The ATSDR (2015) reviewed studies on exposure to perfluoroalkyl substances and uric acid, mortality from kidney disease and biomarkers of renal function.

Studies reviewed

The ATSDR reviewed twelve studies in total, including:

- five studies on uric acid levels and hyperuricemia (Costa et al. 2009; Sakr et al. 2007b; Steenland et al. 2010; Geiger et al. 2013; Shankar et al. 2011b);
- seven studies on chronic kidney disease (Steenland and Woskie 2012; Olsen et al. 1998; Olsen et al. 2003; Sakr et al. 2007b; Costa et al. 2009; Mundt et al. 2007; Shankar et al. 2011a);
- Two studies on biomarkers of kidney disease (Emmett et al. 2006; Watkins et al. 2013).

Considerations and conclusions

In the 'Relevance to public health' section, the ATSDR stated that: "*Epidemiology studies have found statistically significant associations between serum perfluoroalkyl levels (particularly PFOA and PFOS) and a wide range of health effects. When the subjects were categorized by serum perfluoroalkyl levels, dose-response relationships were found for most of the effects. However, findings were not always consistent across studies. However, consistent findings were found for association of serum PFOA and PFOS with increases in .... uric acid levels.*"

Also within this section, the ATSDR stated that: "*Five studies have examined the possible association between serum PFOA and/or PFOS levels and uric acid levels. Based on epidemiology data, an elevated uric acid level appears to be a risk factor for hypertension and possibly renal disease. Significant associations between serum PFOA and uric acid levels were found in PFOA workers, residents highly exposed to PFOA, and adults and adolescents exposed to background levels. Increased risks of hyperuricemia were also associated with higher serum PFOA and PFOS levels in the highly exposed residents and the general population.*"

Summaries of studies reviewed

Uric acid levels and hyperuricemia

Further on in the 'Relevance to public health' section, the ATSDR provided its weight of evidence conclusion and summarised the five studies it reviewed on uric acid levels. The ATSDR stated that: "*Based on the weight of evidence, there is support for identifying several health effects in humans that appear to be related to perfluoroalkyl exposure: increases in serum lipid levels.... The association between serum perfluoroalkyl levels and serum uric acid levels has not been as well investigated as serum lipids. However, the five studies examining this end point have all reported statistically significant findings. Significant associations of serum uric acid levels with serum PFOA levels were found in workers (Costa et al. 2009; Sakr et al. 2007b) and with serum PFOA and PFOS in highly exposed residents (Steenland et al. 2010b) and the general population (Geiger et al. 2013; Shankar et al. 2011b). The study of highly exposed residents also found significant increases in the risk of hyperuricemia (>6.0 mg/dL for women and >6.8 mg/dL for men) in subjects with serum PFOA levels of*

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11.5–20.6 ng/mL and higher or serum PFOS levels of 17.5–23.2 ng/mL and higher (Steenland et al. 2010b). In the general population study, which utilized the NHANES data set, an increased risk of hyperuricemia was observed at serum PFOA levels of 3.5–5.1 ng/mL and higher or serum PFOS levels of 11.2–17.8 ng/mL and higher (Shankar et al. 2011b). It should be noted that serum PFOA or PFOS levels accounted for <1% of the variance in serum uric acid levels (Steenland et al, 2010b)."

Chronic kidney disease

The ATSDR reviewed seven studies on chronic kidney disease, and provided the following summaries: "*In a cohort mortality study of 5801 workers at the DuPont PFOA facility in West Virginia, Steenland and Woskie (2012) found an increase in deaths from chronic renal disease (SMR 3.11, 95% CI 1.66–5.32) when compared to DuPont workers at other regional facilities. When cumulative PFOA exposure was estimated based on the worker's job history and data from a biomonitoring survey conducted from 1979 to 2004, there was a significant positive trend for nonmalignant kidney disease when the workers were divided in estimated cumulative exposure quartiles. Kidney function, assessed by levels of blood urea nitrogen (BUN) and serum creatinine, was not associated with exposure to PFOS and/or PFOA in the occupational exposure studies by Olsen et al. (1998a, 2003a), Sakr et al. (2007b), or Costa et al. (2009) or with exposure to PFNA in the study conducted by Mundt et al. (2007)."*

Of the paper by Shankar et al. (2011a), the ATSDR reported that the authors: "*found a negative association between serum PFOA or PFOS levels and estimated glomerular filtration rate in adults. The likelihood of chronic kidney disease, defined as a glomerular filtration rate of <60 mL/minute/1.73 m2, was significantly higher in adults with the highest serum PFOA (>5.9 ng/mL, OR 1.73, 95% CI 1.04–2.88) or PFOS (>29.5 ng/mL, OR 1.82, 95% CI 1.01–3.27) levels than in adults with serum PFOA or PFOS levels in the lowest quartile after adjustment for age, sex, race/ethnicity, educational level, smoking status, alcohol consumption, BMI, blood pressure, diabetes, serum total cholesterol level, and glycohemoglobin level. The study also investigated whether the association between serum PFOA and PFOS levels and chronic kidney disease was due to reverse causality (i.e. decreased glomerular filtration leads to a decrease in perfluoroalkyl filtration)."*

Biomarkers of kidney disease

For the two studies on biomarkers of kidney disease, the ATSDR reported the following summaries: "*Emmett et al. (2006a) and Watkins et al. (2013) examined biomarkers of renal function in the community living near the DuPont West Virginia facility with high levels of PFOA in the water supply. Emmett et al. (2006a) did not find significant associations between BUN or serum creatinine levels and serum PFOA levels. An examination of 9,660 children aged 1–18 years found significant negative associations between serum PFOA, PFOS, PFNA, and PFHxS levels and estimated glomerular filtration rates (Watkins et al. 2013). Unlike Shankar et al. (2011a), Watkins et al. (2013) suggested that the association between serum perfluoroalkyl levels and estimated glomerular filtration rates may be a consequence of reverse causation because no significant associations were found between estimated serum PFOA levels 3 or 10 years prior to enrolment in the study or at the time of study enrolment and estimated glomerular filtration rates; predicted serum PFOA levels were based on environmental PFOA levels, self-reported residential history, and physiologically based pharmacokinetic (PBPK) modelling."*

New Jersey Drinking Water Quality Institute (DWQI, 2016)

In 2016 the DWQI released its Public Review draft 'Health-based maximum contaminant level support document: Perfluorooctanoic Acid (PFOA)'.

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Studies reviewed

The DWQI reviewed eight studies in total, including:

- seven studies on uric acid and serum PFOA concentrations (Costa et al. 2009; Sakr et al. 2007b; Steenland et al. 2010; Geiger et al. 2013; Shankar et al. 2011b; Gleason et al. 2015; Lin et al. 2013); and
- one study on kidney disease (Steenland and Woskie (2012).

Six studies were the same studies reviewed by the ATSDR (Costa et al. 2009; Sakr et al. 2007; Steenland et al. 2010; Geiger et al. 2013; and Shankar et al. 2011b; Steenland and Woskie 2012).

Considerations and conclusions

In the 'Executive Summary', the DWQI stated: "*Of the end points that were evaluated comprehensively, the evidence for associations with PFOA was strongest for increases in serum levels of ...uric acid.*"

At the end of the section 'Comprehensively reviewed end points: uric acid', the DWQI concluded that: "*Epidemiologic evidence provides evidence of consistency among findings, strength of findings with clinically defined outcomes, and exposure-response with PFOA and uric acid. These findings provide evidence supporting a causal relationship between PFOA and uric acid. However, there are limitations in use of the epidemiologic evidence to draw conclusions regarding temporality and there remain some questions of biologic plausibility due to possible reverse causality explanation.*"

Summaries of studies reviewed

Uric acid levels and hyperuricemia

Of the seven studies the DWQI reviewed, they stated all of them: "*found strong, positively statistically significant associations of uric acid and PFOA (Costa et al. 2009; Geiger et al. 2013; Gleason et al. 2015; Sakr et al. 2007b; Shankar et al. 2011b; and Steenland et al. 2010) with the exception of Lin et al. 2013 which did not find a statistically significant association. Additionally, all three studies which evaluated clinically defined hyperuricemia found strong evidence of a positive statistically significant association (Geiger et al. 2013; Shankar et al. 2011b; and Steenland et al. 2010).*"

The DWQI commented about the wide range of PFOA exposure, age range and serum PFOA concentrations in the studies reviewed, the dose-response observed in the studies and bias of the studies. They noted that: "*Although the six studies with evidence of statistically significant association are mainly crosssectional, they represent the general population, residents from a highly exposed community, and an occupationally exposed population. These studies therefore evaluated a wide range of serum PFOA concentrations – about 4 ng/ml in the general population studies (Geiger et al. 2013; 77 Gleason et al. 2015; Shankar et al. 2011b), a median of about 28 ng/ml in a highly exposed community population (Steenland et al. 2010b), and a median serum PFOA concentration range from 428 ng/ml (Sakr et al. 2007b) to 4,400 -5,700 ng/ml (Costa et al. 2009) among occupationally exposure populations. Also, importantly, these studies evaluated a wide range of age groups as well, including children less than 19 years of age (Geiger et al. 2013), adolescents and adults greater than 11 years of age (Gleason et al. 2015), and adult populations 20 years or older (Costa et al. 2009, Shankar et al. 2011b; and Steenland et al. 2010b).*"

Regarding the mode of action for PFOA and increased uric acid concentrations observed in blood, the DWQI stated that: "*Reverse causality is a potential explanation for increased uric acid with increasing PFOA. It has been proposed that PFOA could be higher in individuals with reduced excretion due to reduced kidney function, and that this would also result in increased uric acid (Kataria et al.*

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2015). Also, Kataria et al. (2015) reviewed toxicology evidence and suggests that PFOA and other PFCs can adversely impact renal function. Unfortunately, a hypothesis of reverse causality cannot be assessed because the six studies which evaluated uric acid and PFOA are limited by their cross sectional design in which exposures and outcomes are measured at the same point in time."

Kidney disease

For kidney disease, the DWQI reviewed the study by Steenland and Woskie (2012) on occupationally exposed workers that was reviewed by the ATSDR (2015). The DWQI made the following observation about this study: "*An important potential confounder in this occupational cohort could be tetrafluoroethylene (TFE) which was also used in the manufacture of fluoropolymers at the Parkersburg, WV facility and has been identified as a rodent kidney carcinogen (NTP, 1997). However, the authors believe that appreciable exposures would have been unlikely, since TFE exposure would have been well controlled due to its explosive and volatile nature."*

United States Environmental Protection Agency (US EPA, 2016)

Also in 2016, the US EPA (2016) released the 'Health effects support documents for PFOA and PFOS', which the US EPA used to establish health advisories for drinking water officials.

Studies reviewed

For PFOA, the US EPA reviewed seven studies in total, including:

- three studies on uric acid: one occupational exposure study (Costa et al. 2009); one study of high community exposure (Steenland et al. 2010); and one general population study (Shankar et al. 2011a);
- two studies on glomerular filtration rate (GFR): the US EPA reviewed one high-exposure community study (Watkins et al. 2013); and one general population study (Shankar et al. 2011a);
- three occupational exposure studies on kidney disease (Steenland et al. 2015; Steenland and Woskie 2012; Raleigh et al. 2014).

For PFOS and kidney function, the US EPA (2016b) reviewed four studies: (Shankar et al. (2011a); Watkins et al. (2013); Steenland et al. (2010); and Geiger et al. (2014b)

Considerations and conclusions

Uric acid levels and hyperuricemia

The US EPA concluded for the studies on PFOA, uric acid and GFR that:"*Overall, studies of occupational cohorts (Costa et al. 2009), a highly exposed community (Steenland et al. 2010; Watkins et al. 2013), and the U.S. general population (Shankar et al. 2011a) that evaluated uric acid levels or eGFR as a measure of kidney function found associations with decreased function, although reverse causality as an explanation cannot be ruled out. Since the URAT transporter functions in the renal resorption of PFOA, the increase in serum uric acid could be a reflection of systemic transport pharmacodynamics rather than formation biochemistry."*

Under the 'Hazard characterization/synthesis and evaluation of major noncancer effects: kidney and other organ effects' section, the US EPA commented that: "*Overall, studies of occupational cohorts (Costa et al. 2009), a highly exposed community (Steenland et al. 2010; Watkins et al. 2013), and the U.S. general population (Shankar et al. 2011a) that evaluated uric acid levels or eGFR as measure of kidney function found associations with decreased function. Reverse causality as an explanation cannot*

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be ruled out in studies using serum PFOA as a biomarker of exposure, as a low GFR would diminish the removal of PFOA from serum for excretion by the kidney."

Associations between PFOA and chronic kidney disease

For PFOA and kidney disease, the US EPA reviewed three occupational exposure studies (Steenland et al. 2015; Steenland and Woskie 2012; Raleigh et al. 2014). These were different studies to those reviewed by the ATSDR and DWQI; the only study reviewed by all three international reports was the study by Steenland and Woskie 2012. The US EPA reported on the three studies they reviewed: "*The occupational mortality studies have produced generally negative results with respect to the association between PFOA and mortality due to chronic kidney disease (Steenland et al. 2015; Steenland and Woskie 2012; Raleigh et al. 2014). The most recent update of incidence of chronic kidney disease in the workers in the C8 West Virginia community identified 43 cases (with medical validation) (Steenland et al. 2015); no association was seen with cumulative exposure when analyzed without a lag (HRs by quartile...), or using a 10-year lag (HRs by quartile...).*"

PFOS and kidney function

In the 'Summary and conclusions from the human epidemiology studies' section, the US EPA noted that: "*Shankar et al. (2011a) and Watkins et al. (2013) analyzed sub-sets or the entire population for an association between PFOS serum levels and either kidney disease or biomarkers that may be associated with kidney function. Shankar et al. (2011a) used NHANES data and showed a positive association between increasing levels of PFOS and chronic kidney disease....and while the possibility of reverse causality could not be excluded, the association between PFOS and eGFR when examined in those with and without chronic kidney disease supports an effect. Watkins et al. (2013) evaluated C8 Health Project children to look at PFOS levels and kidney function in children, as defined as decreased eGFR, and found a dose-related trend...Geiger et al. (2014b) found no association in children between serum PFOS levels and hypertension. Steenland et al. (2010) evaluated C8 Health Project adults and found a positive association between PFOS serum levels and an increase in uric acid...*"

The US EPA reached the following conclusion: "*Overall, studies do suggest an association between chronic kidney disease, as defined by estimated glomerular filtration rate; however, reverse causality cannot be excluded.*"

Dutch National Institute for Public Health and the Environment (RIVM, 2017)

The RIVM reviewed international reports and epidemiological studies that had reported on PFOA exposure and uric acid.

Studies reviewed

RIVM reported the conclusions of the ATSDR (2015), DWQI (2016) and US EPA (2016) and additionally reviewed eight epidemiological studies on uric acid and hyperuricemia (Costa et al. 2009; Geiger et al. 2013; Gleason et al. 2015; Qin et al. 2016; Sakr et al. 2007b; Shankar et al. 2011b; Steenland et al. 2010; Lin et al. 2013a).

Considerations and conclusions

RIVM concluded in their 'Synopsis' that: "*The strength of evidence for the existence of a possible association differs between the observed effects. There are indications of an association with higher blood concentrations of ...uric acid.*"

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Summaries of studies reviewed

RIVM reviewed and reported the conclusions of the ATSDR (2015), DWQI (2016) and US EPA (2016) and additionally reviewed eight epidemiological studies on uric acid and hyperuricemia (Costa et al. 2009; Geiger et al. 2013; Gleason et al. 2015; Qin et al. 2016; Sakr et al. 2007b; Shankar et al. 2011b; Steenland et al. 2010; Lin et al. 2013a).

The findings of these studies, except Qin et al. (2016) have been reviewed in other international reports and mentioned previously in this section. RIVM reported from their review of this study that: "*Qin et al. (2016) examined Taiwanese children aged 12-15 years and observed an association between higher serum PFOA concentration and higher uric acid concentrations in boys only (median serum PFOA concentration: 0.5 ng/ML; 75th percentile: >1.3 ng/mL).*"

Under the 'Discussion and conclusions' section, the RIVM commented on the conclusions of the ATSDR (2015), DWQI (2016) and US EPA (2016a). "*Discussed in the reports is the fact that an association with uric acid has been less frequently examined that was done for, say, blood lipids (ATSDR), that the relationship may be confounded by the presence of kidney disease (US EPA 2016a; DWQI 2016) and that a steep dose-response curve has been observed in the general population, with a flattened slope at higher PFOA concentrations (DWQI 2016).*" Regarding the epidemiological studies the RIVM reviewed, the authors concluded "*The epidemiological studies in which associations were observed were conducted in the general population, high-exposure communities and occupational populations. Differences in uric acid concentrations in blood between the first category (the reference category) and higher categories of PFOA concentrations in blood were in the order of 0.1-0.3 mg/dL. The evidence that PFOA is associated with hyperuricemia is limited. An association with hyperuricemia has been observed in two studies conducted among the general population (relative risks (RR) were 1.62 in the 4th quartile of PFOA concentrations in the blood of adolescents (Geiger et al. 2013) and 1.90 and 1.97 in the 3rd and 4th quartiles in adults (Shankar et al. 2011b)) and one in the high exposed community from the C8 health Project (RR were 1.33 to 1.47 in quintiles 2-5) (Steenland et al. 2010). Although associations were observed, they may be confounded by individual differences in kidney clearance.*"

6.4.3. Systematic reviews

Saikat et al. (2013)

In their literature review of the impact of PFOS on health in the general population, Saikat et al. (2013) reviewed the study by Steenland et al. (2010)[25]. The authors did not provide any comment on the study findings. Their overall conclusion for all of the studies on the general population they reviewed was that: "*the current evidence is inconclusive and further large-scale prospective cohort studies would be useful to assess the association between environmental exposure to PFOS, appropriate biomarkers (e.g. serum levels of PFOS) and health outcomes.*"

Priestly (2016)

Priestly reviewed the epidemiological evidence on PFAS exposure and kidney function under the section 'Altered serum uric acid'.

[25] Saikat et al. report in their Reference section this study was published in 2009; however, it is the study published in 2010 that is referred to throughout this section.

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Studies reviewed

Priestly reviewed five studies (Steenland et al. 2010; Sakr et al, 2007b; Costa et al, 2009; Shankar et al, 2011b; Geiger et al, 2013) under the section 'Altered serum uric acid'. These studies were also reviewed by the ATSDR, US EPA, DWQI, RIVM and Kirk et al.

Priestly also reviewed three papers on kidney function (Shankar et al.2011a; Watkins et al. 2013; Kataria et al. 2015) under 'Miscellaneous end points' section.

Considerations and conclusions

Priestly (2016) stated in the 'Executive Summary' that: "*The epidemiological studies are suggesting, but not yet proving, a possible link between PFOS/PFOA and uric acid disorders [and several other health effects].*"

Summaries of studies reviewed

Of the five studies Priestly reviewed under the section 'Altered serum uric acid', Priestly provided the following summaries:

"*Steenland et al. (2010) analysed PFOA, PFOS and uric acid levels in 54,951 residents in US communities in the mid-Ohio Valley with water contamination from nearby fluorochemical plant (C8 Health Project). Both PFOA and PFOS were correlated with increased serum uric acid, with increases ranging from 0.2 – 0.3 mg/dL from the lowest to the highest decile of PFOS/PFOA blood levels. PFOA effects on uricemia tended to be stronger than PFOS. The mean PFOA in this cohort was 86.4 ng/ml, which is somewhat higher than normal community levels; on the other hand, PFOS blood levels of 23.4 ng/mL were comparable with community norms. The authors cautioned about drawing causative conclusions pending further studies.*

In occupational studies, where PFOS and PFOA blood levels were orders of magnitude higher, a connection with uricaemia was not so clear (Sakr et al. 2007b, Costa et al. 2009).

Conversely, interrogation of the NHANES database, where PFOS and PFOA are at 'background' blood levels, showed a positive association between both PFOA and PFOS with hyperuricemia in both adults (Shankar et al. 2011b) and children (Geiger et al. 2013). In adults (n= 3883 subject from the 1999-2000 and 20032006 NHANES database), the odds ratio for hyperuricemia (serum uric acid >6.8 mg/dL in men and >6.0 mg/dL in women) between the 4th and 1st quartiles was 1.97 (95% CI 1.44 – 2.7) for PFOA and 1.48 (95% CI 0.99 – 2.22), with the multivariate-adjusted increase in serum uric acid in the range 0.14 – 0.44 (PFOA) and 0.18 – 0.27 mg/dL for PFOS. In the same four quartiles in children, the odds ratio for hyperuricemia (>6.0mg/dL) were 1.62 (95% CI 1.1 -2.37) for PFOA and 1.65 (1.1 – 2.49) for PFOS, with the multivariate-adjusted increase in serum uric acid in the range 0.02 – 0.3 (PFOA) and 0.03 – 0.12 mg/dL for PFOS."

Priestly's 'Comment' on these studies was: "*As with some other end points, the discrepancy between findings in populations with essentially 'background' exposures and those with elevated blood levels associated with occupational exposures or pollution sources (C8 Project) are difficult to explain. The main point of consistency was that the effect of PFOA was more marked than PFOS.*"

In reviewing the three papers on kidney function under the 'Miscellaneous end points' section, Priestly provided additional information on the studies by Shankar et al. (2011), Watkins et al. (2013), Kataria et al. (2015).

Of the studies by Shankar et al. (2011a) and Watkins et al. (2013), Priestly reported the following: "*Shankar et al. (2011a) analysed the NHANES dataset for evidence of a link between PFAS and chronic*

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kidney disease, defined as a glomerular filtration rate below 60 mL/min/1.73m2, while Watkins et al. (2013) estimated GFR in a group of children from the PFOA-contaminated water C8 Health Project. The results were similar. The NHANES data on 'normal' exposures suggested an association between PFOS and PFOA and chronic kidney disease. Multivariate odds ratios between the lowest and highest quartiles were: PFOA 1.73 95% CI 1.04-2.88; P for trend 0.015; PFOS 1.82 CI 1.01-3.27, P for trend 0.019. The C8 Health Project data also showed a decrease in eGFR of 0.75 mL/min/1.73m2 for each interquartile increase in PFOA, with similar, but lower findings for PFOS, PFNA and PFHxS. The effect disappeared when compared to PFOA at birth, aged 10 or PFOA predicted from toxicokinetic methods at the time of enrolment in the study. The authors noted that the results could be confounded by reverse causality, in that reduced kidney function could have been responsible for elevating serum levels of the PFAS."

Of the study by Kataria et al. (2015), Priestly noted that: "*In a cross-sectional study of the NHANES database (n=1960; 2003-2010) of adolescents aged 12-19 years, compared to the lowest quartile serum levels, subjects in the highest quartile of had a lower GFR (mL/min/1.73m2) and increased serum uric acid (mg/dL) (Kataria et al. 2015).*

- *PFOA by 6.84 (95% CI 2.19, 11.48); 0.21 mg/dL uric acid (0.056, 0.37)*
- *PFOS by 9.69 (95% CI -4.59, 14.78); 0.19 mg/dL uric acid (0.032, 0.34)."*

Priestly then commented that: "*As noted above, a 'reverse causation' whereby elevated serum PFAS is caused by the reduced GFR cannot be ruled out.*"

Priestly's overall 'Comment' on the 'Miscellaneous End Points' studies was: "*With only one or two studies addressing each end point, and inconsistent findings, it is too early to draw definitive conclusions on whether PFAS have a role in any of the diseases discussed in this section. Many of the studies point out the difficulty of discerning between PFAS causation, or reverse causation, where the condition under study results in a tendency to accumulate higher plasma levels.*"

Rappazzo et al. (2017)

Studies reviewed

In their systematic review of the epidemiologic literature on exposure to perfluorinated alkyl substances and health outcomes in children, Rappazzo et al. reviewed four studies for their section on 'Renal function' (Watkins et al. 2013; Kataria et al. 2015; Qin et al. 2016; Lin et al. 2013b). All these studies were covered in previous reviews.

Considerations and conclusions

Rappazzo et al. (2017) concluded in the 'Abstract' that: "*While there are a limited number of studies for any one particular health outcome, there is evidence for positive associations between PFAS and .... renal function.*"

At the end of the section on 'Renal function', Rappazzo et al. stated: "*While all studies of PFAS and kidney function in children to date have been cross-sectional, results from these studies provide evidence for interesting potential associations between PFAS and renal function in children using, multiple different markers of kidney function.*"

Kirk et al. (2018)

Kirk et al. reviewed the human evidence on PFAS and kidney function reporting on glomerular filtration rate, hyperuricemia, chronic kidney disease, blood urea nitrogen level and creatinine level.

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Studies reviewed

Kirk et al. reviewed 12 papers that investigated the relationship between PFAS exposure and kidney function (Costa et al. 2009; Dhingra et al. 2016; Emmett et al. 2006; Geiger et al. 2013; Kataria et al. 2015; Qin et al. 2016; Rotander et al. 2015; Shankar et al. 2011a; Shankar et al. 2011b; Steenland et al. 2010; Steenland and Woskie, 2012; Watkins et al. 2013). These included:

- two studies on reduced kidney function and glomerular filtration rate in children (Watkins et al. 2013; Kataria et al. 2015);
- seven studies that investigated the association between PFAS exposure and uric acid levels (Costa et al. 2009; Kataria et al. 2015; Steenland et al. 2010; Rotander et al. 2015; Shankar et al. 2011b; Geiger et al. 2013; Qin et al. 2016);
- three studies that investigated the association between PFAS exposure and chronic kidney disease in adults living in the USA (Dhingra et al. 2016; Steenland and Woskie 2012; Shankar et al. 2011a);
- one study on blood urea nitrogen and creatinine[26] levels (Emmett et al. 2006).

Only Rotander et al. (2015) and Dhingra et al. (2016) had not been included in previous reviews.

Considerations and conclusions

In the 'Plain Language Summary', Kirk et al. stated that: "*We found limited*[27] *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.*"

Kirk et al. stated in the 'Executive Summary' that: "*Of the 148 health outcomes investigated ...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....*"

Kirk et al. also noted in the 'Executive Summary': "*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.*"

In the 'Discussion' section, Kirk et al. made two statements about high blood uric acid levels:

- "*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.*"

[26] Concentration of serum creatinine was further used as a biomarker for kidney function in several studies. Creatinine is a chemical waste product carried in the blood until it is filtered by the kidneys and eliminated through urine.

[27] *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. (Source: Kirk et al. page 24)

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- *"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."*

In the 'Conclusion' section, Kirk et al. made the following comment about the public health implications: *"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."*

Kirk et al. made the following observation about PFAS and kidney function in the section on '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. [2016] provides an overview of possible connections between serum uric acid levels and PFAS exposure."*

The following table has been reproduced from Kirk et al. (pg. 81) and reports the associations by PFAS exposure and kidney outcome, following Kirk et al.'s review of the literature.

Associations at a glance (Kidney function)

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

Source: Kirk et al. (2018), p81.

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Summaries of studies reviewed

Reduced kidney function and glomerular filtration rate in children and adolescents

Kirk et al. reported on two studies in children and adolescents that investigated reduced kidney function and changes in glomerular filtration rate (Watkins et al. 2013; Kataria et al. 2015). Of the study by Watkins et al.(2013), Kirk et al. reported that: "*In a study of children enrolled in the C8 Health Project, Watkins et al. [2013] 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)).*"

For the study by Kataria et al. 2015, Kirk et al. reported: "*Kataria et al. [2015] 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. [2015] found no significant association for PFHxS and PFNA.*"

On their review of these two studies Kirk et al. concluded that: "*Although results are conflicting for PFHxS and PFNA, these studies 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 studies were evaluated to have a high risk of bias.*"

Uric acid levels and hyperuricemia

On the seven studies that investigated the association between PFAS exposure and uric acid levels, six were reviewed by the key international reports and systematic reviews, with summaries provided previously in this section. Note that Kirk et al. on page 82 stated they assessed six studies on the association between PFAS exposure and uric acid levels. Kataria et al. (2015) also reported on uric acid concentration (see above), in addition the six studies stated.

Rotander et al. (2015) had not been included in previous reviews and is reported below.

Kirk et al. reported about the study by Rotander et al. (2015): "*Primarily, the papers found a positive association between serum PFOA and increased uric acid levels, except Rotander et al. (2015) who investigated exposed Australian fire-fighters. Rotander et al. (2015) stated a non-significant association for PFOS and PFHxS exposures.*"

Kirk et al. concluded from these studies that: "*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.*"

Chronic kidney disease

On the three studies that investigated the association between PFAS exposure and chronic kidney disease in adults living in the USA, the studies by Steenland and Woskie (2012) and Shankar et al. (2011a) have been mentioned previously in this section. Kirk et al. reported about the study by Dhingra et al. (2016): "*Dhingra et al. [2016] found no association between modelled PFOA exposure and kidney disease in adults enrolled in the C8 Health Project.*"

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Kirk et al. commented on the three studies they reviewed on chronic kidney disease: "*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. [2016] and Shankar et al. [2011a] were evaluated to have a high risk of bias.*"

Kirk et al. also reviewed and reported the findings of the study by Emmett et al. (2006) on blood urea nitrogen and creatinine. This study was reviewed by the ATSDR, the findings of which are under that section.

6.4.4. Summary of key national and international reports and systematic reviews

Recent key national and international reports:

- The ATSDR concluded an association exists between serum perfluoroalkyl levels (mostly PFOA and PFOS and increases in uric acid levels, using a weight of evidence approach).
- The DWQI concluded there was evidence supporting a causal relationship between PFOA and serum levels of uric acid.
- US EPA concluded that for PFOA, studies that evaluated uric acid levels or eGFR as a measure of kidney function found associations with decreased function, although reverse causality could not be ruled out. For PFOS, the US EPA concluded studies suggest an association between chronic kidney disease; however reverse causality cannot be ruled out.
- RIVM concluded there are indications of an association between PFOA and higher blood concentrations of uric acid.

Systematic reviews:

- Priestly concluded there is a possible, but as yet unproven link between PFOS/PFOA and uric acid disorders; the findings were more consistent in populations with background, rather than high exposures and with PFOA more than PFOS.
- Rappazzo et al. concluded while there are a limited number of studies, there is evidence for positive associations between PFAS and renal function in children.
- Kirk et al. concluded there was 'limited' evidence that PFOA and PFOS were associated with higher blood uric acid levels, impaired glomerular filtration rate and chronic kidney disease.

6.4.5. Expert Health Panel synthesis to support advice to the Minister

- There is a clear link to kidney function with consistently shown associations between PFAS and uric acid/kidney function in key reports and reviews.
- There is not strong support for a link between PFAS exposure and kidney pathology; albeit one study linked deaths from kidney disease to estimated high occupational exposures (which may have been due to confounding by other potentially toxic chemicals).
- All associations could be influenced by reverse causation, as it is well known that most PFAS are eliminated by the kidney. Reduced kidney function would cause an increase in both serum uric acid and PFAS.
- The public consultation indicated renal effects were a concern for a small number of those responders exposed to PFAS.

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6.4.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and kidney function:

- An association of PFAS with impaired kidney function and higher serum uric acid is consistently shown. It has not been demonstrated that PFAS causes these problems or indeed is linked to human kidney disease. People with kidney disease are expected to have impaired elimination of PFAS and thus higher levels.

To further investigate the association between PFAS exposure and kidney function in an Australian setting, the Panel suggests the following research priorities:

- This will be a difficult area for researchers to propose and conduct rigorous study designs addressing causal relationships. To reduce the problem created by potential reverse causation, long-term prospective studies (not cross-sectional studies) are required. For example, people with low and high PFAS levels with baseline normal kidney function followed over time to examine the progress of kidney function. Even these study designs might be subject to confounding due to unknown factors affecting both PFAS clearance and rate of decline in kidney function.
- Kidney tissue concentrations would be expected to be higher than concentrations in most tissues due to active reuptake of filtered PFAS. PFAS thus could be selectively causing kidney injury. Studies on mechanisms of kidney PFAS elimination and potential for damage might be useful; these could potentially use human renal cell cultures.
- Research on kidney elimination and kidney disorders might best be nested into broader studies examining mechanisms or long-term health respectively.

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6.5. Thyroid effects and PFAS exposure

The thyroid is an important hormonal gland that plays a major role in human metabolism growth and maturation of the human body. Several recent key international reports and systematic reviews have reviewed the human evidence on exposure to PFAS and thyroid function and thyroid disease.

6.5.1. What evidence did the Expert Health Panel consider?

The Panel considered the findings and conclusions of five published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and five systematic reviews since 2013 that analysed the human epidemiological evidence regarding exposure to PFAS and thyroid effects:

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR, 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **United States Environmental Protection Agency (US EPA, 2016a).** Health effects support document for Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA, 2016b).** Health effects support document for Perfluorooctane Sulphonate (PFOS);
- **New Jersey Drinking Water Quality Institute (DWQI, Public Review draft 2016).** Health-based maximum contaminant level support document: Perfluorooctanoic Acid (PFOA);
- **Dutch National Institute for Public Health and the Environment (RIVM, 2017)**. PFOA exposure and health: A review of scientific literature.

Systematic reviews and reviews

- **Saikat et al. (2013)**. The impact of PFOS on health in the general population: a review;
- **Priestly (2016)**. Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS), Monash University;
- **Ballesteros et al. (2017)**. Exposure to perfluoroalkyl substances and thyroid functions in pregnant women and children: A systematic review of epidemiologic studies;
- **Rappazzo et al. (2017)**. Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature;
- **Kirk et al. (2018).** The PFAS Health Study: systematic literature review. Australian National University.

While the Panel acknowledges that FSANZ (2017) commented on thyroid effects of PFOA and PFOS, this report was not considered further in their section because FSANZ did not review thyroid epidemiological studies in detail; instead they reported on conclusions by other authoritative groups, notably the EFSA (2008), US EPA (2016a,b) and the ATSDR (2015).

6.5.2. Key national and international reports

US Agency for Toxic Substances and Disease Registry (ATSDR, 2015)

In 2015, the ATSDR published the 'Draft Toxicological Profile for Perfluoroalkyls'. In this profile, the ATSDR reported the analysis of the literature on thyroid effects under the 'Endocrine effects' section.

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Studies reviewed

In considering the literature on thyroid effects, the ATSDR referred to 15 studies:

- eight studies with regards to oral exposure (Emmett et al. 2006; Ji et al. 2012; Bloom et al. 2010; Melzer et al. 2010; Dallaire et al. 2009a; Wang et al. 2013; Chan et al. 2011; Inoue et al. 2004).
- seven studies with regards to inhalation exposure (Olsen et al. 1998a, 1998b; Sakr et al. 2007b; Costa et al. 2009; Olsen et al. 2003a; Olsen and Zobel 2007; Mundt et al. 2007).

Considerations and conclusions

The ATSDR did not make statements or comments about PFAS exposure and thyroid effects in the 'Public health statement for perfluoroalkyls' or the 'Relevance to public health' sections of the toxicological profile.

The ATSDR concluded at the end of the section where the eight oral route of exposure studies were reviewed that: "*Based on the results of the studies of adolescents, adults, and pregnant women, exposure to serum perfluoroalkyls does not appear to result in thyroid toxicity.*"

Under the section 'Toxicities mediated through the neuroendocrine axis', the ATSDR provided the following summary of the studies they reviewed: "*Assessments of workers exposed to perfluoroalkyls did not find associations between serum levels of PFOS and PFOA and thyroid hormone levels (Olsen and Zobel 2007; Olsen et al. 2003a; Sakr et al. 2007b). An additional occupational study did not find alterations in thyroid hormones levels in workers exposed to PFNA (Mundt et al. 2007). A study of a population highly exposed to PFOA reported no association between serum PFOA and serum levels of TSH (Emmett et al. 2006). Similarly, several general population studies did not find significant associations between serum perfluoroalkyl levels and thyroid hormone levels (Bloom et al. 2010; Ji et al. 2012; Wang et al. 2013). A study of pregnant women did not find associations between serum PFOA, PFOS, or PFHxS and the risk of hypthyroxinemia (Chan et al. 2011). Utilizing the NHANES data set, Melzer et al. (2010) found a significant association between serum PFOA levels and the risk of thyroid disease.*"

Summaries of studies reviewed

Oral exposure studies:

The ATSDR reviewed eight studies under the oral exposure route.

With regards to Emmett et al. (2006), the ATSDR noted that: "*Serum level of TSH were not correlated with PFOA levels in the health evaluation of 371 individuals whose water supply had high levels of PFOA and whose mean serum PFOA levels were significantly higher than the general U.S. population... Separate analyses of adults and children (≤18 years old) did not change the results. In addition, study individuals with thyroid disease (information provided by the individual) had lower levels of PFOA (387 ng/mL) than individuals without thyroid disease (451 ng/mL), but the difference between the two groups was not statistically significant.*"

The ATSDR reported the study by Ji et al. (2012) as: "*Ji et al. (2012) found no significant associations between serum PFOA, PFOS, PFHpS, PFHxS, PFNA, PFUA, and PFDoA and total T_4 and TSH levels in a Korean general population study.*"

The ATSDR reported the study by Bloom et al. (2010) as: "*A study of 31 New York sport fishermen also did not find significant associations between serum PFOA, PFOS, PFNA, PFHxS and PFDeA levels and free T_4 and TSH levels (Bloom et al. 2010)*", and then commented "*As noted by the investigators, the*

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study did not have sufficient power to detect statistically significant associations at the observed effect sizes."

With regards to Melzer et al. (2010), the ATSDR noted that: "*In contrast to these findings for T_4 and TSH, Melzer et al. (2010) found significant associations among women participating in NHANES between having serum PFOA levels in the highest quartile (mean 9.47 ng/mL) and the likelihood of ever having thyroid disease (OR 1.62, 95% CI 1.09–2.46) or current thyroid disease and taking related medication (OR 1.86, 95% CI 1.12–3.09), as compared to women in the first quartile. No significant associations between thyroid disease and serum PFOS levels were found in women (fourth quartile mean level 0.96 ng/mL). In men, there were also increases in the odds of thyroid disease when comparing men with serum PFOA levels in the fourth quartile (mean 10.39 ng/mL) to men with levels in the first and second quartiles; however, the increase was not statistically significant. A significant increase in the odds of currently having thyroid disease and taking medication was found in men with serum PFOS levels in the fourth quartile (mean 56.45 ng/mL), as compared to men in the first and second quartiles.*"

Dallaire et al. (2009a) undertook a study of the Inuit population in Nunavik Canada, and the ATSDR noted that the authors reported that: "*...serum PFOS levels were negatively associated with serum TSH, T_3, and thyroxine-binding globulin (TBG) levels in adults. Significant positive associations were found between serum PFOS levels and free T_4 levels. Given that most of the subjects (≥95%) had serum TSH, T_4, and T_3 levels within the normal range and the relative high geometric mean serum PFOS level (18.28 ng/mL) for the subjects, the associations may not be biologically relevant.*"

In a study of pregnant women participating in the Norwegian Mother and Child Cohort Study, Wang et al. (2013) reported: "*a significant association was found between serum PFOS levels and TSH levels after adjustment for potential confounders; no significant associations were found for other perfluoroalkyls examined including PFOA, PFHpS, PFHxS, and PFNA (Wang et al. 2013). When the TSH levels were dichotomized (≥7.5 vs <7.5 μIU/L), there was no significant associations; additionally, there were no significant associations with self-reported thyroid abnormalities.*"

The ATSDR reported on Chan et al. (2011) a case-control study of pregnant women undergoing a prenatal screen for trisomy 18, Down's syndrome, and open spina bifida in Canada, and stated that the authors "*did not [find] significant associations between serum PFOA, PFOS, or PFHxS and a higher risk of hypothyroxinemia*[28]*.*" They noted that: "*Similarly, Inoue et al. (2004) did not find significant associations between maternal cord PFOS levels and infant TSH and free T_4 levels.*"

The ATSDR concluded of these eight studies: "*Based on the results of the studies of adolescents, adults, and pregnant women, exposure to serum perfluoroalkyls does not appear to result in thyroid toxicity.*"

Occupationally exposed worker studies

Under 'Endocrine effects', the ATSDR reviewed seven studies that investigated PFAS and thyroid hormones in occupationally-exposed cohorts.

The ATSDR noted that the possible association between serum PFOA and PFOS levels and hormone levels was investigated in two cross-sectional studies of male workers at a PFOA production plant (Olsen et al. 1998a, 1998b). Of these two studies, the ATSDR reported: "*The studies were conducted in 1993 (n=11) and 1995 (n=80). Eleven hormones were assayed: cortisol, dehydroepiandrosterone*

[28] Hypothyroxinemia is a condition in pregnancy where T_4 levels in the mother are low but the TSH levels are normal. (Source: Kirk et al.)

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sulfate, estradiol, follicle stimulating hormone (FSH), 17α-hydroxyprogesterone, free testosterone, total testosterone, luteinizing hormone (LH), prolactin, thyroid stimulating hormone (TSH), and sex hormone-binding globulin. Simple and stratified analysis of variance, Pearson correlation coefficients, and ordinary multivariable regressions were used to evaluate associations between serum PFOA levels and each hormone, with adjustments for potential confounding variables. For stratified analyses, workers were divided into four PFOA categories: 0– 1,000, 1,000–<10,000, 10,000-<30,000, and ≥30,000 ng/mL. The results did not show significant associations between PFOA exposure and hormone levels, but workers with the highest serum PFOA levels had mean estradiol levels 10% greater than workers in other groups. The interpretation of the higher levels of estradiol was limited by the small number of workers in the high-exposure groups (four in 1994 and five in 1995) and the fact that estradiol levels were confounded by BMI. No significant associations between serum PFOS levels and hormone levels were found, with the exception of estradiol levels. However, it was found that one worker influenced the regression model; excluding this employee from the analysis resulted in a nonsignificant association between PFOS and estradiol. Limitations included the cross-sectional design of the study and the fact that the two studies could not be viewed as independent studies because 68 workers were studied in both years."

The ATSDR reviewed Olsen et al.'s (2003a) epidemiological assessment conducted at two perfluorooctanyl-manufacturing locations, and noted that: "*workers did not show evidence of altered thyroid function as assessed by measurements of serum levels of TSH, thyroxine (T4), free T4, triiodothyronine (T3), thyroid hormone binding ratio, and free thyroxine index. Mean concentrations of PFOS and PFOA for employees at one plant were 1320 and 1780 ng/mL, respectively. Mean PFOS and PFOS-serum values at the other plant were approximately 50% lower.*"

ASTDR reported: "*Olsen and Zobel (2007) also found no significant associations between serum PFOA and TSH or T4 values in a study of 506 employees at three fluorochemical production facilities. Serum PFOA levels in this study ranged from 7 to 92,030 ng/mL (arithmetic mean 2,210 ng/mL). Similar results were reported in the cross-sectional study of workers conducted by Sakr et al. (2007b). In that study, serum TSH, T4, and T3 uptake were within normal limits.*"

With regards to Mundt et al.'s (2007) epidemiology study of 630 workers exposed to PFNA previously described by the ATSDR under hepatic effects, the ATSDR noted that: "*there was no indication that exposure to PFNA affected thyroid function as assessed by serum levels of TSH, T4, T3 uptake, and free T4 index five times over a 25-year period. In this study, exposure was ascertained by work histories; levels of PFNA in serum were not available.*"

United States Environmental Protection Agency (US EPA, 2016a,b)

Studies reviewed

The US EPA reviewed the following studies regarding PFOA and Thyroid Effects:

- six studies of occupational exposure (Olsen et al. 1998a; Olsen et al. 2003a; Olsen and Zobel 2007; Steenland et al. 2015 Costa et al. 2009; Sakr et al. 2007b);
- three studies of adults in high exposure communities (Emmett et al. 2006; Winquist and Steenland 2014b);
- five studies of adults in the general population (Bloom et al. 2010; Shrestha et al. 2015; Melzer et al. 2010; Wen et al. 2013; Pirali et al. 2009);
- one study of children – high-exposure communities (Lopez-Espinosa et al. 2012);
- two studies of children – general population (de Cock et al. 2014b; Lin et al. 2013b);

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- four studies of pregnant women – general population (Chan et al. 2011; Wang et al. 2013; Berg et al. 2015; Webster et al. 2014).

Six of these studies had been considered by the ATSDR (Olsen and Zobel 2007; Emmett et al. 2006; Bloom et al. 2010; Melzer et al. 2010; Wang et al. 2013; and Chan et al. 2011).

The US EPA reviewed 14 studies regarding PFOS and thyroid effects:

- one study of occupational exposure (Olsen et al. 2001a);
- one study of children – high-exposure communities (Lopez-Espinosa et al. 2012);
- 13 studies in the general population (Dallaire et al. 2009b; Melzer et al. 2010; Wen et al. 2013; Webster et al. 2015; Bloom et al. 2010; Shrestha et al. 2015; Pirali et al. 2009; Wang et al. 2013; Berg et al. 2015; Inoue et al. 2004; Chan et al. 2011; Webster et al. 2014).

Six of the studies had been considered by the ATSDR (Dallaire et al. 2009b, Bloom et al. 2010; Inoue et al. 2004; Melzer et al. 2010; Wang et al. 2013; and Chan et al. 2011).

Considerations and conclusions

The US EPA stated in the 'Executive Summary' of the 'Health effects support document for PFOA', firstly that: "*Human epidemiology data report associations between PFOA exposure and thyroid disorders [and a range of other health effects]*", and then that:"*Diagnosed thyroid disease in females and female children was increased both in the high-exposure C8 study population and in females with background exposure; thyroid hormones are not consistently associated with PFOA concentration.*"

In the 'Summary and conclusions' of the 'Human epidemiological studies' section, the US EPA stated: "*Three large studies provide support for an association between PFOA exposure and incidence or prevalence of thyroid disease in women or children, but not in men (Lopez-Espinosa et al. 2012; Melzer et al. 2010; Winquist and Steenland 2014b). In addition, associations between PFOA and TSH were seen in pregnant females with anti-TPO antibodies (Webster et al. 2014). In contrast, generally null associations were found between PFOA and TSH in people who had not been diagnosed with thyroid disease.*"

Under the 'Hazard identification –thyroid effects' section of the PFOA document, the US EPA stated that: "*As illustrated above, numerous epidemiology studies have evaluated thyroid function and/or thyroid disease in association with serum PFOA concentrations (Tables 3-7 and 3-8). As noted previously, thyroid disease is more common in females. Several studies provide support for an association between PFOA exposure and incidence or prevalence of thyroid disease, and include large studies of representative samples of the general U.S. population (Melzer et al. 2010) and the high-exposure C8 community population (Lopez-Espinosa et al. 2012; Winquist and Steenland 2014b). Two of these studies are of adults (Melzer et al. 2010; Winquist and Steenland 2014b) and one is of children/adolescents (Lopez-Espinosa et al. 2012). The trend for an association with thyroid disease was seen in females in the C8 population (Winquist and Steenland 2014b) and the general population (Melzer et al. 2010), and in children (Lopez-Espinosa et al. 2012); this was most often hypothyroidism. Association between PFOA and TSH*[29] *also was seen in pregnant females with anti-TPO antibodies*

[29] TSH acts 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; low concentrations of TSH indicate an overactive thyroid gland. (Source: Kirk et al. (2018).

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(Webster et al. 2014). In contrast, generally null associations were found between PFOA and TSH or thyroid hormones (T3[30] or T4[31]) in people who have not been diagnosed with thyroid disease."

For PFOS, the US EPA did not make any statements or conclusions about thyroid effects in the 'Executive Summary' of the 'Health effects support document'.

In the 'Hazard identification – thyroid effects' and 'Summary and conclusions' of the 'Human epidemiology studies' sections of PFOS, the US EPA noted that: *"Numerous epidemiologic studies have evaluated thyroid hormone levels and/or thyroid disease in association with serum PFOS concentrations. These epidemiologic studies provide limited support for an association between PFOS exposure and incidence or prevalence of thyroid disease, and include large studies of representative samples of the general U.S. adult population (Melzer et al. 2010; Wen et al. 2013). These highly powered studies reported associations between PFOS exposure (serum PFOS concentrations) and thyroid disease but not thyroid hormone status. Melzer et al. (2010) studied thyroid disease with medication (PFOS level of 25 ng/mL in males and 19 ng/mL in females) and Wen et al. (2013) studied subclinical thyroid disease (mean serum 14.2 ng/mL). Thyroid function can be affected by iodide sufficiency and by autoimmune disease. People testing positive for the anti-TPO biomarker for autoimmune thyroid disease showed associations with PFOS (4.8 ng/mL) and TSH or T4 (Webster et al. 2014); this association was stronger in people with both low iodide status and positive anti-TPO antibodies, with a PFOS level of 14 ng/mL (Webster et al. 2015). These studies used anti-TPO antibody levels as an indication of stress to the thyroid system, not a disease state. Thus, the association between PFOS and altered thyroid hormone levels is stronger in people at risk for iodine deficiency than those receiving adequate dietary iodine. In people without diagnosed thyroid disease or without biomarkers of thyroid disease, thyroid hormones (TSH, T3, or T4) show mixed effects across cohort."*

Summaries of studies reviewed

PFOA

The US EPA reviewed 20 studies regarding PFOA and thyroid effects. Six of these studies had been considered by the ATSDR (Olsen and Zobel 2007; Emmett et al. 2006; Bloom et al. 2010; Melzer et al. 2010; Wang et al. 2013; and Chan et al. 2011).

For the studies that were not reviewed by the ATSDR, summaries provided by the US EPA are provided below. Where the US EPA has commented on or provided more detail about the studies already reviewed, this information is also included below.

Occupational exposure studies

The US EPA provided additional detail about the occupational exposure study by Olsen et al. (1998a): *"Serum PFOA levels were obtained from volunteer workers of the Cottage Grove, Minnesota, PFOA plant in 1993 (n = 111) and 1995 (n = 80) as part of the medical surveillance program and analyzed to determine a relationship between TSH and PFOA concentration (Olsen et al. 1998a). Employees were placed into four exposure categories based on their serum PFOA levels: 0–1000 ng/mL,*

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[30] T3(Triiodothyronine). Triiodothyronine hormones are produced by the thyroid gland through the same TSH signalling pathway as T4 . but this occurs to a lesser degree. T3 are primarily produced by the breakdown of T4 in tissues of the human body, particularly the liver and exists bound and unbound to carrier-proteins. Testing of free and total T3 hormones in the blood is another method used by medical practitioners to assess thyroid function in the human body. (Source: Kirk et al).

[31] T_4 (Thyroxine). 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. (Source: Kirk et al.)

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1000 – < 10000 ng/mL, 10000 – < 30000 ng/mL, and >30000 ng/mL. Statistical methods used to compare PFOA levels and hormone values included multivariable regression analysis, ANOVA, and Pearson correlation coefficients. TSH was significantly (p = 0.002) elevated in 10000 –<30000 ng/mL exposure category for 1995 only (mean serum TSH level was 2.9 ppm). However, mean TSH levels for the other exposure categories, including the ≥30000 ng/mL category, were all the same (1.7 ppm). In 1993, TSH was elevated in this same exposure category, but was not statistically significant (p = 0.09) when compared to the other exposure categories."

The US EPA reported that: "*Steenland et al. (2015) did not find an association between self-reported thyroid disease and PFOA levels among 3,713 workers at the Washington Works plant in West Virginia who participated in the C8 Health Project.*"

Of the studies by Costa et al. (2009 and Sakr et al. 2007b), the US EPA reported that: "*Two studies measured thyroid hormones in PFOA-exposed workers, but did not present an analysis of the relation between PFOA exposure and hormone levels. Both studies noted that the thyroid hormone values were in the normal range.*"

HIGH EXPOSURE COMMUNITIES

Adults

The US EPA provided additional details about the study by Emmett et al. (2006): "*Emmett et al. (2006) examined the association of serum PFOA with thyroid disease in 371 residents of the Little Hocking, Ohio, water district as described previously. No association was observed between serum PFOA and thyroid disease. Serum PFOA was decreased (not significantly different) in subjects with self-reported disease (e.g., hyperthyroidism, goiter or enlarged thyroid, hypothyroidism) (387 ng/mL; n = 40) compared to subjects without thyroid disease (451 ng/mL; n = 331). No association was seen between serum PFOA and TSH when analyzed with linear regression or by t-test comparison of PFOA in the abnormal TSH (n = 24, 6%) and normal TSH groups (p = 0.59).*"

Of the study by Winquist and Steenland et al. 2014b), the US EPA reported the following details: "*Participants in the C8 Health Project were examined for an association between PFOA levels and thyroid disease (Winquist and Steenland 2014b). The cohort included 28,541 community members and 3,713 workers who had completed study questionnaires during 2008–2011. The median serum PFOA level at enrollment in 2005–2006 was 26.1 ng/mL for the combined cohort, 24.2 ng/mL for the community members, and 112.7 ng/mL for the workers. Retrospective serum levels for the community cohort, estimated from air and water concentrations, residential history, and water consumption rates, were used to estimate yearly intakes. For the workers, yearly serum estimates were modeled from work history information and job-specific concentrations. Cox proportional hazard models, stratified by birth year, were used to assess self-reported adult thyroid disease hazard in relation to time-varying yearly or cumulative (sum of yearly estimates) estimated PFOA serum concentration, controlling for gender, race, education, smoking, and alcohol consumption. For the combined cohort, quintiles for yearly exposure were 0.11-<4.7, 4.7-<8.49, 8.49-<21.6, 21.6-<100, and 100-3303 ng/mL; quintiles for cumulative exposure were 0.1-<115, 115-<202, 202-<497, 497-2676, and 2676-97396 ng/mL year. As expected, the number of thyroid disease cases was higher among females than among males. Positive associations were seen with the cumulative exposure and the per-year exposure metrics for incidence of all thyroid disease (as well as for specific subtypes), with the observations seen primarily in females When limited to disease occurring after the 2005–2006 serum collection, the number of incident cases was reduced from 2,008 to 454, and the patterns of associations were more variable.*"

The US EPA noted: "*No associations between estimated serum PFOA level and thyroid disease were found in the analysis limited to workers in this study population (Steenland et al. 2015).*"

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Children

Lopez-Espinosa et al. (2012) considered children from the C8 cohort who were highly exposed to PFOA. The US EPA noted that they: "*observed positive associations between prenatal PFOA (modeled maternal levels) and any thyroid disease or clinical hypothyroidism; similar results were seen with the child's PFOA level. Associations were not seen with subclinical hypothyroidism or hyperthyroidism, or TSH or total T_4 levels among children without thyroid disease.*"

GENERAL POPULATION STUDIES

Adults

The US EPA provided additional detail to the ATSDR about the study by Bloom et al. (2010): "*Bloom et al. (2010) investigated the associations between serum PFAS, including PFOA, and TSH and free thyroxine (FT_4). The serum samples came from 31 participants (27 males, 4 females; mean age 39 years) of the 1995–1997 New York State Angler Cohort Study Dioxin Exposure Substudy. The study subjects each completed a questionnaire and provided a blood sample for serum analysis. The questionnaire contained questions about sportfish and game consumption, lifestyle, demographic factors, and medical history. The serum samples were analyzed for TSH and FT_4 in 2003 by immunometric chemiluminescent sandwich assay and for PFAS in 2006 by ion pair extraction high-performance LC-MS/MS. Regression models were used to analyze the data and adjust for confounders. No subjects reported use of thyroid medication or physician-diagnosed goiter or thyroid conditions. Mean TSH concentration (range 0.43–15.70 µIU/mL) was within normal range (0.40–5.00 µIU/mL) with the exception of one subject. Mean FT_4 (0.90–1.55 ng/dL) was within normal range (0.80–1.80 ng/dL) for all subjects. The mean serum PFOA concentration was 1.33 ng/mL and ranged from 0.57 to 2.58 ng/mL. The males had a significantly higher serum PFOA concentration than the females (1.47 ng/mL versus 1.05 ng/mL; $p = 0.047$). There was no association between serum PFOA concentration (or PFOS) and TSH or FT_4.*"

Of the study by Strestha et al. (2015), the US EPA reported that: "*The relationship between serum levels of PFOA, PFOS and other persistent organic pollutants and thyroid biomarkers was investigated in older adults (Shrestha et al. 2015). Levels of TSH, FT_4, T_4, and T_3 were measured in 51 males and 36 females with a mean age of 63.6 years. None of the participants had thyroid disease or were taking thyroid medication. Covariates in the analysis included age, gender, education level, the sum of polychlorinated biphenyls (∑PCBs) and polybrominated diphenyl ethers (∑PBDEs), smoking status, and alcohol consumption. The mean PFOA serum level was 10.4 ± 5.7 ng/mL for all participants. In both unadjusted and adjusted models, PFOA was significantly ($p < 0.05$ or 0.01) and positively associated with T_4 and T_3; a possible dose-response was not evaluated in this small sample. A statistical interaction was detected between age and PFOA for effects on FT_4 and T_4 suggesting that the positive associations of PFOA were potentiated by age.*"

The US EPA provided additional detail and commented about the study by Melzer et al. (2010): "*Melzer et al. (2010) examined the association between serum PFOA concentration and thyroid disease in the general population of the United States by analyzing data from the 1999– 2000, 2003–2004, and 2005–2006 NHANES The population included 3,966 adults (2,066 females, 1,900 males) older than 18 years. Each of the participants answered a questionnaire, had a physical examination, and provided blood and urine samples for analysis. Serum samples were analyzed for PFOA concentration by solid-phase extraction coupled to isotope dilution/highperformance LC-MS/MS. Data on diseases diagnosed by a physician and confounding factors, including year of NHANES, age, gender, race/ethnicity, education, smoking status, BMI, and alcohol consumption were obtained from the questionnaire. Individuals were considered to have thyroid disease if they responded on the questionnaire to having a*

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physician-diagnosed disease or if they were taking medication for either hypothyroidism or hyperthyroidism. Regression models were used to analyze the data and adjust for confounders. Serum PFOA concentration was divided into quartiles for each gender. In females, serum PFOA concentration ranged from 0.1–123 ng/mL (Q1 = 0.1–2.6; Q2 = 2.7–4; Q3 = 4.1– 5.7; Q4 = 5.7–123), and in males, serum PFOA concentration ranged from 0.1– 45.9 ng/mL (Q1 = 0.1–3.6; Q2 = 3.7–5.2; Q3 = 5.3–7.2; Q4 = 7.3– 45.9). Females in PFOA Q4 were more likely to report current thyroid disease [OR = 2.24, 95% CI: 1.38–3.65, p = 0.002] compared to females in Q1 and Q2. No association between serum PFOA concentration and thyroid disease was observed in males. With PFOS, the opposite was found, with males in the highest quartile, but not females, more likely to report thyroid disease."

The US EPA made the following comments about this study: "*Data interpretation was limited by the cross-sectional study design, lack of information on the specific thyroid disorder diagnosis in the questionnaire responses, and single serum samples for PFOA measurements taken at the same time disease status was ascertained through the questionnaire. Thus, the possibility of reverse causality cannot be eliminated.*"

Wen et al. (2013) used the NHANES data to examine the association between serum PFOA levels (and 12 other PFASs) and thyroid hormone levels, with the US EPA reporting that: "*Multivariable linear regression models were used with serum thyroid measures as the dependent variable and individual natural log-transformed PFAS concentration as a predictor along with confounders. The geometric mean serum PFOA level was 4.15 ng/mL. A positive association between PFOA level and free T3 (FT3) was found in females as a 1-unit increase in natural log-serum PFOA increased serum total T3 concentration by 6.628 ng/dL (95% CI 0.545, 12.712, p = .035). However, the association was no longer significant when PFOS, PFNA, and PFHxS levels were included in the model.*"

The US EPA noted Pirali et al. (2009) took a different approach in their study: "*The study measured intrathyroidal levels of PFOA (and PFOS) in thyroid surgical specimens to determine if a relationship existed between PFOA and the clinical, biochemical, and histological phenotype of thyroid disease patients. Serum PFOA concentration also was measured to determine if a relationship existed between thyroid tissue and serum PFOA levels. Patients (n = 28; 8 males, 20 females; 33–79 years) with benign multinodular goiters (n = 15), Graves' disease (n = 7), malignant papillary carcinoma (n = 5), and malignant follicular carcinoma (n = 1) were included in the study. Informed consent, clinical examination, work history, thyroid hormone and antibody measurements, thyroid ultrasound, fine-needle aspiration of nodules greater than 1 cm, and serum samples (n = 21) were performed or collected prior to surgery. The control group consisted of thyroid tissues collected at autopsy from subjects with no history of thyroid disease (n = 7; 5 males, 3 females; 12–83 years) and serum samples from 10 subjects with no evidence of thyroid disease. The student's t-test, Mann-Whitney U-test, Pearson and Spearman's correlation tests, and chi-square test with Fisher's correction were used to compare group results. Regression analysis was used to test the effect of different variables independently of a covariate.*"

Pirali et al. (2009) found that: "*The median concentration of PFOA in thyroid tissue was 2.0 ng/g (range = 0.4–4.6 ng/g). The patients were divided into three different groups: group I (toxic and nontoxic multinodular goiter, n = 12), group II (differentiated thyroid cancer, n = 6), and group III (Hashimoto's thyroiditis or Graves' disease, n = 10). Thyroid PFOA concentration for the control group, group I, group II, and group III ranged from 1.0–6.0, 0.4–4.4, 1.4–4.0, and 1.0–4.6 ng/g, respectively. Serum PFOA concentration for the control group, group I, group II, and group III ranged from 4–13.7, 1.2–16.6, 5.1–9.6, and 3.9–12.5 ng/ml, respectively. The concentration of PFOA in the thyroid and serum was similar between control and thyroid patients at the time of measurement. Age, gender, residence, working activity, malignant nonmalignant conditions, antibodies, thyroid hormone concentrations, and*

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ultrasound parameters were not associated with thyroid or serum PFOA concentration. There also was no correlation between serum and thyroid PFOA concentration. Similar results were obtained with PFOS."

Children

The US EPA reviewed a study of 52 males and 31 females from the Netherlands (de Cock et al. 2014b), and reported that: *"increasing T4 levels in females were associated with increasing prenatal PFOA concentrations (as measured in cord blood samples) no associations were reported in males."*

Of the study by Lin et al. (2013b) of adolescents and young adults (aged 12–30 years) from Taiwan, the US EPA reported that the authors: *"did not observe associations between serum PFOA concentrations and TSH or T4 levels."*

Pregnant females

The US EPA reviewed four studies (Chan et al. 2011; Wang et al. 2013; Berg et al. 2015; Webster et al. 2014). They noted that several studies of thyroid have been conducted with pregnant females: *"mostly reporting null associations between maternal PFOA concentration and thyroid status during pregnancy (Berg et al. 2015; Chan et al. 2011; Wang et al. 2013). The exception to these results is the only study that included an analysis stratified by presence of antithyroid peroxidise (anti-TPO) antibodies (Webster et al. 2014), in which associations between PFOA and TSH were seen only among females with high autoantibody levels. This finding supports the importance of further research into the association between PFOA and autoimmunity and autoimmune conditions."*

Summaries of studies by Chan et al. (2011) and Wang et al. (2013) were provided under the ATSDR, above. Where the US EPA provided comment or more detail on these four studies, the information has been included below.

Of the study by Chan et al. (2011), the US EPA provided the following detail: *"Chan et al. (2011) examined the association between hypothyroxinemia and serum PFOA concentration (and PFOS) in pregnant Canadian females (n = 271; 20.1–45.1 years of age, ≥22 weeks of gestation) in a matched case-control study. Maternal serum from the second trimester was collected between December 15, 2005, and June 22, 2006, as part of an elective prenatal screen for birth defects. Serum samples were analyzed for TSH and FT4 concentrations and PFOA. The cases of hypothyroxinemia (n = 96) had normal TSH concentrations and FT4 concentrations in the lowest 10th percentile (≤8.8 pmol/L). The controls (n = 175) had normal TSH concentrations and FT4 concentrations between the 50th and 90th percentiles (12–14.1 pmol/L). Maternal age, weight, and gestational age at blood draw and dichotomized at 50th percentiles were included as confounders, and race was included as a covariate. Chi-square tests and regression models were used to analyze the data. Overall, the geometric mean PFOA level was 1.35 ng/mL. Statistical comparisons used the geometric mean serum PFOA concentration in the cases of 3.10 nmol/L and 3.32 nmol/L in the controls. There was no association between serum PFOA concentration (or PFOS) and hypothyroxinemia in pregnant females."*

Of the study by Wang et al. (2013), the US EPA reported that: *"A cross-sectional study of 903 pregnant females evaluated the association between plasma PFOA levels and plasma TSH (Wang et al. 2013). Twelve other PFASs also were quantified and evaluated. The females were a cohort of the Norwegian Mother and Child Cohort Study and the blood samples were drawn at approximately week 18 of gestation. The median PFOA concentration was 2.2 ng/mL with an interquartile range of 1.57–2.95 ng/mL. No association was found between plasma levels of PFOA and TSH. PFOS was associated with higher TSH levels, but plasma levels of other PFASs were unrelated to TSH."*

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The study by Berg et al. (2015), was not reviewed by the ATSDR. The study was reported by the US EPA as: "*Expanding on the above study, Berg et al. (2015) investigated the association between a number of PFASs, including PFOA, and TSH, T_3, T_4, FT_3, and FT_4. A subset of 375 females in the Norwegian Mother and Child Cohort Study with blood samples at about gestational week 18 and at 3 days and 6 weeks after delivery were included. Seven compounds were detected in more than 80% of the blood samples, with PFOS present in the highest concentration followed by PFOA. The median PFOA level was 1.53 ng/mL, and the females were assigned to quartiles based on the first blood sample at week 18 of gestation. Females in the highest quartile had significantly higher mean TSH than females in the first quartile; however, when PFOS concentration was included as a covariate, the association was not significant.*"

The study by Webster et al. (2014) was reported by the US EPA as: "*A study of Canadian females (n = 152) evaluated maternal serum PFOA levels (and PFHxS, PFNA, and PFOS) for associations with thyroid hormone levels during the early second trimester of pregnancy, weeks 15–18 (Webster et al. 2014). Mixed effects linear models were used to examine associations between PFOA levels and FT_4, total T_4, and TSH; associations were made for all females and separately for females with high levels of TPO antibody, a marker of autoimmune hypothyroidism. Median serum PFOA was 1.7 ng/mL. No associations were found between levels of PFOA (or PFOS and PFHxS), and thyroid hormone levels in females with normal antibody levels. PFNA was positively associated with TSH. Clinically elevated TPO antibody levels were found in 14 (9%) of the study population. In the females with high antibody levels, PFOA, as well as PFNA and PFOS, was strongly and positively associated with TSH. An IQR increase in maternal PFOA concentrations was associated with a 54% increase in maternal TSH compared to the median TSH level. PFNA and PFOS concentrations were associated with 46% and 69% increases, respectively, in maternal TSH.*"

PFOS

The US EPA reviewed 14 studies regarding PFOS and thyroid effects: one in an occupational setting (Olsen et al. 2001a); 12 studies in the general population (Dallaire et al. 2009b; Melzer et al. 2010; Wen et al. 2013; Webster et al. 2015; Bloom et al. 2010; Shrestha et al. 2015; Pirali et al. 2009; Wang et al. 2013; Berg et al. 2015; Inoue et al. 2004; Chan et al. 2011; Webster et al. 2014), and one study of children in a high-exposure community (Lopex-Espinosa et al. 2012).

Six of the studies had been considered by the ATSDR (Dallaire et al. 2009b, Bloom et al. 2010; Inoue et al. 2004; Melzer et al. 2010; Wang et al. 2013; and Chan et al. 2011).

The US EPA's review of the studies not previously reviewed by the ATSDR, or where the US EPA has provided more detail or comment about studies, are provided below.

Occupational exposure

Of the cross-sectional study of production workers by Olsen et al. (2001a), the US EPA stated that: "*In the cross-sectional study described above for production workers, thyroid hormone (TH) levels were also measured in male (n = 215) and female (n = 48) volunteers working at the Decatur, Alabama plant and male (n = 206) and female (n = 49) volunteers working at the Antwerp, Belgium plant (Olsen et al. 2001a). The mean PFOS level in all employees from the Decatur and Antwerp plants was 1400 ng/mL (range: 110–10060 ng/mL) and 960 ng/mL (range: 40–6240 ng/mL), respectively. No significant associations were found for quartile of PFOS level and thyroid-stimulating hormone (TSH), serum thyroxine (T_4), free thyroxine (FT_4), triiodothyronine (T_3), and thyroid hormone binding ratio.*"

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General population exposure

Of the study by Dallaire etal. (2009b), the US EPA provided more detail and comments, including: "*Those using medication for thyroid disease and pregnant females were not included in the study. Concentrations of TSH, FT4, total triiodothyronine (TT3), and thyroxine-binding globulin (TBG) were measured in 623 individuals. Participants were given a survey to indicate smoking status, frequency of alcohol consumption, medications taken, and dietary fish consumption. The study detected PFOS in 100% of individuals, with a mean plasma PFOS concentration of 18 ng/mL (95% CI: 17–19 ng/mL). PFOS was negatively associated with circulating levels of TSH, TT3, and TBG and positively associated with FT4. The results suggest that human thyroid hormone levels could be affected by PFOS exposure. However, because the majority of individuals were reported by the authors to have normal thyroid gland function and the thyroid hormone levels were in the normal range, it is uncertain that these relationships are connected to thyroid disease or are a reflection of hormone variability in the human population.*"

The study by Wen et al. (2013) used the NHANES data to examine the association between serum PFOS levels (and 12 other PFASs) and thyroid hormone levels, with the US EPA noting that: "*Multivariable linear regression models were used with serum thyroid measures as the dependent variable and individual natural log-transformed PFAS concentration as a predictor along with confounders. The geometric mean serum PFOS level was 14.2 ng/mL. No associations between PFOS level and thyroid hormones were found in males and females. However in 23 individuals defined as subclinical hypothyroid (TSH above normal range), a 1-unit increase in natural log-PFOS was positively associated with hypothyroidism (OR = 3.03; 95% CI: 1.14–8.07 in females; OR = 1.98; 95% CI: 1.19–3.28 for males; both p < 0.05).*"

Webster et al. (2015) also used NHANES 2007–2008 data to explore the contribution of PFOS exposure to those with risk factors for thyroid disease, low iodide status and/or high thyroid peroxidase antibody (TPOAb). The US EPA noted that the authors found: "*that people with both elevated TPOAb and low iodide (those at risk for thyroid insufficiency) were more susceptible to PFOS associated disruption of thyroid hormone concentrations than were people without these two risk factors.*"

The US EPA provided more detail about the study by Bloom et al. 201): "*Levels of TSH and FT4 were measured in a subsample of participants in the cross-sectional New York State Angler Cohort Study (27 males and 4 females). A survey was conducted to determine smoking status, history of thyroid disease, medications used, and dietary fish consumption. None of the participants reported a thyroid condition or the use of thyroid medication. PFOS occurred at a high concentration compared to the other PFASs measured with a mean concentration of 19.6 ng/mL (95% CI: 0.0163–0.0235). The results indicated no significant association between PFOS serum concentration (or PFOA) and thyroid hormone levels, potentially due to the study's small sample size.*"

The relationship between thyroid biomarkers and serum levels of PFOS, PFOA, and other persistent organic pollutants was investigated in older adults by Shrestha et al. 2015, with the US EPA noting that: "*Levels of TSH, FT4, T4, and T3 were measured in 51 males and 36 females with a mean age of 63.6 years. None of the participants had thyroid disease or were taking thyroid medication. Covariates in the analysis included age, sex, education level, polychlorinated biphenyl (PCB) and PBDE exposure, smoking status, and alcohol consumption. The mean PFOS serum level was 36.6 ± 23 ng/mL for all participants. In both unadjusted and adjusted models, PFOS was significantly (p < 0.05 or 0.01) and positively associated with FT4 and T4; a possible dose-response was not evaluated in this small sample.*"

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Of the study by Pirali et al. (2009), the US EPA reported that: "*The potential relationship between PFOS exposure and thyroid disease was investigated by Pirali et al. (2009) in a sample of 28 patients undergoing thyroid surgery (22 benign and 6 malignant) and a control group of 7 patients with no evidence of thyroid disease. PFOS was detected in thyroid tissue in 100% of the 8 males and 20 females with thyroid disease, with a median PFOS concentration of 5.3 ng/g, and no significant difference in levels between benign and malignant patients. The median PFOS concentration (4.4 ng/g) in the healthy glands of the control group was similar to that found in the diseased thyroid samples indicating that there was no association between PFOS concentration and thyroid disease.*"

Of the study by Wang et al. (2013), the US EPA reported additional detail to the ATSDR, including: "*A cross-sectional study of 903 pregnant females evaluated the association between plasma PFOS levels and plasma TSH (Wang et al. 2013). Twelve other perfluoroalkyl substances were also quantified and evaluated. The females were a cohort of the Norwegian Mother and Child Cohort Study, and the blood samples were drawn at approximately week 18 of gestation. The median PFOS concentration was 13 ng/mL with an interquartile range of 10–17 ng/mL. A trend was observed for increasing TSH across PFOS quartiles, with females in the third and fourth quartiles having significantly higher TSH levels compared with the first quartile. After adjustment, each 1 ng/mL increase in PFOS concentration was associated with a 0.8% (95% CI: 0.1%–1.6%) rise in TSH. The odds ratio of having an abnormally high TSH, however, was not increased. The plasma levels of other perfluoroalkyl substances were not related to TSH levels.*"

Berg et al. (2015) expanded on Wang et al. (2013)'s study using the Norwegian Mother and Child Cohort Study. The US EPA reported that: "*Berg et al. (2015) investigated the association between a number of perfluoroalkyl substances, including PFOS, and TSH, T_3, T_4, free triiodothyronine (FT_3), and FT_4. A subset of 375 females on the Norwegian Mother and Child Cohort Study with blood samples at about gestational week 18 and at 3 days and 6 weeks after delivery were included. Seven compounds were detected in > 80% of the blood samples with PFOS present in the greatest concentration. The median PFOS level was 8.03 ng/mL and the females were assigned to quartiles based on the first blood sample at week 18 of gestation. After adjustment for covariates (parity, age, thyroxin binding capacity, BMI), TSH was positively associated with PFOS. Females in the highest quartile had significantly higher mean TSH at all three time points compared to females in the first quartile. No associations were found between PFOS and the other thyroid hormone levels.*"

The US EPA provided addition detail about the study by Inoue et al. (2004): "*Maternal and umbilical cord blood concentrations of a number of fluorinated organic compounds, including PFOS, were determined in 15 females (17–37 years of age) and their newborns at Sapporo Toho Hospitals in Hokkaido, Japan from February 2003 to July 2003 (Inoue et al. 2004). PFOS was detected in 100% of the maternal and cord blood samples, with maternal blood PFOS ranging from 4.9 to 17.6 ng/mL, and cord blood PFOS ranging from 1.6 to 5.3 ng/mL. TSH and FT_4 levels in the infants between days 4 and 7 of age were not related to cord blood PFOS concentration in this small study.*"

The US EPA also provided additional detail on the study by Chan et al. (2011): "*Chan et al. (2011) used blood from 974 serum samples collected in 2005–2006 from females in Canada (mean age 31.3 years) at 15–20 weeks gestation and measured thyroid hormones, FT_4 and the level of PFAS to determine whether PFAS levels were associated with hypothyroxinemia. From the samples, there were 96 identified as cases of hypothyroxinemia and 175 identified as controls. The cases had normal TSH concentrations and free T_4 concentrations in the lowest 10th percentile (≤ 8.8 pmol/L). The controls had normal TSH concentrations and free T_4 concentrations between the 50th and 90th percentiles (12–14.1 pmol/L). The geometric mean for PFOS was 7.4 ng/mL. The mean free T_4 levels were 7.7 pmol/L in the cases and 12.9 in the controls. The mean TSH concentrations were 0.69 milli-Units/L in the cases and*

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1.13 in the controls. Analysis by conditional logistic regression indicated that the concentration of PFOS (or PFOA) was not significantly associated with hypothyroxinemia. For PFOS, the odds ratio for association of hypothyroxinemia with exposure to PFOS was 0.88 with a 95% CI of 0.63–1.24."

Of the study by Webster et al. (2014), the US EPA reported that: "*A similar study of 152 Canadian females evaluated maternal serum PFOS levels (and PFHxS, PFNA, PFOA) for associations with thyroid hormone levels during the early second trimester of pregnancy, weeks 15–18 (Webster et al. 2014). Mixed effects linear models were used to examine associations between PFOS levels and FT4, total T4, and TSH; associations were made for all females and separately for females with high levels of thyroid peroxidase antibody, a marker of autoimmune hypothyroidism. Median serum PFOS was 4.8 ng/mL. No associations were found between levels of PFOS (or PFOA and PFHxS), and thyroid hormone levels in females with normal antibody levels. PFNA was positively associated with TSH. Clinically elevated thyroid peroxidase antibody levels were found in 14 (9%) of the study population. In the females with high antibody levels, PFOS, PFNA, and PFOA were strongly and positively associated with TSH. An IQR increase in maternal PFOS concentrations was associated with a 69% increase in maternal TSH compared to the median TSH level. PFNA and PFOA concentrations were associated with 46% and 54% increases, respectively, in maternal TSH."*

The US EPA also noted the study by Lopez-Espinosa et al. (2012), which reported that: "*In children from the C8 cohort, increasing PFOS was associated with increased T4 in children aged 1 to 17 years (LopezEspinosa et al. 2011); PFOS was not associated with hypothyroidism."*

The US EPA concluded for the studies they reviewed that: "*Numerous epidemiologic studies have evaluated thyroid hormone levels, thyroid disease, or both in association with serum PFOS concentrations (Table 3-6). These epidemiologic studies provide limited support for an association between PFOS exposure and incidence or prevalence of thyroid disease, and they include large studies of representative samples of the general U.S. adult population (Melzer et al. 2010; Wen et al. 2013). These highly powered studies reported associations between PFOS exposure (serum PFOS concentrations) and thyroid disease but not thyroid hormone status. Melzer et al. (2010) studied thyroid disease with medication and Wen et al. (2013) studied subclinical thyroid disease. In studies of pregnant females, PFOS was associated with increased TSH levels (Berg et al. 2015; Wang et al. 2013; Webster et al. 2014). Thyroid function can be affected by iodide sufficiency and by autoimmune disease. Pregnant females testing positive for the anti-thyroid peroxidase (TPO) biomarker showed a positive association with PFOS and TSH (Webster et al. 2014). An association with PFOS and TSH and T3 was found in a subset of the NHANES population with both low iodide status and positive anti-TPO antibodies (Webster et al. 2015). These studies used anti-TPO antibody levels as an indication of stress to the thyroid system, not a disease state. Thus, the association between PFOS and altered thyroid hormone levels is stronger in people at risk for thyroid insufficiency.*

In people without diagnosed thyroid disease or without biomarkers of thyroid disease, thyroid hormones (TSH, T3, or T4) show mixed effects across cohorts. Studies of thyroid disease and thyroid hormone concentrations in children and pregnant females found mixed effects. TSH was the indicator most frequently associated with PFOS in studies of pregnant females. In cross sectional studies where thyroid hormones were measured in association with serum PFOS, increased TSH was associated with PFOS exposure in the most cases (Berg et al. 2015; Wang et al. 2013; Webster et al. 2014), but this association was null in a smaller study with 15 participants (Inoue et al. 2004)."

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New Jersey Drinking Water Quality Institute (DWQI, 2016).

The DWQI reviewed the human evidence on thyroid hormones, TSH, hypo-and hyperthyroidism, thyroid disease in general, and/or other thyroid conditions in their 'Health-based maximum contaminant level support document: Perfluorooctanoic Acid (PFOA)'.

Studies reviewed

The DWQI (2016) reviewed 20 studies (Bloom et al. 2010; Chan et al. 2011; de Cock et al. 2014b; Emmett et al. 2006; Jain 2013; Ji et al. 2012; Kim et al. 2011; Knox et al. 2011a; Lin et al. 2013b; Lopez – Espinosa et al. 2012; Melzer et al. 2010; Olsen et al. 1998b; Olsen et al. 2003a; Olsen and Zobel 2007; Shrestha, et al. 2015; Steenland et al. 2015; Wang et al. 2014; Webster et al. 2014; Wen et al. 2013; Winquist and Steenland 2014b.).

Of these studies, four had been reviewed by both the ATSDR and the US EPA (Bloom et al. 2010; Chan et al. 2011; Emmett et al. 2006; Melzer et al. 2010), three by just the ATSDR (Ji et al. 2012; Olsen et al. 1998b; Olsen et al. 2003a), and eight by just the US EPA (de Cock et al. 2014b; Lin et al. 2013b; Lopez – Espinosa et al. 2012; Olsen and Zorbel. 2007; Shrestha, et al. 2015; Steenland et al. 2015; Webster et al. 2014; Wen et al. 2013; Winquist and Steenland 2014b).

Considerations and conclusions

In the 'Executive Summary', the DWQI reported that: "*For some other end points that were comprehensively reviewed, limited evidence of an association with PFOA was found... Other end points with limited evidence of an association include thyroid disease. There was limited or no evidence of association of PFOA with TSH and thyroid hormones.*"

The DWQI concluded that: "*Overall, studies evaluating thyroid hormones, TSH, and thyroid disease provide inconsistent evidence of any associations with PFOA.*"

In the 'Summary of conclusions for epidemiologic information' section, the DWQI concluded that: "*Overall studies evaluating thyroid hormones and TSH provide limited or no evidence of any associations with PFOA.*"

Summaries of studies reviewed

Summaries of the studies except Jain (2013), Kim et al. (2011), and Knox et al. (2011a) are provided above under the ATSDR and US EPA sections. Whereas the ATSDR and US EPA reported on the studies from an exposure perspective (occupational, high-exposure community, general population), the DWQI reported their evaluation by thyroid hormone and thyroid disease. The DWQI information is included below as it presents a different way to 'cut' the human data.

Thyroid stimulating hormone

The DWQI reported: "*Thyroid stimulating hormone (TSH) was the most commonly evaluated thyroid end point, and there was limited evidence of a positive statistically significant relationship with PFOA. Three general population studies which include a cross-sectional U.S. population study (Jain, 2013), a South Korean prospective birth cohort (Kim et al. 2011), and a prospective cohort study in Canada (Webster et al. 2014) found some evidence of a positive statistically significant association of elevated TSH and PFOA. The remaining 12 studies found limited or no evidence of a positive association. These 12 studies are all cross-sectional study design, which include six general population studies (Bloom et al. 2010; Ji et al. 2012; Lin et al. 2013b; Shrestha et al. 2015; Wang et al. 2014; and Wen et al. 2013), three studies of residents in a highly exposed community (Emmett et al. 2006; Knox et al. 2011a; Lopez-Espinosa et al. 2012), and three occupational studies (Olsen et al. 1998b; Olsen et al. 2003a; Olsen and*

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Zobel 2007). Three of the 12 studies also included components of other study designs in addition to the cross-sectional 74 design: birth cohort (Lopez-Espinosa et al. 2012), longitudinal (Olsen et al. 1998b), and prospective birth cohort (Wang et al. 2014)."

Total thyroxine (TT4)

For TT4, the DWQI reported: "*Additionally, total thyroxine (TT4) has been extensively evaluated with little evidence of a positive statistically significant association. Only two studies found some evidence of statistically significant positive association (de Cock et al. 2014b, and Knox et al. 2011a), while 11 others found no evidence of a statistically significant association (Jain 2013; Ji et al. 2012; Kim et al. 2011; Lin et al. 2013b; Lopez-Espinosa et al. 2012; Olsen et al. 2003a; Olsen and Zobel 2007; Shrestha et al. 2015; Wang et al. 2014; Webster et al. 2014; and Wen et al. 2013). A case-control study of hypothyroxemic pregnancy matched with non-hypothyroxemic pregnant women in Canada evaluated the association of PFOA and maternal hypothyroxemia, a common condition in pregnant women characterized by low maternal free thyroid hormone (fT4) and normal TSH levels, and found no evidence of a statistically significant association (Chan et al. 2011)."*

Total triiodothyronine (TT3)

The DWQI reported: "*Eight studies evaluated PFOA and associations with total triiodothyronine (TT3). Four of these studies found some evidence of a statistically significant positive association, including two larger (n=1,540 and 1,180) cross-sectional studies of the U.S. general population (Jain 2013 and Wen et al. 2013, respectively) as well as both of the occupational studies (Olsen et al. 2003a; Olsen and Zobel 2007). Three studies did not find any statistically significant evidence of an association (Kim et al. 2011; Shrestha et al. 2015; and Wang et al. 2014), while a large (n=50,113) cross-sectional study of the mid-Ohio Valley which found some evidence of an inverse association (Knox et al. 2011a). Two of these studies also evaluated free triiodothyronine (FT3) and neither found evidence of a statistically significant association (Jain, 2013; and Wen et al. 2013). These same two studies also evaluated associations of PFOA and thyroglobulin and found no evidence of a statistically significant association (Jain, 2013 and Wen et al. 2013)."*

Hypo- and hyperthyroidism

The DWQI reported: "*Three studies evaluated the association of PFOA and hypo- and hyperthyroidism, with mixed results. Hypothyroidism is a condition in which the thyroid gland is under-active and is characterized by elevated TSH serum levels combined with low serum FT4. Hyperthyroidism is a condition involving an over-active thyroid gland and is characterized by very low TSH hormone and raised FT4. Lopez-Espinosa et al. (2012) found a borderline statistically significant positive association with measured PFOA concentrations and self-reported subclinical hypothyroidism, but found non-statistically significant results for modeled PFOA, including modeled in utero exposure to PFOA, and subclinical measures of hypothyroidism. Odds ratio for PFOA and hyperthyroidism were mixed and not statistically significant. A study by Wen et al. (2013) of the U.S. adult general population found a statistically significant positive association of hypothyroidism among women but not men, and a statistically significant negative association of hyperthyroidism among men and not women. Winquist and Steenland (2014b) found increasing hazards with increasing PFOA exposure for hypothyroidism, although the trend was not statistically significant, while retrospective and prospective analyses were statistically significantly positively associated among men. A statistically significant trend of hyperthyroidism and increasing PFOA exposure was found overall and for women."*

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Thyroid disease

For thyroid disease, the DWQI reported that: "*Five studies evaluated thyroid disease in general, which may also include hypo- and hyperthyroidism. Three studies found some evidence of a statistically significant positive association with PFOA and thyroid disease. A large study of highly exposed children in the midOhio Valley found a positive statistically significant association among measured PFOA concentrations, median of 29 ng/ml, and parent-reported thyroid disease, but this association was not statistically significant with modeled PFOA (Lopez-Espinosa et al. 2012). A cross-sectional study of the U.S. general population found increasing odds ratio of self-reported thyroid disease, both ever and current, with increasing quartiles of PFOA among women but not men (Melzer et al. 2011). A large retrospective cohort study with prospective analyses found evidence of a positive association with thyroid disease and increasing quintiles of PFOA which was strongest among women for retrospective analyses, but prospective analyses found no clear associations with PFOA and thyroid disease (Winquist and Steenland, 2014b). The remaining two studies, a small study in a highly exposed community with median serum PFOA concentration of 354 ng/ml and a relatively narrow range of exposures (Emmett et al. 2006), and a retrospective occupational cohort with a median PFOA exposure of 113 ng/ml (Steenland et al. 2015), found no evidence of a statistically significant association with thyroid disease and PFOA.*"

DWQI comments about certain studies.

The DWQI commented on the limitations of some of the studies they reviewed, including:

- "*Selection bias may be an issue in Lin et al. (2013b) which included individuals with an abnormal urinalysis from a population-based screening program*";
- "*Reliance on recall for studies assessing thyroid disease, hypo-, and hyperthyroidism may bias results (LopezEspinosa et al. 2012)*"; and
- "*Small sample sizes in some studies may have limited their power to detect associations (Bloom et al. 2010; Kim et al. 2011b; Mundt et al. 2007; and Webster et al. 2014).*"

Dutch National Institute for Public Health and the Environment (RIVM, 2017)

The RIVM reviewed the conclusions of previous international reviews and epidemiologic studies to inform their conclusion on PFOA and thyroid effects.

Studies reviewed

The RIVM considered the conclusions of

- four international reviews (C8 Science Panel, 2012; ATSDR, 2015); ECHA-RAc (2015a); US EPA (2016a); and
- twenty-five epidemiological studies, including:
  - sixteen studies that examined the general population, among newborns, children, pregnant women or adults with plasma and serum PFOA concentrations ranging from 0.05 to 123 ng/mL (Berg et al. 2015; Bloom et al. 2010; Chan et al. 2011; de Cock et al. 2014b; Jain, 2013; Ji et al. 2012; Kim et al. 2016; Lin et al. 2013b; Melzer et al. 2010; Shah-Kulkarni et al. 2016; Shrestha et al. 2015; Wang Y. et al. 2014; Wang Y. et al. 2013; Webster et al. 2014; Wen et al. 2013; Yang et al. 2016).
  - three studies performed in high-exposure communities (interquartile range of serum PFOA concentrations: 184-571 ng/mL (Emmett et al. 2006); full range of serum PFOA concentrations: 0.05-3,987 ng/mL (Lopez-Espinosa et al. 2012); and full range of serum PFOA concentrations: 0.25-564.3 ng/mL (Knox et al. 2011a)).

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- one study that examined both workers and a high-exposure community (Winquist and Steenland, 2014b).
- five studies that examined populations that were occupationally exposed to PFOA, where serum PFOA concentrations ranged between 7-92,030 ng/mL (Olsen and Zobel, 2007), 5-9,550 ng/mL (Sakr et al. 2007a), 10-12,700 ng/mL (Olsen et al. 2003a) and 0.00-114,100 ng/mL (Olsen et al. 1998b). Steenland et al. (2015) did not report a range, but did report a median-measured serum PFOA concentration of 113 ng/mL.

Only two of these above studies (Shah-Kulkarni et al. 2016 and Yang et al. 2016) have not been discussed in any of the preceding reviews.

Considerations and conclusions

The RIVM reported in their 'Synopsis' that: "*The strength of evidence for the existence of a possible association differs between the observed effects.*" Under the associations the RIVM examined and concluded are "*less clear", RIVM stated: "Furthermore, associations have been found between exposure to PFOA and ... changes in concentrations of thyroid hormones in blood and thyroid disease."*

In the 'Discussion and conclusions' section under 'Thyroid effects', the RIVM reported the conclusions of the C8 Science Panel, the US EPA (2016a), the ATSDR (2015) and the DWQI (2016) and commented that: "*the organizations drew contradictory conclusions.*" RIVM then concluded: "*PFOA in relation to thyroid effects has been the topic of 25 studies in either the general population, high-exposure communities, or occupational populations. These studies provide inconsistent evidence, i.e. positive associations, negative associations and no associations were observed with various thyroid effects. An association with thyroid disease has been studied less. Of the four studies in which thyroid disease has been examined, two observed an association with PFOA concentrations in the blood in relatively large study populations (RR=1.44 per IQR of 13.1-67.7 ng/ml in children from a high-exposure community population, i.e. the C8 population (Lopez-Espinosa et al. 2012); RR=1.24, 1.27, 1.36 and 1.37 in quintiles 2(114.7-202.2 ng/ml) through 5 (2,670-97,396 ng/ml) in women in a high-exposure community and an occupational study population (Winquist and Steenland, 2014b)."*

Summaries of studies reviewed

Of the general population study by Shah-Kulkarni et al. (2016), RIVM reported the study was one of eight studies (i.e. among newborns, children, pregnant women, or adults), where no association was observed with any of the thyroid effects they examined, which were TSH, (total) T_3, (free or total)T_4.

The RIVM made only one note about Yang et al. 2016, in the summary of 'Thyroid effects': "*In three studies that examined adults, associations were found with greater occurrence of thyroid disease in women only (Melzer et al. 2010), changes in T_3 in women only (Wen et al. 2013) and free T_3 (Wen et al. 2013; Yang et al. 2016)."*

6.5.3. Systematic reviews

Saikat et al. (2013)

Studies reviewed

Saikat et al. reviewed five papers (Meltzer et al. 2010; Dallaire et al. 2009b; Pirali et al. 2009; Bloom et al. 2010; Chan et al. 2011) on 'Thyroid hormones'. All of these papers were considered by the US EPA and other previous reviews.

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Considerations and conclusions

In their literature review of the impact of PFOS on health in the general population, Saikat et al. (2013) stated in the 'Executive Summary': "*Small but statistically significant associations have been reported with PFOS and thyroid function [and a range of other health effects].*" The authors did not make a specific conclusion about PFOS exposure and thyroid disease in the general population, only making conclusions about the studies they reviewed.

In the 'Coherence with evidence' section, Saikat et al. commented that: "*Animal studies show that chronic PFOS exposure causes disruption of thyroid hormones, specifically an increase in TSH and a decrease in total T3. Two studies were identified that investigated this association of which Dallaire et al. [2009b] demonstrated an effect on thyroid hormones that was different from that seen in animals. Specifically it showed that PFOS was related to a decrease in TSH, T3 and TBG and an increase in T4. However, the majority of study participants had normal thyroid hormone levels; so the clinical significance of this association is difficult to determine. Another study [Chan et al. 2011] did not observe any association between exposure to PFOS and hypothyroxinemia.*"

Summaries of studies reviewed

All of the studies reviewed by Saitkat et al. have been reviewed and reported above.

Priestly (2016)

Studies reviewed

In the section 'Thyroid dysfunction', Priestly provided a summary of 16 epidemiological studies on the effects of PFAS exposures on thyroid disease and function (Pirali et al. 2009; Melzer et al. 2010; Jain 2013; Webster et al. 2016; Lewis et al. 2015; Knox et al. 2011a; Lopez-Espinosa et al. 2012; Lin et al. 2013b; Bloom et al. 2010; de Cock et al. 2014b; Shrestha et al. 2015; Berg et al. 2015; Webster et al. 2015; Wang et al. 2014; Chan et al. 2011; and Kato et al. 2016).

All of these studies have been discussed in previous reviews, except for Lewis et al. (2015) and Kato et al. (2016).

Considerations and conclusions

Priestly (2016) stated in the 'Executive Summary' that: "*The epidemiological studies are suggesting, but not yet proving, a possible link between PFOS/PFOA and thyroid disease.*"

Priestly's comment about the literature on thyroid effects he reviewed was that: "*Five of the studies in Table 3 (Chan et al. 2010, Wang et al. 2014, Berg et al, 2015; Webster et al. 2015, and Kato et al. 2016) focussed on dysfunction of the thyroid hormone system in pregnancy, because of the possible effect on embryonic and foetal development. The evidence that maternal thyroid hormone disturbances could account for postnatal developmental effects is somewhat unconvincing. The overall conclusion is that thyroid and hormone status may be altered by exposure to PFOS and/or PFOA, and possibly other PFAS, but the evidence is currently inconsistent in regards to which hormones are affected, and by which PFAS congeners. Different studies have suggested movements in T3 and T4 in different directions and there is also inconsistency as to whether it is the free or total forms of the thyroid hormones that are more susceptible to modification.*"

Priestly noted that a possible link with thyroid disease was sparked by a study by Melzer et al. in 2010. Regarding this study Priestly reported that: "*The authors used standard statistical methods to assess the relationship between serum PFOA & PFOS and self-reported thyroid disease in the cohorts. It was not possible to determine the nature of the thyroid disease, which can include either increased to*

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decreased thyroid function associated with quite different mechanisms, nor was it possible to relate the PFOA/PFOS or disease data to serum thyroid hormone levels. The increased risk comparison between the highest and lowest quartiles of the PFOA & PFOS serum measurements was highest in women (especially >5.7 ng/mL for PFOA), where the baseline incidence of thyroid disease is roughly five times higher than in men. Similar trends for increased risk associated with PFOA (and to a lesser extent PFOS) could be seen across both genders. When the data were adjusted for factors also shown to influence PFOA/PFOS exposure patterns (e.g. smoking, age, drinking patterns, educational status) the strength of the association with thyroid disease appeared to become stronger. An alternative explanation cannot be excluded – that thyroid disease could result in a reduced clearance of PFOS or PFOA, resulting in higher blood levels." Priestly then noted: "*The findings of the Melzer et al. (2010) have not been replicated in a range of other studies, nor have there been consistent findings in studies where the primary focus has been on whether exposures to PFAS have resulted in changes in circulating thyroid hormones in various population groups, including children and pregnant women.*"

Regarding occupationally exposed workers, Priestly stated that: "*To date, there has been no confirmation of an increased thyroid disease risk in workers manufacturing PFOA/PFOS, despite reports that serum levels in these workers are orders of magnitude higher than the subjects in the NHANES samples. While there is some confirmatory evidence that high PFOA doses in animals can alter thyroid function, the mechanisms (including hormone carrier protein and/or receptor displacement and altered thyroid hormone metabolism) are complex and likely to be relevant only at exposure levels well above those in the human population. The authors concede that the findings in their study simply point to an association between PFOA (but not PFOS) serum levels (measured on a single sample) and thyroid disease. While the findings merit further study, including further exploration of biologically plausible mechanisms, they do not definitively prove that PFOA/PFOS exposures cause thyroid disease in humans.*"

Summaries of studies reviewed

Priestly provided little detail about Lewis et al. (2015), stating in the table titled 'Summary of epidemiological studies on the effects of PFAS exposures on thyroid disease and function' that in using the NHANES database to test serum levels of thyroid hormones TSH, T3 (F/T)[32], T4 (F/T) and testosterone, they found: "*No effect on testosterone; exposure to PFAS may be associated with increased FT3, TT3 and FT4 among adult females, and that during adolescence, PFAS were associated with increased TSH in males, but decreased TSH in females. The majority of the associations studied (for individual PFAS, different age groups/genders and different hormones) failed to show statistical significance.*"

In the same table, Priestly reported on Kato et al.'s (2016) longitudinal cohort study of pregnant Japanese women and their infants. The study considered serum thyroid hormones TSH and fT4 measured at ~11 weeks and from infants at 4-7 days. Priestly noted that they found "*Median concentrations (ng/mL) were PFOS 5.2 and PFOA 1.2. Maternal PFOS (but not PFOA) was associated with lower maternal and higher infant TSH; there were no associations with fT4.*"

Ballesteros et al. (2017)

In 2017, Ballesteros et al. reviewed the human evidence on exposure to perfluoroalkyl substances and thyroid function in pregnant women and children in a systematic review.

[32] F/T= Free/total

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Studies reviewed

Ballesteros et al. (2017) reviewed 10 studies (Lewis et al. 2015; Berg et al. 2015; de Cock et al. 2014b; Wang et al. 2014; Webster et al. 2014; Lin et al. 2013b; Wang et al. 2013; Lopez-Espinosa et al. 2012; Chan et al. 2011; Kim et al. 2011).

All these studies have been reviewed in previous key international reports and/or systematic reviews, above.

Considerations and conclusions

In the 'Abstract', Ballesteros et al. reported that: "*We found some evidence of a positive association between PFHxS and PFOS exposure and TSH levels measured in maternal blood, and PFNA and TSH levels measured in the blood of boys aged ≥ 11 years.*" They concluded "*Although there is a small number of studies with comparable data, we found some consistency of a positive association between maternal or teenage male exposure to some PFAS and TSH levels based on the current literature. However, further studies are required to confirm these possible relationships.*"

In the 'Discussion', the authors noted: "*In sum, there were insufficient numbers of studies in each population group to make comparisons except in two cases: mothers (n = 4) and 11–19-year-old children (n=3). In both cases, no consistent associations between four PFAS and THs or thyroid dysfunctions were found except for TSH levels. There was some evidence of a positive association between PFHxS and PFOS exposure and levels of TSH measured in the blood of mothers, as well as PFNA and TSH levels measured in the blood of teenage boys. Differences in the expression of the results and/or effect estimates, as well as the treatment of the outcome and exposure variables (e.g., log transforming or not of data, continuous or categorical PFAS, etc.), prevented us from combining effect estimates in a meta-analysis. Therefore, due to the small number of studies with comparable data, further studies are warranted to confirm the possible relationships outlined above. In order to draw our conclusion, we have assessed the evidence of a possible association between PFAS and thyroid function impairment by assessing the exposure and outcomes, and, by using the Bradford-Hill Criteria of consistency and coherence, strength of the association, temporality, biological gradient, and biological plausibility (Hill, 1965).*"

Under 'Biological Plausability', Ballesteros et al. made the following observations: "*Interactions between the hypothalamic–pituitary–thyroid axis can be inhibited or stimulated by natural physiological responses or by exposure to chemical pollutants with endocrine disrupting properties, such as PFAS (Jensen and Leffers, 2008). Although further investigation is warranted, it has been proposed that PFAS may interfere with thyroid homeostasis through various mechanisms, including regulation of hepatic glucuronidation enzymes and deiodinases in the thyroid gland, as reported in studies of exposed rat tissues (Yu et al. 2009), by competition with T4 for binding to protein TTR*[33] *as seen in studies of exposed rat tissues (Weiss et al. 2009), by altering the expression of genes involved in TH signaling, as reported in salmon embryos and larvae (Spachmo and Arukwe, 2012), or by altering the function of nuclear hormone receptors, as reported in zebrafish embryos (Du et al. 2013). Leaving aside the inter-species diversity due to differences in modes of action and the generally high exposure in the experimental studies, some animal evidence on the interference of these substances with the thyroid system exists. For example, decreased T3 and T4 levels after short-term or long-term PFOS/PFOA exposure were found in animal studies (Boas et al. 2012). Experimental studies on PFHxS and PFNA exposure are scarcer, although both altered TH function in in vitro tests (Long et al. 2013), PFHxS*

[33] 'TTR' is not defined in the paper but presumed to be Transthyretin.

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reduced plasma TH levels in a concentration-dependent manner in an in ovo study (Cassone et al. 2012), and long-term PFNA exposure raised T3 levels in zebrafish (Liu et al. 2011).

According to the existing scientific understanding of the functioning of the hypothalamic–pituitary–thyroid axis, TSH levels should be inversely proportional to T4 and T3 levels at the same lifestage. TSH regulates the synthesis and secretion of THs by the thyroid gland. In turn, THs negatively influence TSH secretion from the anterior pituitary gland through a negative feedback loop (Dietrich et al. 2012). However, this relationship between these hormone levels was not observed consistently in the epidemiological studies reviewed, since an increase in TSH was not always associated with a reduction in T4 and/or T3 levels or vice versa, when data were available."

In the 'Conclusion' of the systematic review, Ballesteros et al. stated that: "*In conclusion, heterogeneity was found across studies in terms of study design, study setting, timing of PFAS exposure assessment, timing and type of thyroid-related outcome assessment, adjustment for potential confounders, and statistical approach. As a consequence, there were insufficient numbers of comparable studies in each population group except for two cases: mothers and 11–19-year-old children. Based on the current literature, we found some consistency of a positive association between PFHxS and PFOS in relation to TSH levels measured in maternal blood and PFNA and TSH levels measured in the blood of boys aged ≥11 years. However, further studies are warranted to confirm these possible relationships. Future studies should measure FT4 as well as TSH in order to yield more comprehensive information concerning any effects on the functioning of the hypothalamic-pituitary-thyroid axis. They should preferably be longitudinal, and should include, if possible, repeated measures of PFAS and thyroid outcomes in order to identify any periods of extra vulnerability.*"

Summaries of studies reviewed

As all of the studies reviewed by Ballesteros et al. have been reviewed and summarised by the key international reports or systematic reviews, above, summaries are not provided here.

Rappazzo et al. (2017)

In 2017 Rappazzo et al. published a systematic review on exposure to perfluorinated alkyl substances and health outcomes in children, including thyroid effects.

Studies reviewed

Rappazzo et al. reviewed five studies regarding thyroid function in children (Lopez-Espinosa et al. 2012; Lin et al. 2013b; de Cock et al. 2014b; Tsai et al. 2017; Kim et al. 2011; Kim et al. 2016; Kato et al. 2016; Melzer et al. 2010).

The two studies that Rappazzo et al. reviewed that were not reviewed by previously published key international reports or systematic reviews were by Tsai et al. (2017) and Kim et al. (2016). Priestly in 2016 reported on the study by Kato et al. (2017), but the systematic review by Ballesteros et al. (2017) did not. Ballesteros et al. also did not review the study by Kim et al. (2016).

Considerations and conclusions

Rappazzo et al. did not report a conclusion specifically on thyroid effects in the 'Abstract'. The authors found an association between PFAS and a number of health effects in children, but thyroid was not included in the list. At the end of the section on 'Thyroid function', Rappazzo et al. did state that: "*While some associations are observed between thyroid hormones and PFAS, no clear patterns emerge. There is some evidence for hypothyroidism, a finding that has also been observed in an adult NHANES population [Melzer et al. 2010], but not in other studies of PFAS and thyroid function. Given*

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the limited number of studies and the variability in the responses, no conclusions can be reached with certainty."

In the 'Discussion', Rappazzo et al. noted: "*In addition, the potential exists for non-monotonic dose response curves for PFAS, some of which are known endocrine disrupting compounds. It is possible that lower concentrations/exposures may have a more disruptive effect than high concentrations/exposures, in particular with outcomes connected to the endocrine system such as thyroid function or pubertal development.*"

Summaries of studies reviewed

Rappazzo et al. reported the study by Tsai et al. (2017) as: "*In a cross-sectional analysis of the Taiwan Birth Panel study, doubling of cord blood plasma PFOS was associated with decreased T_4 in boys, and increased TSH in both boys and girls though effects appeared to be non-linear and magnitude of effects was higher in boys [Tsai et al. 2017]. PFOA, PFNA, PFUnDA were generally not associated with thyroid hormone concentrations [Tsai et al. 2017].*"

Of the study by Kim et al. (2016), Rappazzo et al. reported: "*In another small South Korean study, infants with congenital hypothyroidism had higher mean serum levels of PFOA, PFNA, PFDA, PFUnDA, and total PFASs compared to healthy infants [Kim et al. 2016].*"

Kirk et al. (2018)

Studies reviewed

Kirk et al. (2018) evaluated 25 publications investigating the effect of PFAS exposure on the functioning of the thyroid gland in neonates, children, adults and pregnant women.[34]

Infants

- Nine studies examined the association between prenatal and early infancy exposure to PFAS and TSH levels in neonates and infants (0-5-years old) (Emmett et al. 2006; Kim et al. 2011; Berg et al. 2017; Kato et al. 2016; Lopez-Espinosa et al. 2012; Shah-Kulharni et al. 2016; Tsai et al. 2017; Wang et al. 2014; Yang et al. 2016).
- Seven studies considered prenatal PFAS exposure and concentrations of T_4 in neonates and infants (0-5 years) (Kim et al. 2011; Lopez-Espinosa et al. 2012; Shah-Kulharni et al. 2016; Tsai et al. 2017; Yang et al. 2016; Wang et al. 2014; de Cock et al. 2014b).
- Three prenatal studies considered Free Thyroxine (T_4) level (Kato et al. 2016; Yang et al. 2016; Wang et al. 2014).
- Five studies considered Total Triiodothyronine (T_3) level (Shah-Kulharni et al. 2016; Tsai et al. 2017; Wang et al. 2014; Yang et al. 2016; Kim et al. 2011).
- One study examined free Triiodothyronine (T_3) level (Yang et al. 2016).
- One study was on Thyroid disease – Congenital hypothyroidism (Kim et al. 2016).

Children

- Two studies investigated the association between PFAs exposure and TSH levels during childhood (6-17 years) (Emmett et al. 2006; Lopez-Espinosa et al. 2012).
- One paper investigated the association between PFAS exposure concentrations and total T_4 in children aged 6-17 years (Lopez-Espinosa et al. 2012).

[34] Note that papers may be in more than one of the categories below.

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Pregnant women

- Seven papers considered Thyroid Stimulating Hormone (TSH) (Berg et al. 2017; Kato et al. 2016; Yang et al. 2016; Wang et al. 2014; Wang et al. 2013; Berg et al. 2015; Webster et al. 2014).
- Four studies investigated PFAs exposures and concentrations of total T_4 in pregnant women (Berg et al. 2017; Yang et al. 2016; Wang et al. 2014; Webster et al. 2014).
- Five studies considered Free Thyroxine (T_4) leve (Berg et al. 2017; Kato et al. 2016; Yang et al. 2016; Wang et al. 2014; Webster et al. 2014).
- Four studies considered Total Triiodothyronine (T_3) level (Berg et al. 2017; Yang et al. 2016; Wang et al. 2014; Berg et al. 2015).
- Three studies examined free Triiodothyronine (T_3) level (Berg et al. 2017; Yang get al. 2016; Berg et al. 2015).
- One study was on Thyroid disease – Hypothyroxinemia (Chan et al. 2011).

Adults

- Seven studies considered Thyroid Stimulating Hormone (TSH) (Emmett et al. 2006; Bloom et al. 2010; Jain, 2013; Knox et al. 2011a; Lin et al. 2013b; Shrestha et al. 2015; Webster et al. 2016).
- Five papers looked at the association between elevated PFAS levels and T_4 concentrations in adults (Jain, 2013; Knox et al. 2011a; Shrestha et al. 2015; Webster et al. 2016; Wen et al. 2013).
- Six studies considered Free Thyroxine (T_4) level (Bloom et al. 2010, Jain, 2013 Lin et al. 2013b; Shresha et al. 2015; Webster et al. 2016; Wen et al. 2013).
- Five studies were on Total Triiodothyronine (T_3) level (Jain, 2013; Knox et al. 2011a; Shrestha et al. 2015; Webster et al. 2016; Wen et al. 2013).
- Three studies were on Free Triiodothyronine (T_3) level (Jain, 2013; Webster et al. 2016; Wen et al. 2013).
- Three studies reported the effect of elevated PFAS exposure levels on the development of thyroid disease in adults (Melzer et al. 2010; Winquist and Steenland., 2014b; Steenland et al. 2015).
- One paper investigated the association between PFAS exposure and thyroglobulin levels (Wen et al. 2013).

All of the papers have been discussed in previous reviews. Additional information about Tsai et al. (2017) is provided under the 'Summaries of studies reviewed' section below.

Considerations and conclusions

Associations at a glance: Thyroid Stimulating Hormone (TSH) level

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

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Health outcome	PFAS exposure	Evaluation of 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

Source: Kirk et al. (2018), pp87.

Associations at a glance: Thyroxine (T_4) level

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

Source: Kirk et al. (2018), pp 92.

Associations at a glance: Triiodothyronine (T_3) level

Health outcome	PFAS exposure	Evaluation of evidence
Total T_3		
Total T_3 in infants		
	Umbilical cord; PFOA, PFOS, PFHxS, PFNA,	Inadequate evidence

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Health outcome	PFAS exposure	Evaluation of evidence
	PFDA, PFUdA, PFDoA, PFTrDA, PFPeA	
	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

Source: Kirk et al. (2018), pp97.

Associations at a glance: Thyroid disease

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

Source: Kirk et al. (2018), pp102

Associations a glance: Thyroglobulin levels

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

Source: Kirk et al. (2018), pp103

Kirk et al. reported the following conclusions or comments about the studies they reviewed under each thyroid end point for infants, children, pregnant women and adults:

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INFANTS:

Thyroid Stimulating Hormone (TSH): *"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."*

Total Thyroxine (T_4) level: "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."

Free Thyroxine (T_4) level: "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."

Total Triiodothyronine (T_3) level: "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."

Free Triiodothyronine (T_3) level: "... 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."

Thyroid disease – Congenital hypothyroidism: From the one paper Kirk et al. evaluated of a case-cohort study of 40 newborn infants that visited one hospital in Seoul, South Korea (Kim et al. 2016), they stated: *"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. [2016], 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."*

CHILDREN:

Thyroid Stimulating Hormone (TSH): "Neither study suggested a potential association between exposure to PFAS and concentrations of TSH in children."

Total Thyroxine (T_4) level: "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

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total T_4 levels have not been investigated in other studies to date, and would benefit from further research."

PREGNANT WOMEN:

Thyroid Stimulating Hormone (TSH): "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 effcct. The results for Webster et al. [2014] relating only to women with high TPOAb concentrations makes it difficult to compare to the significant positive finings reported by Berg et al. (173) and Wang et al. [2013]. Although Wang et al. [2014] and Yang et al. [2016] 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."

Total Thyroxine (T_4) level: "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."

Free Thyroxine (T_4) level: "Overall, the findings reported for free T_4 were similar to those reported for total T_4... The negative association between PFDoA and free T_4 in pregnant women may warrant further investigation, as Wang et al. [2014] and Yang et al. [2016] both reported this finding. Both studies were evaluated to have high risk of bias, which suggests that results should be interpreted with caution."

Total Triiodothyronine (T_3) level: "...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."

Free Triiodothyronine (T_3) level: "Thus, the results presented by the Berg et al. [2017; 2015] studies and Yang et al. [2016] are conflicting, specifically related to PFUdA exposure levels. As Yang et al. [2016] 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."

Thyroid disease- Hypothyroxinemia: *"Chan et al. [2011] 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."*

ADULTS:

Thyroid Stimulating Hormone (TSH): "The findings of six of seven studies suggest that there is no association between PFAS and TSH levels in adults." Kirk et al. reported on the study (Webster et al. 2016) that did find significant results when considering a subpopulation of adults who had several other stressors affecting their thyroid gland and impairing its production of T_3 and T_4 hormones. Kirk et al. stated: *"Webster et al. [2016] 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."*

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Total Thyroxine (T_4) level: "*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.*"

Free Thyroxine (T_4) level: "*...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.*"

Total Triiodothyronine (T_3) level: "*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. [2013] and Webster et al. [2016], 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 Triiodothyronine (T_3) level: "*...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: "*...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.*"

Thyroglobulin levels: Kirk et al. reported on the one paper: "*Wen et al. [2013] investigated the association between PFAS exposure and thyroglobulin levels in adults, and reported no significant association for PFOA, PFOS, PFNA and PFHxS.*"

Summaries of studies reviewed

Kirk et al. discussed the Tsai et al. (2017) study in the section on 'Umbilical cord blood studies', noting that the authors: "*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. [2017] only found a significant association for boys (adjusted regression coefficient β (CI); 0.333 (0.012, 0.678))*."

Kirk et al. further noted that: "*Tsai et al. [2017] also reported no association for PFNA, PFDA, PFUdA and PFDoA*" When discussing the Total t_4 levels in infants, Kirk et al. refer to Tsai et al. (2017) and noted, "*Tsai et al. [2017] 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. [2017] also reported non-significant results for PFOA, PFDA and PFUdA levels and total T_4.*" For Total t_3 levels in infants, Kirk et al. noted that "*Kim et al. [2011] and Tsai et al. [2017] further reported non-significant associations between umbilical cord measurements of PFOA and PFOS, PFHxS (Kim et al. [2011] only), PFDA (Tsai et al. [2017] only), PFTrDA (Kim et al. [2011] only) and PFUdA (Tsai et al. [2017] only) and total T_3 concentrations in infants at birth.*"

Differing conclusions

The Dutch National Institute for Public Health and the Environment (RIVM) noted that the C8 Science Panel, the US EPA, the ATSDR and the DWQI "*drew contradictory conclusions*" regarding PFOA and thyroid effects. While the C8 Science Panel in 2011 and 2012, had found a '*probable link*', the DWQI found limited evidence with thyroid disease and limited or no evidence with TSH and thyroid hormones. While the US EPA concluded an association was observed for thyroid disease in

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women, the ATSDR concluded perfluoroalkyls (including PFOA) do not appear to result in thyroid toxicity.

Two systematic reviews on PFAS exposure and thyroid effects in children were published in 2017. Ballesteros et al. (2017) concluded there was some consistency of a positive association between maternal or teenage male exposure to some PFAS and TSH levels, while Rappazzo et al. concluded that while some associations are observed between thyroid hormones and PFAS, no clear patterns emerge, and given the limited number of studies and the variability in the responses, no conclusions can be reached with certainty.

Kirk et al. concluded the literature suggest a positive association between PFOA and thyroid disease in women, noting the evidence is inconsistent. However, no more mention of this positive association is made in the 'Executive Summary' or 'Discussion' of the systematic review. Priestly concluded the epidemiology studies suggest an association but this is not yet proven, with the evidence being currently inconsistent as to which hormones are affected and by which PFAS.

6.5.4. Summary of key national and international reports and systematic reviews

Recent key national and international reports:

ATSDR concluded PFAS are not associated with thyroid toxicity based on studies in adolescents, adults, workers involved in manufacturing PFAS and pregnant women.

The US EPA concluded that PFOA was associated with thyroid disease (in women) but not with thyroid hormones. For PFOS, the US EPA advised limited support exists for an association between PFOS and thyroid disease and study findings on thyroid hormones are inconsistent in people without diagnosed thyroid disease or biomarkers of thyroid disease. For those findings where significant differences in thyroid hormone levels were found, the differences were small and generally all fell within the normal reference range, so any differences are of uncertain clinical significance.

DWQI concluded there was limited and inconsistent evidence of an association with PFOA and thyroid disease, with associations more common in women (in whom thyroid disease is more common than men) and limited or no evidence with TSH and thyroid hormones.

RIVM concluded the evidence is less clear for PFOA exposure and changes in concentrations of thyroid hormones in blood and thyroid disease.

Systematic reviews:

Saikat et al. concluded that small but statistically significant associations have been reported with PFOS and thyroid function, but that the pattern of change was different from that seen in animal studies.

Priestly concluded that while there is a possible, but as yet unproven link between PFOS/PFOA and thyroid disease; evidence of maternal exposure to PFAS and postnatal developmental effects is unconvincing; thyroid and hormone status may be altered by PFOS and/or PFOA and other PFAS but the evidence is inconsistent about which hormones are affected and by which PFAS. He also considered that reverse causality could also be an alternative explanation for any associations. Priestly also noted that there is little evidence for an association with thyroid disease in manufacturing workers (mainly men), despite their exposure levels being substantially higher than in exposed communities.

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Ballesteros et al. found some evidence of a positive association between maternal and teenage exposure to PFHsX and PFOS and TSH levels in males older than 11 years, but noted inconsistent results in most studies and recommended further longitudinal studies with a stronger study design, including repeated measures of thyroid function, are required to confirm the possible relationship.

Rappazzo et al. concluded that, for children, some associations were observed between thyroid hormones and PFAS, but no clear patterns emerged; given the limited number of studies and the variability in the responses, no conclusions can be reached with certainty.

Kirk et al. noted limited and inconsistent evidence of a positive association between PFOA and thyroid disease in women while there was no evidence of effects between PFOS and thyroid disease in men. For TSH, T_3 and T_4 levels Kirk et al. found the evidence to be inconsistent or show no association with PFAS among infants, children, pregnant women and adults. The concluded that there was inadequate evidence of an association for all outcomes.

6.5.5. Expert Health Panel synthesis to support advice to the Minister

There are no consistent associations between any particular PFAS and thyroid hormones and in those studies where small associations were found, the pattern of changes in levels of the different hormones was not consistent and there were often differences within the normal range, which is of uncertain clinical significance. This applied to infants, children and adults.

For thyroid disease, there is limited evidence of an association between PFOA in women (in whom thyroid disease is much more common), but not in men.

Studies of workers involved in the manufacture of PFAS, for whom exposure levels were considerably higher than community members in population studies, were largely negative for thyroid function and thyroid disease.

If there are any causal associations, it is difficult to disentangle which PFAS is likely to be involved because of high correlations between the different exposures. Reverse causation may also be an alternative explanation.

Thyroid effects was not a major concern among those who responded to the community consultation.

6.5.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and thyroid effects:

PFAS exposure is unlikely to be a major contributor to the burden of thyroid dysfunction or disease in the community among infants, children or adults.

To further investigate the association between PFAS exposure and thyroid effects in an Australian setting, the Panel suggests the following research priorities:

- If further studies of thyroid function and thyroid disease are to be undertaken, these would best be nested into longitudinal studies of a range of health effects and focus on groups where alterations in thyroid function would be most critical (e.g. pregnancy and early childhood).

Studies that explored the potential causal mechanisms of associations would also be useful – e.g. does thyroid function change PFAS elimination, do PFAS affect thyroid hormone related transcription.

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6.6. Neonatal, infant and maternal outcomes from exposure during pregnancy

Evidence from human data that PFAS (particularly PFOA and PFOS) can cross the placenta has raised potential concerns about their effect on fetal growth and development, given the fetal stage is a period of high vulnerability to toxicological impacts. The epidemiological evidence base on the association between PFAS and fetal growth has been accumulating. The majority of the recently published key national and international reports considered by the Panel extensively reviewed the epidemiological studies, particularly on birth weight; in addition, seven systematic reviews have reviewed the human evidence on fetal and/or maternal outcomes.

6.6.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of the following five key international authority/intergovernmental/governmental reports published between 2015 and 2017 and eight systematic reviews from 2013 that reported on exposure to PFAS and pregnancy, prenatal and birth outcomes.

Key national and international reports

- **US Agency for Toxic Substances and Disease Registry (ATSDR, 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **United States Environmental Protection Agency (US EPA. 2016a).** Health effects support document for Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA, 2016b).** Health effects support document for Perfluorooctane Sulphonate (PFOS);
- **Dutch National Institute for Public Health and the Environment (RIVM 2017).** PFOA exposure and health: A review of scientific literature;
- **Food Standards Australia New Zealand (FSANZ, 2017).** Hazard Assessment report (PFOS, PFOA, PFHxS).

Systematic reviews

- **Saikat et al. (2013).** The impact of PFOS on health in the general population: a review;
- **Lam et al. (2014).** The navigation guide – evidence-based medicine meets environmental health: integration of animal and human evidence for PFOA effects on fetal growth;
- **Johnson et al. (2014).** The navigation guide – evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth;
- **Bach et al. (2015).** Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: a systematic review;
- **Priestley (2016)** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS), (Monash University);
- **Negri et al. (2017).** Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data;
- **Kirk et al. (2018)**. The PFAS Health Study. Systematic Literature Review **(Australian National University)**.

While the Panel acknowledges that the DWQI (2016) commented on birth weight, they did not review epidemiological studies and make their own evaluation. They reported on the systematic

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review by Johnson et al. (2014) and stated in the 'Executive Summary' that: "*The Health Effects Subcommittee found that the basis for this conclusion [the conclusion of Johnson et al. 2014] is reasonable and supportable.*" As the DWQI did not undertake their own evaluation of the human epidemiological literature, the 'Health-based maximum contaminant level support document: perfluorooctanoic acid (PFOA) public review draft' is not considered further in this section.

6.6.2. Key national and international reports

US Agency for Toxic Substances and Disease Registry (ATSDR, 2015)

In 2015, the ATSDR in its 'Draft toxicological profile for perfluoroalkyls' considered the human evidence on prenatal effects under 'Developmental effects'.

Studies reviewed

The ATSDR reviewed the following studies on perfluoroalkyl exposure and pregnancy related effects, including:

- four studies on pregnancy-induced hypertension and pre-eclampsia (Savitz et al. 2012b; Darrow et al. 2013; Stein et al. 2009; Savitz et al. 2012a);
- sixteen studies on PFOA and birth measurement (Savitz et al. 2012b; Darrow et al. 2013; Nolan et al. 2009; Fei et al. 2007; Maisonet et al. 2012; Lee et al. 2013; Hamm et al. 2010; Washino et al. 2009; Monroy et al. 2008; Whitworth et al. 2012b; Kim et al. 2011; Fei et al. 2008a,b; Anderson et al. 2010; Apelberg et al. 2007b; Chen et al. 2012a);
- fifteen studies on PFOS and birth measurements (Maisonet et al. 2012; Fei et al. 2007; Darrow et al. 2013; Monroy et al. 2008; Whitworth et al. 2012b; Lee et al. 2013; Hamm et al. 2010; Washino et al. 2009; Inoue et al. 2004; Kim et al. 2011; Grice et al. 2007; Fei et al. 2008a,b; Chen et al. 2012a; Apelberg et al. 2007b);
- four studies on PFHxS and birth weight (Maisonet et al. 2012; Monroy et al. 2008; Lee et al. 2013; Kim et al. 2011);
- four studies on PFOA exposure and birth defect incidence (Darrow et al. 2013; Nolan et al. 2010; Savitz et al. 2012b; Stein et al. 2009);
- three studies on PFOA exposure and increased risk of still births or premature birth (Darrow et al. 2013; Hamm et al. 2010; Savitz et al. 2012b);
- one study on inhalation exposure and pregnancy outcomes (Grice et al. 2007).

Considerations and conclusions

The ATSDR made three major statements about prenatal and birth outcomes in the 'Public health statement' and 'Relevance to public health' sections of the report.

In the 'Public health statement – how can perfluoroalkyls affect children?' section, the ATSDR advised that: "*No associations between serum PFOA and birth defects were observed in children of mothers living in an area with high PFOA levels in the water. Some studies of the general population and people living near a PFOA manufacturing facility have found that higher levels of serum PFOA or PFOS are associated with lower infant birth weights. However, the decrease in birth weight is small and may not affect the infant's health.*"

In the 'Relevance to public health – summary of health effects' section, the ATSDR advised on the evidence for both maternal outcomes and birthweight. For maternal outcomes the ATSDR reported that: "*Additionally, a study of highly exposed residents found significant associations between serum PFOA and PFOS levels and the odds of pregnancy-induced hypertension. However, another study that*

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used predicted serum PFOA levels did not find a significant association. Two studies of highly exposed residents also found an increased risk of pre-eclampsia among women with higher serum PFOA levels."

Regarding the evidence on birth weight, the ATSDR advised: "*There is evidence to suggest that high serum PFOA or PFOS levels are associated with lower birth weights. The significant associations have come from general population studies and a study of highly exposed residents. Studies of populations with lower serum PFOA or PFOS levels have not found significant associations for birth weight. Although significant associations were found, decreases in birth weight were small and may not be biologically relevant. No studies found an increased risk of low birth weight in infants (<2,500 g) in highly exposed areas."*

In the 'Relevance to public health – minimal risk levels' section, the ATSDR issued the following advice about birth weight: "*Based on the weight of evidence, there is support for identifying several health effects in humans that appear to be related to perfluoroalkyl exposure: ...small decreases in birth weight. The magnitude of the changes in birth weight ...observed in the human studies are small and not likely biologically relevant."*

Summaries of studies reviewed

Maternal outcomes – pre-eclampsia and pregnancy induced hypertension

The ATSDR reviewed four studies on the possible associations between PFOA and PFOS and pregnancy-induced hypertension and pre-eclampsia (Savitz et al. 2012b; Darrow et al. 2013; Stein et al. 2009; Savitz et al. 2012a).

Of the study by Savitz et al. (2012b), the ATSDR reported that: "*Using birth record data and serum PFOA levels predicted from addresses, Savitz et al. (2012b) found no consistent associations between serum PFOA and the occurrence of pregnancy-induced hypertension in participants in the C8 Health Project."*

For the study by Darrow et al. (2013), the ATSDR reported that: "*Another study of participants in the C8 Health Project that used measured serum perfluoroalkyl levels found significant increases in the ORs for pregnancy-induced hypertension in women with higher PFOA (≥6.9 ng/mL) or PFOS (≥12.1 ng/mL) levels."*

The study by Stein et al. (2009) investigated pre-eclampsia. The ATSDR noted that the: "*study of highly exposed residents reported a weak association between serum PFOA and PFOS and pre-eclampsia in subjects whose PFOA and PFOS levels were above the median (Stein et al. 2009); however, there was no dose-response gradient. A significant increase in the risk of pre-eclampsia was also found in subjects with PFOS levels above the 90th percentile (≥120.6 ng/mL)."*

Of the study by Savitz et al. (2012a), the ATSDR reported the findings as: "*Savitz et al. (2012a) also found an increased risk of self-reported pre-eclampsia in C8 Health Project participants with elevated PFOA levels."*

Neonatal outcomes – birth measurements and diagnoses at birth

Under the 'Oral exposure – health effects – developmental effects' section, the ATSDR noted that a number of epidemiological studies have examined the potential of perfluoroalkyls to adversely affect birth outcome in the general population and in populations living in an area with high PFOA drinking water concentration.

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The studies in this section, and reviewed by the ATSDR, reported on a number of end points including, birth weight, birth length, preterm birth, gestational age, head circumference, chest circumference.

High-exposure communities

Of the study by Nolan et al. (2009), the ATSDR reported the following summary: "*Nolan et al. (2009) examined birth outcomes in women living in areas of Ohio exclusively or partially serviced by the Little Hocking Water Association (LHWA), which has been shown to have high levels of PFOA, and compared the birth outcomes (taken from the Ohio Department of Health) to outcomes from women living in areas not serviced by LHWA. The incidence of low birth weight (<2,500 g) infants was significantly lower in the exclusive and partial LHWA groups, as compared to the national average, and the likelihood of low birth weight was significantly lower in the partial LHWA group, as compared to the no LHWA group; however, no association was found among the group exclusively serviced by LHWA. Additionally, no associations between residence location and mean birth weights, gestational age, or the likelihood of preterm birth were found.*"

The ATSDR discussed a later study by this group (Nolan et al. 2010), and noted that it: "*did not find significant differences in the likelihood of congenital anomalies in infants of mothers living in the partially or exclusively LHWA serviced areas, as compared to infants of mothers living in areas not serviced by LHWA. A major limitation of these studies is the lack of biomonitoring data, which would allow for a more accurate examination of possible associations between maternal PFOA exposure and birth outcome.*"

The ATSDR noted that other studies conducted with residents Ohio and West Virginia (including participants in the C8 Health Project) used estimates of maternal PFOA and PFOS levels (Darrow et al. 2013; Savitz et al. 2012b; Stein et al. 2009).

Of the study by Stein et al. (2009) on PFOA and PFOS, the ATSDR reported that it: "*examined the self-reported outcomes of pregnancies that occurred within 5 years preceding blood sample collections for the C8 Health Project. No associations were found between serum PFOA levels in 1,845 pregnancies and the likelihood of miscarriage, preterm birth, low birth weight, or birth defects; the investigators did note that there was a decreased risk of low birth weight among infants of mothers with higher serum PFOA levels (75th – 90thpercentile), but there was no apparent dose-response relationship. Stein et al. (2009) also examined the possible association between maternal serum PFOS levels and birth outcomes from 5,262 pregnancies; the likelihood of preterm birth was significantly increased in women with PFOS levels above the 90th percentile (23.2–83.4 ng/mL). The likelihood of low birth weight was increased in women with serum PFOS levels above the median (12.8 ng/mL), in the 75th–90th percentile (17.7–<23.2 ng/mL) and above the 90th percentile (23.2–83.4 ng/mL). No association between maternal PFOS levels and the risk of miscarriage or birth defects were found.*"

Of the study by Savitz et al. (2012b), the ATSDR noted: "*Similarly, Savitz et al. (2012b) found no association between estimated maternal serum PFOA levels and the risk of stillbirths, preterm birth, low birth weight, or birthweight in 8,253 singleton infants born to mothers living in areas of the Mid-Ohio valley with known PFOA contamination from 1990 to 2004.*"

Of the study by Darrow et al. (2013) the ATSDR reported that: "*In the Darrow et al. (2013) study of 1,330 women participating in the C8 Health Project and giving birth between 2005 and 2010, blood samples collected in 2005–2006 were used to estimate maternal serum levels. No associations between estimated maternal serum PFOA or PFOS levels and the likelihood of preterm birth, low birth weight, or*

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small for gestational age were found in the entire cohort or in a subcohort of 783 women whose first pregnancy occurred after blood samples were collected. In the entire cohort, there was a non-significant trend for decreased birth weight among full-term infants with increasing PFOS levels; this trend was statistically significant in the subcohort of nulliparous women. No association between maternal PFOA levels and birth weight was found."

General population studies

Of the studies by Fei et al. (2007, 2008a,b) and Andersen et al. (2010), the ATSDR reported: "*In a study of 1,400 pregnant women participating in the Danish National Birth Cohort, maternal PFOA levels were inversely associated with birth weight; birth weights were significantly lower in infants of mothers with serum PFOA levels in the second (3.91–5.20 ng/mL), third (5.21–6.96 ng/mL), and fourth (≥6.97 ng/mL) quartiles, as compared to the first quartile (Fei et al. 2007). Maternal serum PFOA levels were also inversely associated with birth length and abdominal circumference (Fei et al. 2008a); however, in stratified analysis (after adjustment for potential confounding variables), only birth lengths in the infants of mothers with serum PFOA levels in the second and fourth quartile were significantly lower than in the first quartile. Grouping the infants by sex resulted in significant inverse associations between serum PFOA and birth weight in the male and female infants (Andersen et al. 2010). No significant associations between maternal serum PFOS levels and birth weight, birth length, or abdominal circumference were found (Fei et al. 2007, 2008a). However, when the infants were grouped by sex, maternal PFOS levels were inversely associated with birth weight in female infants (Andersen et al. 2010). No associations between serum PFOA or PFOS levels and gestation length, likelihood of low birth weight, or small for gestational age were found in this cohort (Fei et al. 2008a). The study also found no associations between maternal serum PFOA or PFOS levels and Apgar scores (assessed 5 minutes after birth) (Fei et al. 2008b)."*

For the study by Whitworth et al. (2012b), the ATSDR reported that: "*A study of 901 pregnant women participating in the Norwegian Mother and Child cohort study found inverse associations between maternal serum PFOA (median of 2.2 ng/mL) and PFOS (median of 13.0 ng/mL) and the likelihood of preterm birth. No associations (after adjustment for potential confounding variables) were found between maternal serum PFOA or PFOS levels and birth weight, small for gestational age, or large for gestational age."*

The study by Maisonet et al. (2012) was summarised by the ATSDR as: "*Significant negative trends between maternal serum PFOA, PFOS, and PFHxS levels and birth weight were found in a study of 447 female infants of mothers participating in the Avon Longitudinal Study of Parents and Children in Great Britain; birth length was also negatively associated with maternal serum PFOS and PFHxS."*

The study by Washino et al. (2009) was summarised as: "*A negative correlation between maternal serum PFOS levels and birth weight was also observed in a study of 428 pregnant Japanese women (geometric mean PFOS level was 4.9 ng/mL); when the infants were grouped by sex, the significant association was only found in females No significant association was found between birth weight and maternal serum PFOA levels (geometric mean level of 1.2 ng/mL) and no associations were found between serum PFOA or PFOS levels and birth length, chest circumference, or head circumference."*

The study by Hammet al. (2010) involved 252 pregnant women in Canada undergoing screening for Down's syndrome, trisomy 18, and open spina bifida. The ATSDR reported the authors found: "*no association between maternal serum levels of PFOA, PFOS, or PFHxS and birth weight, small for gestational, or preterm delivery; the mean serum concentrations of PFOA, PFOS, and PFHxS were 2.1, 9.0, and 2.1 ng/mL, respectively."*

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The ATSDR also reviewed several smaller-scale studies of pregnant women in Canada (Monroy et al. 2008), South Korea (Kim et al. 2011; Lee et al. 2013), and Japan (Inoue et al. 2004). The ATSDR reported these studies: "*did not find significant associations between maternal serum PFOS levels (Inoue et al. 2004; Kim et al. 2011; Lee et al. 2013; Monroy et al. 2008) or PFHxS (Kim et al. 2011; Lee et al. 2013) and birth weight. Kim et al. (2011) and Monroy et al. (2008) also did not find a significant association between serum PFOA and birth weight; however, Lee et al. (2013) found significantly higher maternal blood PFOA levels in infants whose birth weights were below the median level. The Lee et al. (2013) study also found significantly higher maternal PFOA levels in infants with birth length and ponderal index below the median level and an inverse association between maternal PFOS levels and ponderal index.*"

The study by Apelberg et al. (2007b) was summarised as: "*Cord blood serum PFOA and PFOS levels were inversely associated with birth weight, ponderal index, and head circumference in a study of 341 singleton births in Baltimore, Maryland.*"

The ATSDR noted, in its review of Chen et al. (2012a): "*Similarly, Chen et al. (2012a) found a significant inverse association between cord blood PFOS levels and birth weight, head circumference, and gestational age in a study of 492 infants whose mothers were participating in the Taiwan Birth Panel study. The likelihood of preterm birth and small for gestational age were also significantly associated with PFOS; low birth weight was not associated with cord blood PFOS levels. Cord blood PFOA, PFNA, and PFUA levels were not associated with birth weight or the likelihood of preterm birth, low birth weight, or small for gestational age; an inverse association between PFNA and ponderal index was found.*"

At the end of the section Oral exposure – health effects – developmental effects', the ATSDR made the following conclusions about the studies they reviewed: "*Epidemiology studies have examined the potential developmental toxicity of perfluoroalkyls in studies of populations living in areas with high PFOA contamination and in the general population. Birth weight was the most studied end point in these studies. Although it is difficult to compare across studies due to the differences in study design and the characterization of perfluoroalkyl exposure, ranking the studies by the upper end of the blood perfluoroalkyl levels provides some suggestion of an effect on birth weight (Table 3-8).*"

Below is Table 3-8 reproduced from pages 214/215 of the 'Draft toxicological profile for perfluoroalkyls' (ATSDR, 2015), titled 'Possible Associations Between Perfluoroalkyl Exposure and Alterations on Birth Weight in Humans'.

Reference	Timing of blood collection	Mean (ng/mL)	Range (ng/mL)	Effect on birthweight
PFOA				
Savitz et al. 2012b.	2012b Estimated	13.4 (median)	3.9–921.3	-18.55 g per 100 ng/mL increase in PFOA levels
Darrow et al. 2013	2005-2006	31.0	0.6-459.5	Nonsignificant trend, significant in women whose first pregnancy was conceived after sample collection
Nolan et al. 2009	No biomonitoring data	No biomonitoring data	No biomonitoring data	NS

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Reference	Timing of blood collection	Mean (ng/mL)	Range (ng/mL)	Effect on birthweight
Fei et al. 2007	First Trimester	5.6	<LLOQ (1.0)–41.5	- 10.63g per 1ng/mL increase in PFOA
Maisonet et al. 2012	Gestation	3.7 (median)	1.0–16.4	Negative trend 2012
Lee et al. 2013	Delivery	2.73	1.2-5.72	PFOA levels significantly higher in infants below the median birth weight
Hamm et al. 2010	Early 2nd Trimester	2.1	<LOD (0.25)-18	NS
Washino et al. 2009	Second timester	1.4	ND-5.3	NS
Monroy et al. 2008	Delivery	2.24	1.33–2.64	NS
Whitworth et al. 2012b Gestation 2.2 (median) NR NS	Gestation	2.2 (median)	NR	NS
Kim et al. 2011	Third trimester	1.46 (median)	1.15–1.91	NS
PFOS				
Maisonet et al. 2012	Gestation week 15	19.6 (median)	3.8–112.0	Negative trend
Fei et al. 2007	First trimester	**35.3**	6.4–106.7	NS
Darrow et al.	2005-2006	15.6	LOD (0.25)– 92.9	-49 g per log unit increase in PFOS levels (nulliparous women)
Monroy et al. 2008	Delivery	16.19	9.19–20.22	NS
Lee et al. 2013	Delivery	10.77	2.38–35.18	NS
Hamm et al. 2010	Early second trimester	9	<LOD (0.25)–35	NS
Washino et al. 2009	Second trimester	5.6	1.3-16.2	-148.8 g per 10 ng/mL increase in PFOS levels
Inoue et al. 2004	Gestation weeks 38-71	-	4.9–7.6	NS
Kim et al. 2011	Third trimester	2.93 (median)	2.08–4.36	NS
PFHxS				
Maisonet et al. 2012	Gestation week 15	1.6 (median)	0.2–54.8	Negative trend
Monroy et al. 2008	Delivery	1.62	1.33–2.66	NS
Lee et al. 2013	Delivery	1.35	0.53–3.67	NS
Kim et al. 2011	Third trimester	0.55 (median)	0.46–0.85	NS

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The ATSDR also concluded that: "*Overall, the studies suggest that higher maternal blood levels of PFOA, PFOS, and PFHxS are associated with lower birth weights. However, the magnitudes of the decreases in birth weight are small and the biological significance of the finding is not known. Although low birth weight can be associated with increased infant mortality and morbidity, the deceases in birth weight were not great enough to result in an increased risk of low birth weight infants. In general, studies of highly exposed individuals did not find an increased risk for low birth weight infants (<2500 g) associated with high maternal PFOA levels (Darrow et al. 2013; Savitz et al. 2012b; Stein et al. 2009). Two studies (Nolan et al. 2009; Stein et al. 2009) reported lower risks of low birth weight infants at the highest maternal PFOA levels.*"

The ATSDR then provided their conclusion about the evidence on other developmental effects, reporting that: "*Other developmental end points have not been as widely investigated. The available data do not suggest an association between PFOA exposure and birth defect incidence (Darrow et al. 2013; Nolan et al. 2009[reported as 2010 in the text, page 196 and 208]; Savitz et al. 2012b; Stein et al. 2009) or PFOA exposure and increased risk of stillbirths or premature birth (Darrow et al. 2013; Hamm et al. 2010; Savitz et al. 2012b).*"

Inhalation exposure

Of the one study reported under 'Inhalation Exposure', by Grice et al. (2007), the ATSDR reported: "*In the study of self-reported health conditions and exposure to PFOS, conducted by Grice et al. (2007), mentioned earlier under Gastrointestinal Effects, the women were asked to fill a questionnaire that assessed pregnancy outcome history including number of pregnancies, the month and year the pregnancy ended, the outcome of the pregnancy, and the weight of the live-born children, as well as tobacco use. The results of the analyses showed that birth weight of singleton births, adjusted for maternal age at birth, gravidity, and smoking status did not vary between exposure groups.*"

United States Environmental Protection Agency (2016 a, b)

Studies reviewed

For PFOA, the US EPA reviewed the following studies:

- four studies on pregnancy-induced hypertension and preeclampsia (Stein et al. 2009; Savitz et al. 2012a; Savitz et al. 2012b; Darrow et al. 2013);
- one study on PFOA and birthweight in a high-exposure community (Darrow et al. 2013);
- two studies on PFOA and birth weight in the general population (birth weight among term births) (Fei et al. 2007; Monroy et al. 2008);
- seven studies of PFOA and birth weight in the general population (birth weight or low birth weight among all births) (Fei et al. 2007; Hamm et al. 2010; Whitworth et al. 2012a; Maisonet et al. 2012; Washino et al. 2009; Apelberg et al. 2007; Chen et al. 2012);
- two studies on PFOA and gestational age and preterm birth and risk of miscarriage in the high-exposure community (Darrow et al. 2014; Nolan et al. 2009, 2010);
- one study on duration of breast feeding (Fei et al. 2010);
- one study on PFAS (PFOA and PFOS) and cerebral palsy (Liew et al. 2014);
- three studies on preeclampsia, low birth weight and glomerular filtration rate (GFR) (Morken et al. 2014; Verner et al. 2015; Vesterinan et al. 2014).

The US EPA also commented on the systematic review by Johnson et al. (2014).

For PFOS, the US EPA reviewed the following studies:

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- two studies on pregnancy-related outcomes (Stein et al. 2009; Darrow et al. 2013);
- nine studies on PFOS and fetal growth (Grice et al. 2007; Apelberg et al. 2007; Fei et al. 2007; Andersen et al. 2010; Monroy et al. 2008; Washino et al. 2009; Hamm et al. 2010; Stein et al. 2009; Darrow et al. 2013);
- one study on PFOS and congenital cerebral palsy (Liew et al. 2014);
- one study on duration of breast feeding (Fei et al. 2010a).

Considerations and conclusions

In the 'Executive Summary' of the 'Health effects support document (PFOA)', the US EPA stated that: "*Human epidemiology data report associations between PFOA exposure and...pregnancy-induced hypertension and preeclampsia.*"

Further on in the 'Executive Summary', the US EPA stated that: "*Studies in the high-exposure community reported an association between serum PFOA and risk of pregnancy-related hypertension or preeclampsia, conditions related to renal function during pregnancy; this outcome has not been examined in other populations. An inverse association between maternal PFOA (measured during the second or third trimester) or cord blood PFOA concentrations and birth weight was seen in several studies.*"

In the 'Executive Summary' of the 'Health effect support document (PFOS)', the US EPA stated that: "*Numerous epidemiology studies have examined occupational populations at large-scale PFOS production plants in the United States and a residential population living near a PFOA production facility in an attempt to determine the relationship between serum PFOS concentration and various health outcomes. Epidemiology data report associations between PFOS exposure and developmental parameters [and other health effects]....Data also suggest a correlation between higher PFOS levels and ... decreased body weights in offspring, and other measures of postnatal growth.*"

Summaries of studies reviewed

Maternal outcomes – pregnancy-induced hypertension and preeclampsia (PFOA and PFOS)

For PFOA, the US EPA reviewed the same four studies as the ATSDR (Stein et al. 2009; Savitz et al. 2012a; Savitz et al. 2012b; Darrow et al. 2013). Summaries of these studies are included under the ATSDR section above.

The US EPA did make the following comment about all of the studies they reviewed: "*Each of these studies provides some evidence of an association between PFOA exposure and risk of pregnancy-induced hypertension or preeclampsia, with the most robust findings from the methodologically strongest study (Darrow et al. 2013). Maternal serum PFOA levels were positively associated with pregnancy-induced hypertension, with an adjusted OR per log unit increase in PFOA of 1.27 (95% CI: 1.15, 1.55). PFOS also was positively associated with pregnancy-induced hypertension.*"

Neonatal outcomes – birth measurements and diagnoses at birth (PFOA and PFOS)

The US EPA provided the following background and context about fetal growth in the 'Health effects support document (PFOA)': "*Many different measures of fetal growth can be used in epidemiology studies. Birth weight is widely available (as it is routinely collected in medical records and birth certificates). Low birth weight (defined as < 2,500 g) can be a proxy measure for preterm birth (particularly when accurate gestational age dating is not available). Other measures of fetal growth such as small for gestation age might more accurately reflect fetal growth retardation. Both birth weight and gestational age are characterized as two-part distributions, with a larger Gaussian portion representing term births and a longer tail representing preterm births. Increased risks of complications,*

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including infant mortality, are seen in preterm births (or low birth-weight births). When analyzed as a continuous measure, changes in birth weight might not be clinically significant, as small changes in the distribution among term infants do not result in a shift into the distribution seen in preterm infants (Savitz 2007; Wilcox 2010). This consideration differs from that of some other types of continuous measures, such as neurodevelopment scales, blood pressure, or cholesterol, in which shifts in the distribution are expected to move a greater proportion of the population into an "at risk" or "abnormal" level."

The US EPA reviewed nine studies on PFOA and birthweight (Darrow et al. 2013; Fei et al. 2007; Hamm et al. 2010; Whitworth et al. 2012a; Maisonet et al. 2012; Washino et al. 2009; Apelberg et al. 2007; Chen et al. 2012; Monroy et al. 2008). All of these studies were reviewed by the ATSDR, with summaries provided above.

In addition, the US EPA commented on the systematic review of human evidence for PFOA effects on fetal growth (Johnson et al. 2014). The US EPA reported that: "*The results from the meta-analysis showed that a 1 ng/mL increase in serum or plasma PFOA was associated with a -18.9 g (95% CI -29.8, -7.9) difference in birth weight.*"

For PFOS, the US EPA reviewed and summarised the findings of nine studies on PFOS and birth weight (Grice et al. 2007; Apelberg et al. 2007; Fei et al. 2007; Andersen et al. 2010; Monroy et al. 2008; Washino et al. 2009; Hamm et al. 2010; Stein et al. 2009; Darrow et al. 2013). These studies were reviewed by the ATSDR with summaries of studies provided above.

The US EPA reported that: "*Gestational age and preterm birth and risk of miscarriage were not associated with PFOA in the studies examining pregnancy outcomes in the high-exposure community (Darrow et al. 2014; Nolan et al. 2009, 2010). In contrast, PFOS was positively associated with miscarriage (Darrow et al. 2014).*"

Of the one study on PFAS exposure and congenital cerebral palsy Liew et al. (2014), the US EPA reported in the 'Health effects support document for PFOA': "*A subset of the Danish National Birth Cohort was evaluated for an association between prenatal PFAS exposure and the risk of cerebral palsy (Liew et al. 2014). A total of 156 cases of cerebral palsy were identified and matched to 550 randomly selected controls. Stored maternal plasma samples were analyzed for 16 PFAS and six compounds were quantifiable in >90% of the samples. For the cerebral palsy cases and matched controls, median maternal PFOA levels were 4.56 and 4 ng/mL, respectively, for males and 3.9 and 4.04 ng/mL, respectively, for females. Per natural-log unit increase in maternal PFOA level, the risk of developing cerebral palsy in males was significantly increased (RR=2.1; 95% CI 1.2, 3.6). Positive associations were also found with PFOS and perfluoroheptane sulfonate. No association was found between any PFAS level and risk of cerebral palsy in females.*"

On the same study, the US EPA reported in the 'Health effects support document for PFOS': "*For the cerebral palsy cases and matched controls, median maternal PFOS levels were 28.9 and 27.6 ng/mL, respectively, for boys and 27.5 and 26.2 ng/mL, respectively, for girls. A statistically-significant increased risk of developing cerebral palsy in boys (rate ratio [RR] = 1.7; 95% CI: 1.0–2.8) was detected per each natural-log unit increase in maternal PFOS level. A dose-response relationship between cerebral palsy and categorical PFOS exposures was detected in boys. Positive associations were also found with PFOA and perfluoroheptanesulfonate (PFHpS), and the results for PFOS remained unchanged after adjusting for multiple PFAS in the regression models. No association was found between any PFAS level and risk of cerebral palsy in girls, although this analysis was much more limited by smaller numbers.*"

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Of the one study on PFOS (and other PFAS, including PFOA) and duration of breastfeeding (Fei et al. 2010a), the US EPA summarised the study and commented about the findings: "*Fei et al. (2010a) reported on the effects of PFOS and PFOA on the length of breastfeeding. Self-reported data on the duration of breastfeeding were collected during the telephone interviews at 6 and 18 months after birth of the child. Statistically significant higher levels of PFOS were associated with a shorter duration of breastfeeding following adjustment for confounding.*"

The US EPA also commented that: "*This is an expected consequence because PFOS is transferred from the mother during breast feeding; thus, the shorter the lactation period the greater the proportion of the serum PFOS at the time of birth remains with the mother.*"

The US EPA provided more findings and observations, including reverse causality, about this study: "*A 20% increase risk for the mother in weaning before 6 months was noted in both primiparous [OR = 1.20; 95% CI: 1.04–1.37] and multiparous females, [OR = 1.20; 95% CI: 1.06–1.37]) for each 10 ng/mL increase in PFOS concentration in the maternal blood. A dose-response relationship was noted only among multiparous females (OR range: 1.55–2.64) based on categorical PFOS exposures, as only the highest PFOS quartile showed an elevated effect estimate [OR = 1.52; 95% CI: 0.89–2.60]) among primiparous females. For analyses based on termination of exclusive breastfeeding before 4 months, associations were only seen among multiparous females for both PFOS and PFOA exposures. Given that the associations between length of breastfeeding and PFOA and PFOS exposures were largely only seen among multiparous females, reverse causality is a possible explanation since reductions of current PFOS and PFOA levels may have resulted from longer lactation periods for previous children.*"

The US EPA provided the following context and evidence in relation to confounding by GFR. "*Preeclampsia is a condition that causes the pregnant female to be hypertensive because of reduced renal excretion associated with a decrease in GFR. Preeclampsia is often accompanied by low birth weight (Whitney et al. 1987). Morken et al. (2014) used a subset of the Norwegian Mother and Child Cohort to evaluate the relationship between GFR and fetal size. Participants included 470 preeclamptic patients and 483 nonpreeclamptic females; plasma creatinine measured during the second trimester was used to estimate GFR. For the overall cohort, for each mL/min increase in GFR, infant weight at birth increased 0.73–0.83 g, depending on the method used to calculate GFR. The increases were greater and statistically significant in females with preeclampsia. Differences were not statistically significant for the non-preeclamptic group. Morken et al. (2014) was not a study of perfluorochemicals and there were no serum measurements of any PFASs. However, because PFOA/PFOS serum levels are expected to be higher with a lower GFR, the finding stimulated examination of the GFR as it relates to serum PFAS levels and the low birth weight identified in the epidemiology studies (Verner et al. 2015; Vesterinen et al. 2014).*"

The US EPA made the following comments about the pharmacokinetic model by Verner et al. (2015): "*Verner et al. (2015) modified the human pregnancy/lactation PK model of PFOA/PFOS by Loccisano et al. (2013) described in section 2.6.1 to evaluate the association between GFR, serum PFOA levels, and birth weight. When GFR was accounted for in the model simulations, the reduction in birth weight associated with increasing serum PFOA/PFOS was less than that found by the author's meta-analysis of the same data. This finding suggests that a portion of the association between prenatal PFOA and birth weight is confounded by maternal GFR differences within the populations studied. The true association for each 1 ng/mL increase in PFOA could be closer to a 7-g reduction (95% CI -8, -6) compared to the 14.72-g reduction (95% CI: - 8.92, -1.09) predicted by meta-analysis of the epidemiology data without a correction for low GFR as observed in individuals with pregnancy-induced hypertension or evidence of preeclampsia..... The true association for each 1 ng/mL increase in PFOS*

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could be closer to a 2.72 g reduction (95% CI: −3.40 to −2.04) in body weight compared to the 5.00 g[35] *reduction (95% CI: −21.66 to −7.78) predicted by meta-analysis of the epidemiology data without a correction for low GFR.."*

Dutch National Institute for Public Health and the Environment (RIVM 2017).

The RIVM reviewed international authority reports, systematic reviews and epidemiological studies that examined the relationship between serum PFOA concentration and birth weight. The RIVM also considered the findings of international authorities and epidemiological studies on PFOA exposure and pregnancy-induced hypertension and preeclampsia.

Studies reviewed

For PFOA and birth weight, the RIVM reviewed:

- five international authority reports (C8 Science Panel, 2011; ATSDR, 2015; ECHA-RAC, 2015; DWQI, 2016; US EPA, 2016,a) that had reviewed the evidence on PFOA and birth weight;
- two systematic reviews on PFOA and birth weight (Lam et al. 2014; Bach et al. 2015a).

The RIVM also reviewed fifteen studies that examined a relationship between serum PFOA concentration (of the mother during pregnancy or the umbilical cord) and birth weight, including:

- ten studies in the general population (Apelberg et al. 2007; Chen et al. 2012; Hamm et al. 2010; Lee et al. 2013; Monroy et al. 2008; Washino et al. 2009; Whitworth et al. 2012b; Fei et al. 2007; Ashley-Martin et al. 2016);
- four studies in high-exposure communities (the C8 Health Project community) (Darrow et al. 2013; Savitz et al. 2012a; Savitz et al. 2012b; Stein et al. 2009);
- one study in an occupational study population (Wu et al. 2012).

For PFOA exposure and pregnancy-induced hypertension and preeclampsia, the RIVM reviewed:

- four international authority reports (C8 Science Panel, 2011; ATSDR, 2015; ECHA-RAC, 2015; US EPA, 2016,a); and
- six epidemiological studies that examined the relationship between plasma, serum or full blood PFOA concentrations and pregnancy-induced hypertension and preeclampsia, including:
  - two studies in the general population (Starling et al. 2014a; Starling et al. 2014b);
  - four studies in a high-exposure community (the C8 Health Project community) (Darrow et al. 2013; Savitz et al. 2012 b; Savitz et al. 2012a; Stein et al. 2009).

Considerations and conclusions

The RIVM stated in their 'Synopsis': "*The clearest evidence has been found for a relationship between exposure to PFOA and [several health effects] and a lower birth weight*", and that "*Indications have been found for a higher risk of pregnancy-induced hypertension and preeclampsia.*"

In the 'Discussion and conclusions' section, for birth weight, the RIVM concluded: "*Therefore, most organizations agree that associations with birth weight have been found in the general population, but there is some debate as to whether or not these associations can be explained by other factors. In*

[35] The Panel believes the figures to be wrong as these two point estimates do not lie within their 95%CI – It seems a possible explanation is transposition: the 95%CI for the 14.72 g reduction is −21.66 to −7.78 and the 95% CI for the 5g reduction is - 8.92, -1.09.

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addition, inconsistent results were produced in studies examining an association between serum PFOA concentrations and birth weight in high-exposure communities (that reflected much larger exposure contrasts of PFOA and had more statistical power than the general population studies). As a consequence, the association with birth weight observed in the general population cannot be extrapolated to higher blood concentrations of PFOA."

Summaries of studies reviewed

Birth weight

Of the 15 studies reviewed by the RIVM (Apelberg et al. 2007; Chen et al. 2012; Hamm et al. 2010; Lee et al. 2013; Monroy et al. 2008; Washino et al. 2009; Whitworth et al. 2012b; Fei et al. 2007; Ashley-Martin et al. 2016; Darrow et al. 2013; Savitz et al. 2012a; Savitz et al. 2012b; Stein et al. 2009; Wu et al. 2012), 13 were reviewed by ATSDR (2015) and US EPA (2016a) with summaries of the studies reported earlier in this section. The two studies that RIVM reviewed that the ATSDR and US EPA did not review were the studies by Ashley-Martin et al. (2016) and Wu et al. (2012).

Of the study by Ashley-Martin conducted in the general population, the RIVM reported: "*Ashley-Martin et al. (2016) found that babies with a higher gestational weight gain were more likely to have above-median cord serum PFOA concentration (i.e. >0.39 ng/mL), compared with below-median cord blood PFOA concentrations.*"

Of the study by Wu et al. (2012), the RIVM reported that: "*One study examined an occupational study population (serum PFOA concentrations range: 5.5-58.5 ng/mL) and found an association between higher serum PFOA concentrations in pregnant women (selected from workers at an electronic waste recycling area and from the general population; serum PFOA concentrations ranged from 4.4 to 58.5 ng/mL) and lower birth weight (Wu et al. 2012).*"

Pregnancy-induced hypertension and preeclampsia

Of the six studies on pregnancy-induced hypertension and preeclampsia (Starling et al. 2014a; Starling et al. 2014b; Darrow et al. 2013; Savitz et al. 2012 b; Savitz et al. 2012a; Stein et al. 2009), four (Darrow et al. 2013; Savitz et al. 2012 b; Savitz et al. 2012a; Stein et al. 2009) were reviewed by the ATSDR(2105) and US EPA (2016a), with summaries provided above.

Of the two general population studies (Starling et al. 2014a; Starling et al. 2014b) reviewed by the RIVM, the following summaries were reported: "*Two studies were performed among the general population (i.e. both in the Norwegian Mother and Child Cohort Study), in which plasma PFOA concentrations were measured up to 5.15 ng/mL (i.e. 95th percentile). Both found no association with validated preeclampsia (Starling et al. 2014a) or the biomarkers of preeclampsia (Starling et al. 2014b).*"

The RIVM made the following comment about the measurement of pregnancy-induced hypertension and preeclampsia in studies: "*Pregnancy-induced hypertension and preeclampsia can be measured by self-reports or by birth certificate codes. It has been discussed that pregnancy-induced hypertension and preeclampsia are often reported incorrectly, either through self-reports or retrieved from birth certificates (Savitz et al. 2012b). For example, Darrow et al. (2013) discussed the fact that pregnancy-induced hypertension recorded on the birth record generally does not specify whether it concerns pregnancy-induced hypertension or preeclampsia.*"

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Food Standards Australia New Zealand (FSANZ, 2017)

FSANZ, in their 'Hazard assessment report for PFOS, PFOA and PFHxS', conducted a literature review to attempt to answer the question: Is blood PFOA or PFOS concentration related to infant birthweight?

Studies reviewed

In 'Appendix 1: Observational studies of PFAS and birthweight,' FSANZ examined and reviewed international authority reports, systematic reviews and epidemiological studies including:

- three international authority reports (EFSA (2008); US EPA PFOS(2014); US EPA PFOA (2014);
- two systematic reviews (Johnson et al. 2014; Bach et al. 2015a). FSANZ noted that a third systematic review by Verner et al. (2015) was examined but: "*did not have additional studies compared to Johnson et al. (2014)*";
- thirteen studies included in the literature review (Apelberg et al. 2007; Fei et al. 2007; Washino et al. 2009; Kim S and Choi, 2011; Hamm et al. 2010; Whitworth et al. 2012b; Maisonet et al. 2012; Chen et al. 2012; Fromme et al. 2010, Wu et al. 2012; Darrow et al. 2013; Bach et al. 2016; Wang et al. 2016);
- ten studies included in the literature review but only for qualitative, not quantitative assessment (Grice et al. 2007; Monroy et al. 2008; Kim SK & Lee, 2011; Arbuckle et al. 2013; Lee et al. 2013; Robledo et al. 2015; de Cock et al. 2016; Lauritzen et al. 2016; Shi et al. 2017[36]; Alkhalawi et al. 2016).

Considerations and conclusions

With regard to birth weight FSANZ concluded in the 'Executive Summary', under 'Human Studies': "*FSANZ has reviewed the available human epidemiological information and concluded that while there is evidence of these associations [decreased birth weight], it is not possible to determine whether PFOS or PFOA causes the changes, or whether other factors are involved. As these are observational studies, FSANZ considers that the meaning and clinical significance of the associations for PFOS and PFOA for decreased birth weight in humans are uncertain and should be treated with caution.*"

In the 'Discussion and conclusions' PFOS section, FSANZ made the following comment and conclusions on the human evidence base for birth weight: "*FSANZ has reviewed the available epidemiological information regarding PFOS and PFOA and birthweight. It is noted that the blood concentrations in the human studies is orders of magnitude lower than that found in animal studies showing an effect on birthweight. Overall the studies with numerical data report an association, but missing quantitative data from studies reporting no effect raises the possibility of selective reporting or publication bias affecting the body of evidence. FSANZ has concluded that it is currently not possible to determine whether the association reflects a causal relationship or is the result of a third factor that alters both PFAS concentration and birthweight. For example, changes in GFR that occur during pregnancy would be expected to affect both birthweight and the rate of excretion of PFAS. This may require further investigation.*"

While FSANZ did not review epidemiological studies on pregnancy-induced hypertension, preeclampsia and congenital abnormalities, in the 'PFOS – human data – fertility, pregnancy and birth outcomes' section of the report, FSANZ made the following statements: "*A positive association with gestational hypertension/pre-eclampsia has been found in some studies, while others did not find a*

[36] Note the FSANZ referenced this as Shi et al. 2016, as they used a Epub before print version.

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statistically significant effect. No association between PFOA exposure and risk of congenital abnormalities or complications of labour has been found. An association between PFOA exposure and significant reduction in the duration of breastfeeding was reported in one study."

In the 'PFOA – human data – fertility, pregnancy and lactation' section, FSANZ commented on the human evidence base for birth weight, and provided an overall summary of the studies they reviewed: *"FSANZ has reviewed the evidence for an association between PFOA or PFOS and birthweight. There were two systematic reviews of PFOA and FSANZ has updated these reviews by replicating one of the search strategies in PubMed to find more recently published studies (Appendix 1). A number of inconsistencies in the analysis and presentation of data were identified, for example, data were typically log transformed, using either base 10 or natural logarithms, suggesting that the association was not linear, but most authors did not describe examining regression diagnostics to determine if the transformation was appropriate. Some authors also presented results for linear or categorical analyses, but generally did not comment on which was the best fit for the data. It was noted that some papers stated that there was no association but did not provide usable data describing this. One systematic review conducted a quantitative meta-analysis that assumed that the relationship between PFOA and birthweight was linear, although this assumption was not justified or explained by the authors. FSANZ has identified and added additional studies to the above-mentioned meta-analysis. As a result of including these studies, the effect of PFOA on birthweight was reduced. Most of the studies included in the meta-analysis examined populations with PFOA concentrations <20 ng/mL. The other systematic review did not conduct a meta-analysis.*

Neither of the systematic reviews considered how the results of studies that they excluded owing to data format problems, would have affected their conclusion. However, in the case of PFOA, FSANZ is of the opinion that these excluded studies reflect the range of results shown in the meta-analysis. Most studies examined associations for PFOA and PFOS separately and did not conduct a mutually-adjusted analysis despite often noting a substantial correlation between PFOA and PFOS. Overall the results show a steep decline in birthweight at low blood concentrations of PFAS, which levelled off to a plateau or near-plateau at higher concentrations. The mechanism by which PFASs could lead to such a dose-response curve is not clear.

FSANZ notes that the concentration in blood in the human studies described above is approximately 1,000-fold lower than that found in animal studies showing an effect on birthweight. It is not certain whether the association observed reflects a causal relationship between PFAS and birthweight or is the result of a third factor. For example, Verner et al. (2015) suggest that both would be affected by the changes in GFR that occur during pregnancy.

In summary, FSANZ has found that overall the studies with numerical data report an association between blood PFAS concentration and decreased birth weight. Missing quantitative data from studies reporting no effect raises the possibility of selective reporting or publication bias affecting the body of evidence. The shape of the association is not clear. It is not possible to determine whether the association reflects a causal relationship or is the result of a third factor that alters both PFAS concentration and birthweight, or may have been overstated owing to selective reporting or publication."

In the 'Discussion and conclusions' PFOA section, FSANZ made the following comments: *"PFOA is highly persistent in human beings, with an elimination half-life measured in years. This persistence gives rise to some concern, although PFOA appears to have few adverse effects. Toxic mechanism(s) in humans are unclear, but epidemiological evidence suggests that PFOA may be positively associated with serum levels of .....PFOA may also be positively associated with risk of gestational hypertension,*

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and with a risk of decreased birth weight.....FSANZ has identified a number of deficiencies in the available epidemiological studies and meta-analysis. It is noted that gestational hypertension is a known risk factor for decreased birth weight, and also decreases GFR, which would lead to decreased renal excretion of PFOA."

Summary of studies reviewed

FSANZ commented on the systematic reviews by Johnson et al. (2014) and Bach et al. (2015a), and updated the literature review, including providing graphs. The reader is referred to pages 103 -113 of the 'Hazard assessment report' for more detail.

Confounding was raised by several of the key reports and systematic reviews. FSANZ made the following commentary on residual confounding: *"One notable feature in the papers is that many authors describe the effect of PFOA and PFOS on birthweight after adjusting for various factors which might confound the relationships, such as gestational age, parity, maternal smoking of body habitus. These analyses are performed separately for PFOS and PFOA. Authors sometimes describe the correlation between PFOS and PFOA in their data sets (Table A1.9). However, there does not seem to have been any consideration of whether the analysis examining PFOA should be adjusted for PFOS concentrations and vice versa. For example, in the study of Chen et al. (2012) PFOS has a much larger coefficient than PFOA and so it is possible that the PFOA result might be confounded by PFOS.*

An exception is the analysis of the C8 cohort by Darrow et al. (2013) who found that simultaneously including both PFOS and PFOA in the same model halved the small effect on birthweight observed for PFOA, [but] did not change the effect for PFOS importantly (Table A1.10). In other words, the effect seen for PFOA was partly due to PFOA acting as a surrogate for PFOS. The correlation between the two PFAS in this study was lower than any other shown in Table A1.9 and raises questions about whether there may be confounding of the PFOA result shown in the Johnson meta-analysis. This study is unusual among the available studies in that the median concentration of PFOS and PFOA was almost the same in their subjects and it has a larger sample size than any study included in the meta-analysis of Johnson et al. (2014).

Furthermore, some authors have measured other PFAS and sometimes other chemicals such as PCBs in the same blood sample and these may or may not have associations with birthweight. Only rarely do authors comment on whether any of these other contaminants confound the relationships of PFOS and PFOA with birthweight. For example, Lauritzen et al. (2016) state that only the odds ratio for the association between PFOA and being born small-for-gestational age remained statistically significant when PFOA, PFOS and five organo-chlorine chemicals were included in the same model."

FSANZ also provided a brief summary of birthweight in Australia. FSANZ reported that Table A1.11 (see page 114 of the 'Hazard assessment report'): *"shows that boys are about 200 g heavier than girls at any gestational age. Moreover, birthweight increases by nearly one kilogram across the range of gestational ages that are regarded as full term (greater than 36 weeks and less than 42 weeks) and between the mean birthweight of singleton and twin babies. Consequently, small variations in proportion of boys and girls or gestational ages or the presence of twins in the groups being compared could potentially have lead to the small difference in birthweight found in some of the studies. The meta-analysis result of -11.9 g per ng/mL PFOA predicts -238 g birthweight for a concentration of 20 ng/mL PFOA compared to zero concentration. It is equivalent to a shift of more than half a standard deviation in the birthweight distribution. This is a similar order of magnitude as the difference in birthweight between boys and girls or between the babies born to Indigenous and non-Indigenous in the Northern Territory. As noted elsewhere, the data on which the calculation is based do not necessarily allow a causal association to be drawn."*

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FSANZ provided the following 'Summary' at the end of the literature review: 'Observational studies of PFAS and birthweight': "*FSANZ's update of an existing meta-analysis found that a 1ng/mL increment in PFOA was associated with a decrease of 11.9 g in birthweight. Most studies contributing to the analysis examined populations with PFOA concentrations <20 ng/mL and FSANZ does not believe the result should be extrapolated to higher PFOA concentrations on a per ng/mL basis. The graphical data indicate that the association of birthweight both PFOA and PFOS may attenuate at higher blood concentations. This analysis excludes a number of studies which did not report their results in a suitable format for inclusion. It also assumes that the relationship is linear whereas many of the authors of the underlying paper used a logarithmic transformation when analysing their data. It is possible that the body of evidence contains selective reporting or publication bias in the body of literature leading to an overrepresentation of studies reporting significant adverse effects on birthweight. Furthermore most studies examined associations for PFOA and PFOS separately and did not conduct a mutually-adjusted analysis despite often noting a substantial correlation between PFOA and PFOS. Other explanations of the association are also possible, such as the presence of a physiological change leading to increases in blood PFAS and decreases in birthweight.*"

6.6.3. Systematic reviews

Johnson et al. (2014)

Johnson et al. (2014) applied The Navigation Guide[37] systematic review methodology to determine whether developmental exposure to PFOA affects fetal growth.

Studies reviewed

Johnson et al. identified 18 human studies, published up to May 2012, including:

- ten studies that were included in the meta-analysis of the association between PFOA exposure and measures of fetal growth (Apelberg et al. 2007; Chen et al. 2012; Fei et al. 2007; Fei et al. 2008a; Fromme et al. 2010; Hamm et al. 2009; Kim et al. 2011; Maisonet et al. 2012; Washino et al. 2009; Whitworth et al. 2012b). The authors noted that two studies (Fei et al. 2007; Fei et al. 2008a) are studies of the same population.
- Nine studies were excluded from the meta-analysis (Arbuckle et al. 2013; Halldorsson et al. 2012; Kim et al. 2011; Monroy et al. 2008; Nolan et al. 2009; Savitz et al. 2012a; Savitz et al. 2012b study I; Savitz et al. 2012b study II; Stein et al. 2009).

With respect to the meta-analysis, Johnson et al. reported: "*We combined data from 10 studies in the meta-analyses of the association between PFOA exposure and measures of fetal growth. Within the 10 studies, there were 9 data sets on birth weight, 5 data sets on length, 4 data sets on ponderal index, and 4 data sets on chest circumference.*"

Considerations and conclusions

In the 'Results' section of the 'Abstract', Johnson et al. stated: "*Through meta-analysis, we estimated that a 1-ng/mL increase in serum or plasma PFOA was associated with a -18.9 g (95% CI: -29.8, -7.9) difference in birth weight. We concluded that the risk of bias was low, and we assigned a "moderate" quality rating to the overall body of evidence.*"

In the 'Results' section of the paper, Johnson et al. also included other measures of fetal growth in a smaller meta-analysis and reported: "*We found through meta-analysis that PFOA exposure was also*

[37] Please refer to the information about Johnson et al. (2014) in Section 5 for further information about the Navigation Guide methodology.

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associated with lower values of other fetal growth measures at birth (Table 5). A 1 ng/mL increase in serum or plasma PFOA was associated with a –0.1 (95% CI: –0.1, –0.02) cm change in birth length, a –0.01 (95% CI: –0.03, 0.01) change in ponderal index, and a –0.03 (95% CI: –0.1, 0.01) cm change in head circumference."

In the 'Discussion' section of the paper, Johnson et al. stated: "*Our conclusion that the human data were sufficient was based on "moderate" quality evidence, a meta-analysis estimating a decrement in birth weight in relation to PFOA exposure in which we judged that the confidence bounds were narrow, and our confidence that a new study would be unlikely to have an effect estimate that would change the overall effect estimate of the meta-analysis. The smaller meta-analyses of other fetal growth measures were also consistent in the direction of the effect estimate."*

In the 'Conclusion' section of the paper, the authors stated: "*On the basis of our evaluation and the Navigation Guide criteria, we concluded that there is sufficient evidence of an association between PFOA exposure and reduced fetal growth. There may be remaining uncertainty. However, we investigated residual confounding and evidence for reverse causality via reduced renal clearance, and despite the cross-sectional nature of the human evidence, our judgment was that chance, bias, and confounding could be ruled out with reasonable confidence."*

Summaries of studies reviewed

Birth weight

In the 'Results' section of the paper, the authors noted that the nine data sets in the meta-analysis included 4,149 births. Johnson et al. made a number of comments about the papers they reviewed, including: "*We judged the study of Savitz et al. (2012b) to have "probably high" risk of bias for the exposure assessment domain based on its retrospectively modeled maternal serum PFOA"*; and that: "*Only one study that we included in the meta-analysis for birth weight was assigned a high risk of bias for confounding (Fromme et al. 2010). This study was small and contributed little weight (< 1%) to the overall effect estimate."*

In the 'Discussion' section, Johnson et al. considered confounding, stating that: "*The existence of unmeasured confounders will always be possible with observational studies, but we decided to not let this undermine our ability to make a statement about the available data. Additional information that may arise can and should inform future conclusions. We felt that we could rule out confounding "with reasonable confidence" (Table 3, definition of "Sufficient evidence of toxicity") based on our assessment of the available data. We did not find any evidence suggesting substantial residual confounding. To get an idea of how residual confounding may influence the effect estimate of the association between PFOA exposure and birth weight, we conducted a meta-analysis using unadjusted estimates. Although the unadjusted meta-analysis had a larger effect estimate (i.e., adjusting for confounders attenuated the estimate), the CIs were wider and there was substantial hetero geneity among the unadjusted studies. As in the Bradford Hill considerations for causation, the GRADE approach considers consistency in effect estimates when evaluating confidence in the association and rating the quality of evidence (Schunemann et al. 2011). Because the effect estimates were more homogeneous after adjustment, we considered it more likely that the adjusted estimate was closer to the true association. If adjustment resulted in more hetero-geneity, we would have been more concerned with potential residual confounding. Although this analysis does not prove that residual confounding does not exist, it did not uncover any evidence of unmeasured confounders, and we considered this as support for our interpretation that substantial effects of residual confounding are unlikely."*

In the 'Discussion' section, Johnson et al. reported that they: "*also considered that studies of the population that was highly exposed to PFOA through groundwater contamination found little evidence*

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of an association with low birth weight (Nolan et al. 2009; Savitz et al. 2012a, 2012b; Stein et al. 2009) and on the continuous scale of birth weight (Savitz et al. 2012b). However, these studies differed from the studies included in our main metaanalysis with respect to exposure estimation as described in the risk of bias assessment; that is, these studies estimated exposure based on residence (ecological exposure), retrospective modeling of several parameters, or maternal post natal exposure, and these studies primarily examined odds of low birth weight (< 2,500 g) rather than a change in birth weight on a continuous scale. We did not conduct a metaanalysis with odds ratios for low birth weight because so few studies (three populations) provided this measure and because a continuous change in birth weight provides more information than dichotomized birth weight. We did, however, conduct a meta-analysis including an effect estimate from one of the studies that retrospectively modeled exposure (Savitz et al. 2012b) and found minimal change in the results (Table 5); these results did not change our conclusions."

Comment on confounding by glomerular filtration rate

Johnson et al., again in the 'Discussion' section, reported they considered alternative hypotheses for the relationship between PFOA exposure and birth weight and commented on the study by Whitworth et al. (2012b): "*...an author of one of the studies included in our metaanalysis proposed that the pharmacokinetics of PFOA during pregnancy may influence the relationship between PFOA body burdens and fetal growth such that associations may be due to reverse causality (Whitworth et al. 2012b). That is, mothers of lower-birth-weight babies might experience less plasma volume expansion and therefore reduced clearance of PFOA through glomerular filtration.*" Johnson et al. investigated the plausibility of an alternative hypothesis of reverse causation explaining "*we searched for evidence on the relationship between fetal growth and glomerular filtration rate, including relationships within the hypothesized causal pathway (i.e., between fetal growth and plasma volume expansion, and between plasma volume expansion and glomerular filtration rate). Overall we found limited and inconsistent data that were inadequate to draw conclusions on the association between fetal growth and glomerular filtration rate. Thus, although we did not find evidence to suggest that the observed association between PFOA exposure and fetal growth can be explained, wholly or partially, by reverse causality, we cannot disprove this hypothesis. Nevertheless, we decided at this time there was no compelling evidence of reverse causation to justify altering our conclusions about the strength of the evidence.*"

Lam et al. (2014)

Lam et al. (2014) is an extension of Johnson et al. (2014), with the same authors and studies considered.

Studies reviewed

Lam et al. (2014) identified the same 18 epidemiology studies that Johnson et al. (2014) identified.

Considerations and conclusions

Lam et al. reported in the 'Results' section of the 'Abstract' that: "*We rated both the human and nonhuman mammalian evidence as "moderate" quality and "sufficient" strength. Integration of these evidence ratings produced a final strength of evidence rating in which review authors concluded that PFOA is "known to be toxic" to human reproduction and development based on sufficient evidence of decreased fetal growth in both human and non-mammalian species.*"

In the 'Conclusion' of the 'Abstract', Lam et al. stated: "*We concluded that developmental exposure to PFOA adversely affects human health based on sufficient evidence of decreased fetal growth in both human and non-human mammalian species.*"

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In the 'Results' section of the paper, Lam et al. reported again the findings of the meta-analysis on birth weight and of smaller meta-analysis on other fetal growth measures as reported above under Johnson et al. (2014).

Also in the 'Results' section, Lam et al. reported: "*Our consensus for the human evidence was that the overall quality of evidence was "moderate," and we had a high level of confidence in an association between decreased birth weight and increased exposures to PFOA. Comparing our consensus on these considerations to the definitions of "sufficient evidence of toxicity," "limited evidence of toxicity," "inadequate evidence of toxicity," or "evidence of lack of toxicity," we agreed that a) our findings met the definitions for "sufficient evidence of toxicity" (i.e., a positive relationship was observed between exposure and outcome where chance, bias, and confounding could be ruled out with reasonable confidence); b) the available evidence included results from one or more well-designed, well-conducted studies; and c) the conclusion was unlikely to be strongly affected by the results of future studies.*"

In the 'Discussion' section, Lam et al. commented again on the human data: "*For the human data, we concluded that there was sufficient evidence of an association based on a) a transparent collective rating of the evidence as "moderate" quality; b) a meta-analysis estimating a reduction in birth weight in relation to PFOA exposure for which confidence bounds were sufficiently narrow and did not include zero; and c) our confidence that it would be unlikely for a new study to have an effect estimate that could substantially change the overall effect estimate of the meta-analysis (Johnson et al. 2014).*"

The authors also commented about the method they used: "*Application of the method produced a clear and concise conclusion by the authors of this review: that exposure to PFOA is "known to be toxic" to human reproduction and development based on sufficient evidence of decreased fetal growth in both human and non-human mammalian species.*"

Comment on confounding by glomerular filtration rate

Lam et al. also discussed the hypothesis of reverse causation and lower birth weight taking into account the animal evidence, stating the following: "*In recent years, several scientists have hypothesized that maternal and fetal physiology may influence measured blood levels indicating an exposure; in particular for PFOA and reduced birth weight, these associations may be due to reverse causality whereby women who have smaller babies have higher measures of PFOA as a result of a lower glomerular filtration rate caused by lower plasma volume expansion (Loccisano et al. 2013; Savitz 2007; Whitworth et al. 2012b). If this reverse causality hypothesis were true, it would explain some or all of the relationship observed in human cross-sectional studies documenting an inverse association between fetal growth and prenatal exposure to exogenous chemicals with renal clearance, such as PFOA.*

We considered this hypothesis and its supporting scientific evidence in the context of the final conclusion of our review and decided that it did not undermine our findings for two reasons. First, this hypothesis is not relevant to associations found in animal studies. In our review of PFOA, the experimental animal evidence was robust and mirrored the human evidence, lending support for the association between PFOA exposure and low birth weight (Koustas et al. 2014). Second, we systematically reviewed the literature for evidence of the relationship between birth weight and maternal glomerular filtration rate (see Supplemental Material, "List of studies included in systematic review of the relationship between birth weight and maternal glomerular filtration rate") and concluded that there is currently insufficient evidence to support the reverse causality hypothesis for associations between fetal growth and maternal glomerular filtration rate in humans. Additional research is needed to confirm or disprove this hypothesis. Thus, although we cannot disprove reverse causality, we have found no conclusive evidence currently available to justify altering our conclusions regarding the strength of human evidence. However, review authors were cognizant of the potential for these

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physiological factors associated with pregnancy to account for the negative association of PFOA with low birth weight. A preliminary study based on physiologically based pharmacokinetic (PBPK) modeling of a meta-analysis of seven published epidemiology studies suggested that a portion of the association between PFOA and low birth weight was attributed to confounding by glomerular filtration rate (Verner et al. 2014). Another study investigating hematologic changes and pregnancy outcomes similarly showed that low hemoglobin in late pregnancy was associated with low birth weight, but the association disappeared after adjusting for plasma volume (Whittaker et al. 1996). However, there remains a lack of human evidence that this is indeed the case for external chemical exposures. Although the reverse causation hypothesis is reasonable and warrants further investigation, without stronger evidence—and in light of the strength of the animal data—we believe that downgrading the final conclusion for "sufficient" for the human evidence was not justifiable at this time."

Roth and Wilks (2014)

Roth and Wilks reviewed studies published after 2006, that evaluated the neurodevelopmental end point of head circumference in children, in their systematic review of the epidemiological literature on neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals, using a quality assessment scheme. The PFCs[38] (PFAS) in these studies included PFOS, PFOA, PFNA, PFUA, PFHxS.

Studies reviewed

Roth and Wilks (2014) evaluated five studies (Apelberg et al. 2007; Chen et al. 2012; Fei et al. 2008b; Lee et al. 2013; Washino et al. 2009), including

- two studies that used umbilical cord blood samples of PFAS (Apelberg et al. 2007; Chen et al. 2012); and
- three studies that used maternal blood samples of PFAS (Fei et al. 2008b; Lee et al. 2013; Washino et al. 2009)

Roth and Wilks assigned an overall quality rating of 'high' to two studies (Apelberg et al. 2007; Fei et al. 2008b), a 'moderate' quality rating to two studies (Chen et al. 2012; Washino et al. 2009), and a 'low' quality rating to the study by Lee et al. (2013).

Considerations and conclusions

In the 'Results' section of the paper, Roth and Wilks stated that: "*Collectively, the epidemiological evidence currently does not support a strong causal association between PBDEs and/or PFCs and adverse neurodevelopmental and neurobehavioural out-comes in infants and children. However, despite some limitations (dose–response, strength of association, sample size, consistency), these studies raise questions that require further investigation.*" The authors also noted that: "*Measurement of head circumference is inherently associated with a larger degree of error than other types of anthropometric measurements, e.g. due to head molding during vaginal deliveries (Apelberg et al. 2007). Besides, as noted by Savitz (2007), small biological variations in the normal range of distribution for birth parameters such as weight, length or head circumference are common in a population without necessarily bearing clinical significance.*"

In the 'Conclusion' section of the paper, Roth and Wilks stated that: "*In some instances associations were only observed for specific chemicals for a given health outcome, and nearly all studies could have been confounded by exposures to other environmental contaminants. There is little evidence for "class effects", and chemicals have to be evaluated on a case-by- case basis, though many inconsistencies*

[38] PFC - Perfluorinated (perfluoroalkyl) chemicals

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have to be reported for some PBDE congeners and to a lesser degree PFOS. The only consistent results were obtained for PFOA, for which none of the studies evaluated have shown any developmental or behavioural effects on all the different functional domains assessed."

Summaries of studies reviewed

Roth and Wilks stated of the five studies they reviewed: "*Five PFC studies included head circumference as a neurodevelopmental end point. There are many inconsistencies in the outcome, regardless of the study quality rating. Apelberg et al. (2007) found that both PFOA and PFOS were significantly associated with reduced head circumference, but this was only observed for vaginal deliveries, after adjustment for the delivery mode in the model. Fei et al. (2008b) did not report any statistically significant association between PFOS and head circumference, but a negative non-significant association for PFOA. Chen et al. (2012) reported that only PFOS was significantly inversely associated with head circumference, but not PFOA, perfluorononanoic acid (PFNA) or perfluoroundecanoic acid (PFUA). These findings contrast with the results from Washino et al. (2009) who found no statistically significant association between head circumference and PFOA but a negative non-significant association for PFOS, or from Lee et al. (2013) who reported no statistically significant association for PFOS, PFOA and perfluorohexane sulfonate (PFHxS)."*

Regarding study methodology and analysis, Roth and Wilks commented that: "*Only one study reported a dose–response for PFOS after categorization of PFOS levels into quartiles (Chen et al. 2012). Four studies added smoking during pregnancy as a potential confounder to their statistical model, and only a single study controlled for alcohol (Fei et al. 2008b), but none adjusted for co-exposures to other environmental contaminants (see supplementary material S5)."*

Bach et al. (2015)

Bach et al. in their systematic review considered PFOS and PFOA concentrations with respect to birth weight, low birth weight and small for gestational age.

Studies reviewed

Bach et al. reviewed 14 studies (Inoue et al. 2004; Apelberg et al. 2007; Fei et al. 2007; Monroy et al. 2008; Stein et al. 2009; Washino et al. 2009; Hamm et al. 2010; Chen et al. 2012; Maisonet et al. 2012; Whitworth et al. 2012b; Wu et al. 2012; Arbuckle et al. 2013; Darrow et al. 2013; Lee et al. 2013).

Considerations and conclusions

In the 'Results' section of the 'Abstract', Bach et al. reported: "*In utero PFOA exposure was associated with decreased measures of continuous birth weight in all studies, even though the magnitude of the association differed and many results were statistically insignificant. PFOS exposure and birth weight were associated in some studies, while others found no association."*

The authors' 'Conclusion' in the 'Abstract' was: "*Higher PFOS and PFOA concentrations were associated with decreased average birth weight in most studies, but only some results were statistically significant. The impact on public health is unclear, but the global exposure to PFASs warrants further investigation."*

In the 'Conclusion' section of the paper, Bach et al. provided more context and detail about the impact on public health: "*While high PFOA and PFOS exposures in pregnancy were associated with lower average birth weights in human newborns in most studies, not all results were statistically significant. The existing data is insufficient to confirm or reject a certain association between PFASs*

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exposure and fetal growth. Knowledge on the influence of PFASs other than PFOS and PFOA on fetal growth is sparse and needs to be investigated in future studies. Although any risk to the individual pregnant woman and her child due to PFOS and PFOA exposures seems small based on the limited information available, the widespread environmental presence of these and other PFASs warrants continued investigation."

Summaries of studies reviewed

In the 'Discussion', Bach et al. made the following comments on the studies they reviewed: "*Higher PFOA levels were associated with lower average birth weight in eight studies of a total of 5046 pregnancies, even though the magnitude and significance of associations differed. Data are insufficient to determine a safe lower PFOA exposure level, but statistically significant associations were only demonstrated when median serum or plasma levels during pregnancy were above approximately 3 ng/mL (Fei et al. 2007, Maisonet et al. 2012, Wu et al. 2012). However, one study with median levels above this level found no significant association (Darrow et al. 2013). The value of 3 ng/mL is similar to the present day average PFOA exposure in US women in the fertile age (Jain 2013).*

Six out of eight studies equivalent to 4627 out of 4894 pregnancies found lower average birth weight with higher levels of PFOS, but most of the results were not statistically significant, and in studies with high average exposure levels, there was not a higher proportion of significant associations. Studies that examined birth weight as a predictor of PFOA and PFOS levels provided little evidence of an association (Lee et al. 2013, Monroy et al. 2008). We found some suggestion that PFOS might be associated with LBW, but overall, the evidence concerning associations between PFOS or PFOA and other proxy measures of fetal growth restriction such as LBW, SGA, and birth weight z-scores was limited. This may be due to relatively small associations with birth weight as well as underpowered samples that were insufficient to demonstrate observable differences in dichotomized outcomes."

Regarding confounding, Bach et al. made the following observations: "*We considered parity, body mass index (BMI), and socioeconomic status to be the most important potential confounders, as these are associated with both exposure and outcome in the literature. Most included studies considered several potential confounders (Table 5), but as in all observational studies, residual confounding cannot be excluded. The magnitude of observed associations was small and therefore more likely to be explained by confounding or bias than strong associations, even if the extent of this was modest.*

Overall, crude estimates failed to change substantially when adjustments were made in multivariate models. In most studies, associations became somewhat stronger with adjustments. However, a few studies did not include some of the potential confounders we considered to be important. Apelberg et al. (2007), Arbuckle et al. (2013), Hamm et al. (2010), Inoue et al. (2004), Lee et al. (2013), Maisonet et al. (2012), and Monroy et al. (2008) failed to consider socio-economic status in their analyses of PFOA or PFOS and birth weight. It was previously demonstrated that women in higher socio-economic groups tend to have higher PFAS levels (Brantsæter et al. 2013). As women with high socio-economic status often give birth to children with higher birth weights (Luo et al. 2004, Moser et al. 2003), a lack of adjustment for socio-economic status may potentially explain the higher birth weight associated with higher PFOS (although statistically insignificant) in the study by Hamm et al. (2010). A lack of adjustment could have obscured a potential decrease in birth weight with PFOA exposure (Apelberg et al. 2007, Arbuckle et al. 2013, Hamm et al. 2010, Lee et al. 2013, Maisonet et al. 2012, Monroy et al. 2008) or PFOS exposure (Apelberg et al. 2007, Inoue et al. 2004, Lee et al. 2013, Maisonet et al. 2012, Monroy et al. 2008). Adjusting for socio-economic status is likely to be insufficient to control for

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behavioral factors such as smoking and alcohol consumption, but associations for PFASs with such behaviors have not been established."

Animal evidence

Bach et al. discussed several pathways by which PFASs may affect fetal growth based on animal studies, before concluding that these mechanisms had not been established in humans: "*Different pathways for the biological effects of PFASs have been suggested, for example hormone disruption.*

Estrogen has been demonstrated to be important in promoting fetal growth (Kaijser et al. 2000). PFASs influence the expression of estrogen-responsive genes in animal studies (Benninghoff et al. 2011, Tilton et al. 2008, Wei et al. 2007), and PFAS-induced changes in sex hormone biosynthesis have been reported in vitro (Du et al. 2013, Kraugerud et al. 2011). PFASs, including PFOS and PFOA, have been shown to interfere with the estrogen receptor in human in vitro studies (Benninghoff et al. 2011, Henry and Fair 2011, Kjeldsen and Bonefeld-Jørgensen 2013).

Thyroid hormones are pivotal for normal fetal growth and development, and maternal hypothyroidism is related to low birth weight (Blazer et al. 2003). Animal studies demonstrated alterations in thyroid hormone signaling with PFAS exposure (Du et al. 2013, Lau et al. 2003, Luebker et al. 2005, Martin et al. 2007, Thibodeaux et al. 2003, Yu et al. 2009a, b). Long et al. (2013) showed that PFASs interfered with thyroid hormone function. However, human studies concerning PFAS exposure and adult or fetal thyroid hormone function are not consistent (Emmett et al. 2006, Inoue et al. 2004, Kim et al. 2011, Olsen et al. 2003, Olsen and Zobel 2007, Wang et al. 2013).

Some animal studies have demonstrated changes in lipid metabolism with exposure to PFOS and PFOA (Haughom and Spydevold 1992, Kennedy et al. 2004, Loveless et al. 2006, Thibodeaux et al. 2003). However, Apelberg et al. (2007) found no association between PFOS and PFOA concentrations in cord serum and total serum cholesterol, triglycerides or total lipids.

Finally, immunotoxicity and susceptibility to infections in pregnant women may be a potential mechanism of fetal growth impairment. Adverse effects on the immune system have been demonstrated in vitro, in animals, and in children (DeWitt et al. 2012, Grandjean et al. 2012), but to our knowledge, such effects in pregnant women have not been evaluated.

Overall, several pathways by which PFASs may impair fetal growth are plausible, but the mechanisms have not been established in humans."

Priestly (2016)

Priestly, in his literature review and report on the potential health effects of PFAS (mainly PFOS), considered the evidence on effects of exposure during pregnancy.

Studies reviewed

Priestly reviewed:

- two systematic reviews (Olsen et al. 2009; Bach et al. 2015a);
- ten studies on birthweight (Maisonet et al. 2012; Fei et al. 2007; Hamm et al. 2010; Washino et al. 2009; Lien et al. 2013; Kishi et al. 2015; Verner et al. 2015; Bach et al. 2016; Callan et al. 2016; De Cock et al. 2016);

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- six studies on miscarriages, still births and birth defects, pregnancy-induced hypertension and preeclampsia (Stein et al. 2009; Savitz et al. 2012b; Darrow et al. 2014; Jensen et al. 2015; Liew et al. 2014; Louis et al. 2016[39]); and
- five studies on pregnancy outcomes or associated effects, including breast feeding (Ashley-Martin et al. 2016; Lyngsø et al. 2014; Bae et al. 2015; Lind et al. 2016; Timmermann et al. 2016).

Priestly commented that: "*It should be noted that only one of the studies relates to women exposed to PFAS in Australia (Callan et al. 2016).*" Details of this study are provided below under 'Summaries of studies reviewed'.

Considerations and conclusions

In the 'Executive Summary', Priestly stated that: "*The epidemiological studies are suggesting, but not yet proving, a possible link between PFOS/PFOA and foetal development and disturbances or normal birth characteristics. ...It would be reasonable to draw the conclusion that the evidence for all these effects needs further corroboration, since the evidence is somewhat inconsistent as to which specific PFAS are responsible and in some cases, the direction of the change attributed to specific PFAS is different across different studies.*"

Priestly made the overall 'Comment' regarding the studies he reviewed under the section 'Altered fetal development and effects on pregnancy': "*The overall conclusion is that female reproductive performance and foetal development may be altered by exposure to PFOS and/or PFOA, and possibly other PFAS, but at this time, there is no overarching consensus, nor a clear mode of action.*

The evidence for PFAS being associated with reduced birth weight is more consistent, but there are inexplicable sex differences, both in specific and direction of change, and different PFAS have been in[p]licated across several studies. While several studies have noted a possible link between PFOS and PFOA and the incidence of pregnancy loss (miscarriage), the evidence is tentative at best (Jensen et al. 2015)."

Summaries of studies reviewed

Birthweight

The studies on birthweight by Bach et al. (2015a) Maisonet et al. (2012), Fei et al. (2007), Hamm et al. (2010), Washino et al. (2009), and Verner et al. (2015) were reviewed by other key reports and systematic reviews with summaries of those studies provided earlier in this section. For the studies that Priestly reviewed, that have not been reported on previously, the following summaries are provided.

Of the systematic review by Olsen et al. (2009), Priestly reported that the authors: "*reviewed the evidence that PFOS/PFOA could alter human foetal development. They reviewed eight epidemiological studies, including two with occupational exposures. Overall, they found no evidence for any association between PFOS blood levels and altered birth weight, head circumference or gestational age. They noted the strengths and weaknesses of the methodologies used in the various epidemiological studies, and suggested that mere replication of the studies would not help to resolve the question of whether PFAS can cause foetal developmental effects unless the methodological deficiencies are appropriately addressed.*"

Of the following studies, Priestly provided the following details in a Table (Table 5, pages 36-39):

[39] Priestly references this paper as Louis et al. 2016, whereas other studies have referenced this as Buck Louis et al. 2016. Please refer to Buck Louis et al. 2016 in the reference list.

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- Lien et al. (2013): "*Subjects from Taiwan Birth Panel Study; n=486 infant-mother pairs; 2004-05 enrolment; Median serum levels (ng/mL) PFOA 1.86; PFOS 5.67, PFNA 3.0, PFUA 13.5; multiple linear regression suggested only PFOS was negatively correlated with birth weight; adjusted coefficient 0.011 ± 0.003 (SE)*";
- Kishi et al. (2015): "*Subjects from Hokkaido Study on Environment & Children's Health; n=306 mother-child pairs; 2002-05 enrolment; Median (ng/mL) PFOS 5.6 & PFOA 1.4; female infants weight reduction -186.6g (95% CI -9,8, 363.4) comparing 1st & 4th quartiles; PFOA effect smaller & NS; no change for male infants for either PFOS or PFOA*";
- Verner et al. (2015): "*Published data on maternal PFOS, PFOA blood levels and birth weight; Birth weight reductions per 1 ng/mL increase in PFOS 2.72g (95% CI -2.04, -3.4) and PFOA 7.13g (CI -5.8, -8.46); a meta analysis of literature estimated comparable changes (-5g PFOS, -14.7g PFOA)*";
- Bach et al. (2016): "*Subjects from Aarhus Birth Cohort (Denmark); n=1,507; 2008-13; Birth weight reduced for PFOS, difference 1st vs 4th quartiles; -50g (95% CI -23, -123), all births, and -62g (-3, -126) for term births. No consistent associations for other PFAS; no associations for any PFAS with birth length or head circumference*";
- Callan et al. (2016): "*Pregnant women from Western Australia; n=98; 2008-11; Median (ng/mL) serum PFOS 1.99, PFHXS 0.33, PFOA 0.86, PFNA 0.3, PFDA 0.12, PFUnDA 0.08; comparison of 1st & 3rd tertiles showed increased OR reduced birth weight (<95% optimal) 3.5 (95% CI 1.1 – 11.5) for PFHXS only; OR for 5 with increased birth weight +4.7% (CI -.7, 8.8) for PFUnDA; regression analysis for all PFAS showed NS trends for changes in birth weight; no associations for other PFAS; lower PFAS levels associated with mutiparity*";
- De Cock et al. (2016): "*Prospective cohort study of pregnant Dutch women; n=91; enrolled 2011-13; Linear regression analysis showed associations with lower birth weight for DDE (boys -326g 95% CI -17.6, -634 but trend to higher birth weight in girls); lesser effects associated with MECPP (DEHP metabolite) and PFOA; on the other hand, PFOS and MEHHP associated with higher birth weights in boys, no effect girls; authors noted the results should be interpreted with caution because of the small sample size.*"

Miscarriages, still births and birth defects

The following four studies (Stein et al. 2009; Savitz et al. 2012b; Darrow et al. 2014; Liew et al. 2014) have been reviewed by other key reports and systematic reviews, with summaries of those studies provided earlier in the section.

Of the following studies that have not been reviewed by other key reports or systematic reviews, Priestly provided the following summaries (see Table 6, page 36 of Priestly's report):

- Jensen et al. (2015): "*Case-control study from Odense Child Cohort, Denmark; n=2874; enrolled 2010-12; 56 cases with serum PFAS compared to 336 controls, also with serum PFAS; Comparing the highest and lowest tertiles for serum levels of PFNA & PFDA, odds ratio for miscarriage 16.5 (95% CI 7.4 – 36.5) and 2.67 (CI 1.31 – 5.44); an association with PFHxS was in a similar direction, but not statistically significant. No association found with PFOS or PFOA*";
- Louis et al. (2016): "*Prospective study of pregnant women from Michigan & Texas (LIFE Study); n=501; recruited pre-conception 2005-09; Median (ng/mL) PFOS 12.2; PFOA 3.3; other PFAS 0 to 1.2; No significant odds ratios for pregnancy loss with any PFAS.*"

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Pregnancy outcomes and associated effects, including breastfeeding

Priestly provided the following summaries of the studies reviewed (Ashley-Martin et al. 2016; Lyngsø et al. 2014; Bae et al. 2015; Lind et al. 2016; Timmermann et al. 2016) under this section (see Table 5 Priestly report):

- Ashley-Martin et al. (2016): "*MIREC study Canadian women; 2008-11; Interquartile increases in maternal weight gain associate with increased cord blood PFOA (OR 1.33 95% CI 1.13 – 1.56) and PFOS (OR 1.2 95% CI 1.03 – 1.4), but not PFHxS*";
- Lyngsø et al. (2014): "*INUENDO cohort of 3833 pregnant women from Greenland, Poland & Ukraine; 1743 sub-group with menstrual cycle data; 2002-04; Higher exposure to PFOA associated with longer menstrual cycle on pooled estimates from 3 countries; OR for long cycles 1.8 (95% CI 1.0, 1.33) in highest tertile; no effects seen for PFOS, although tendency for more irregular cycles OR 1.7 (0.8, 3.5); authors noted variability across countries on participation rates and lacking data on possible confounders (stress, disease and gynaecological disorders)*";
- Bae et al. (2015): "*Prospective study of pregnant women from Michigan & Texas (LIFE Study); n=233; recruited pre-conception 2005-09; No apparent dose-response relationship for the ratio of male: female births (secondary sex ratio, or SSR). For 5 of the 7 PFAS measured, including the two most prominent (PFOS and PFOA); paternal (but not maternal) levels of N-methyl-perfuorooctane sulfonamidoacetic acid (MePFOSAA), suggested an increased in female births. The authors did not rule this out as a chance finding despite the odds ratio achieving statistical significance (0.53 CI 0.26-1.1 2nd vs 1st tertile; 0.33 CI 0.13-0.86 for; 3rd vs 1st tertile)*";
- Lind et al. (2016): "*Odense Child Cohort, Denmark; n=649; enrolled 2010-12; Dose-related trend for decreased anogenital distance (AGD) in girls (not boys) for PFOS, PFHxS, PFNA & PFDA; in highest quartile PFOS, AGD reduced by 2.8mm (95% CI 1.1, 4.5); trend for increased birth weight in girls and reduced birth weight in boys; authors suggested this is evidence of endocrine disrupting effects of PFAS; sex-dimorphism was not explained*";
- Timmermann et al. (2016): "*Two birth cohorts from Faroe Islands; n=605 1997-2000; n=487 2007-09; Adjusted linear regression suggested doubling of maternal PFAS associated with reduction of duration of breastfeeding (total & exclusive); PFOS had strongest effect on total breastfeeding 1.4 mo (95% CI 0.6, 2.1) but PFOA doubling only reduced exclusive breastfeeding by 0.5 mo (0.3, 0.7); effect seen in primiparous and multiparous women and not confounded by previous breastfeeding or timing of PFAS sampling; no speculation on possible mechanism for reduced lactation, if this is the cause.*"

Comment on Confounding by GFR

Priestly provided the following comment: "*The apparent relationship between rising maternal PFAS blood levels and infant birth weights may be confounded by a number of factors, including gestational age, ethnicity, multiparity, smoking and dietary factors. In some of the studies in Table 5, attempts have been made to adjust for these confounders. A more intriguing explanation of confounding comes from the PBPK simulation study of Verner et al. (2015). Noting that GFR increases by around 50% in early pregnancy, then declines in the second half, and that where GFR does not rise as much in the second phase is associated with smaller babies, they showed that similar modifications of the GFR in their PBPK model could account for at least part of the association between birth weight and PFOS and PFOA blood levels. Effect of GFR changes on PFAS blood levels were predicted to be less during the 1st half of pregnancy, when some of the blood samples were drawn in other published studies. The potential effects of GFR changes was also confirmed by Sagiv et al. (2015).*"

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Negri et al. (2017)

Negri et al. examined the toxicological and epidemiological evidence on the association between PFOA or PFOS and birth/fetal weight.

Studies reviewed

Negri et al. considered 16 epidemiological studies which were presented in Table 6 (p494-495) of the paper (Apelberg et al. 2007; Kim et al. 2011; Wu et al. 2012; Lee et al. 2013; Lee et al. 2016; Fei et al. 2007; Monroy et al. 2008; Washino et al. 2009; Fromme et al. 2010; Hamm et al. 2010; Chen et al. 2012; Maisonet et al. 2012; Whitworth et al. 2012b; Darrow et al. 2013; Bach et al. 2015a; Lenters et al. 2016; Robledo et al. 2015).

Negri et al. also considered 25 toxicological studies of PFOA or PFOS on mice. The animal study evidence is not considered further here, but the conclusions of authors based on this are noted as they appear to influence overall conclusions.

Considerations and conclusions

Negri et al. stated in the 'Abstract', under 'Epidemiological Evidence' that: "*The pooled LRC [linear regression coefficient] for a 1ng/mL increase in untransformed PFOA (12 studies) in maternal plasma/serum was -12.8g (95% CI -23.2; 2.4), and -27.1g (95% CI -50.6; -3.6) for an increase of 1 $\log_e$ ng/mL PFOA (nine studies). The pooled LRC for untransformed PFOS (eight studies) was -0.92g (95%CI -3.4; 1.6), and for an increase of 1 $\log_e$ ng/mL was -46.1(95% CI -80.3; -11.9). No consistent pattern emerged for study location or timing of blood sampling.*"

The authors concluded: "*Epidemiological and toxicological evidence suggests that PFOA and PFOS elicit a decrease in birthweight both in humans and rodents. However, the effective animal extrapolated serum concentrations are $10^2 - 10^3$ times higher than those in humans. Thus, there is no quantitative toxicological evidence to support the epidemiological association, thus reducing the biological plausibility of a causal relationship.*"

Summaries of studies reviewed

In the 'Combination of human and animal evidence, and placing in a causal relationship grid' section, Negri et al. reported, regarding PFOA: "*There was a significant inverse relationship when untransformed values were considered, although with significant moderate heterogeneity; for log-transformed values, there was a significant inverse relationship, but with low heterogeneity. There were 16 epidemiologic studies from different areas of the world, mostly with low risk of bias, although the different methods did not allow to consider more than 12 studies together. These studies encompassed a wide range of human blood concentrations (disregarding the sample time). However, in humans, the shape of the dose–response curve has not been sufficiently investigated, particularly for what concerns a possible threshold effect. Also, the clinical relevance of the observed effect needs to be better understood. Overall, therefore, we evaluated the epidemiological evidence for an inverse association between PFOA maternal blood levels and birth weight as moderately likely, at least for the highest blood levels.*"

For PFOS, Negri et al. reported that: "*There was a non-significant inverse relationship [with birth weight] when untransformed values were considered, with significant heterogeneity, while for log-transformed values, there was a significant inverse relationship, with low heterogeneity. There were 13 available epidemiologic studies from different areas of the world, and mostly with low risk of bias, although the different methods did not allow to consider more than eight studies together. The most recent largest study (Bach et al. 2016) did not find any association. The same considerations as for*

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PFOA are valid, i.e. studies encompassed a wide range of concentrations but the shape of the dose–response curve, the possibility of a threshold effect and the clinical relevance of the observed effect, need further investigation. Overall, therefore, we evaluated the epidemiological evidence for an inverse association between PFOS maternal blood levels and birth weight as insufficient tending to moderately likely. As compared with PFOA, the uncertainty of the evaluation is further increased for PFOS by the fact that there does not appear to be a dose-response relationship when untransformed blood levels are analysed."

In the 'Application of framework for the integration of toxicology and epidemiology for causal inference and risk assessment' section, Negri et al. considered and commented on the weight of evidence, including: "*Taking into consideration all reviewed animal data, the overall toxicological evidence for a dose dependent effect of PFOA and PFOS on birth weight is judged plausible. Hence, combining toxicological and epidemiological evidence in a qualitative way, the causal relationship falls in the "likely" category (Figures 11 and 12). However, this is only a qualitative judgment which does not take into account information on Mode of Action (MoA), including quantitative analysis of the dose–response in animals compared with human exposure. For these compounds, an MoA has not yet been clearly identified and agreed, besides the hypothesized PPARs involvement in animals' development, although there appears to be qualitative concordance of the apical effect (i.e. birth weight) between animals and humans.*

Further, a refinement was done by comparing human and animal PFOA and PFOS serum concentrations associated with the effect. Given the strong discrepancy in terms of effective serum concentrations in rodents compared with the concentrations found in epidemiological studies, the uncertainty regarding the biological plausibility of a causal relationship between PFOA or PFOS exposure and lower birth weight in humans is increased. In fact, the 2–3 orders of magnitude difference in serum concentration between rodents and humans suggests that there might be some, not yet identified, confounding factors that lead to a spurious association."

Comment on confounding by GFR

Negri et al. stated in the 'Risk of bias' section: "*Another important potential confounder, related to both exposure and outcome, is glomerular filtration rate (GFR). Some studies have shown that women whose GFR fails to rise sufficiently during pregnancy tend to have smaller babies (Verner et al. 2015). On the other hand, GFR is likely to influence the urinary excretion of xenobiotics like PFAS. Indeed, higher blood PFAS levels have been observed in people with lower GFR (Verner et al. 2015). As renal elimination in humans seems to be negligible and no study adjusted for GFR, the influence of GFR on the results remains undefined."*

Kirk et al. (2018)

Kirk et al. (2018) reviewed the epidemiological evidence published up to January 2017 that investigated the effect of prenatal PFAS exposure on health outcomes in infants and their mothers. The authors noted that: "*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."*

Studies reviewed

Kirk et al. reviewed 38 papers that investigated the effect of prenatal PFAS exposure on health outcomes in infants and their mothers (Andersen et al. 2010; Apelberg et al. 2007; Arbuckle et al. 2013; Bach et al. 2016; Bae et al. 2015; Buck Louis et al. 2016; Chen et al. 2012; Darrow et al. 2013;

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Darrow et al. 2014; De Cock et al. 2016; Fei et al. 2007; Fei et al. 2008a; Govarts et al. 2016; Grice et al. 2007; Hamm et al. 2010; Jensen et al. 2015; Kim et al. 2011; Kishi et al. 2015; Kwon et al. 2016; Lee et al. 2013; Lee et al. 2016; Lenters et al. 2016; Liew et al. 2014; Maisonet et al. 2012; Monroy et al. 2008; Nolan et al. 2009; Nolan et al. 2010; Robledo et al. 2015; Savitz et al. , 2012a; Savitz et al. 2012b; Shi et al. 2016; Starling et al. 2014; Stein et al. 2009; Stein et al. 2014a; Wang et al. 2016; Washino et al. 2009; Whitworth et al. 2012b; Wu et al. 2012).

Under 'Measurements at birth', Kirk et al. reviewed:

- twenty-nine studies under birth weight measurements (Anderson et al. 2010; Apelberg et al. 2007; Arbuckle et al. 2013; Bach et al. 2016; Chen et al. 2012; Darrow et al. 2013; De Cock et al. 2016; Fei et al. 2007; Govarts et al. 2016; Grice et al. 2007; Hamm et al. 2010; Kim et al. 2011; Kishi et al. 2015; Kwon et al. 2016; Lee et all., 2013; Lee et al. 2016; Lenters et al. 2016; Maisonet et al. 2012; Nolan et al. 2009; Robledo et al. 2015; Savitz et al. 2012a; Savitz et al. 2012b; Shi et al. 2017; Stein et al. 2009; Wang et al. 2016; Washino et al. 2009; Whitworth et al. 2012b; Wu et al. 2012; Monroy et al. 2008);
- six studies on small for gestational age (Chen et al. 2012; Fei et al. 2007; Hamm et al. 2010; Savitz et al. 2012b; Wang et al. 2016; Whitworth et al. 2012b);
- one study on large for gestational age (Whitworth et al. 2012b);
- one study on placental weight (Fei et al. 2008a);
- eleven studies on birth length (Apelberg et al. 2010; Bach et al. 2016; Chen et al. 2012; Lee et al. 2013; Maisonet et al. 2012; Robledo et al. 2015; Shi et al. 2017; Wang et al. 2016; Washino et al. 2009; Wu et al. 2012; Fei et al. 2008a);
- eight studies on neonatal head circumference (Apelberg et al. 2007; Bach et al. 2016; Chen et al. 2012; Lee et al. 2013; Robledo et al. 2015; Wang et al. 2016; Wahino et al. 2009; Fei et al. 2008a);
- one study on abdominal circumference (Fei et al. 2008a);
- one study on chest circumference (Washino et al. 2009); and
- one study on APGAR score (Wu et al. 2012).

Under 'Delivery outcomes', Kirk et al. reviewed:

- ten studies on preterm birth (Arbuckle et al. 2013; Chen at al., 2012; Darrow et al. 2013; Fei et al. 2007; Hamm et al. 2010; Nolan et al. 2009; Savitz et al. 2012a; Savitz et al. 2012b; Stein et al. 2009; Whitworth et al. 2012b);
- five studies on gestational age (Apelberg et al. 2007; Hamm et al. 2010; Maisonet et al. 2012; Nolan et al. 2009; Wu et al. 2012);
- four studies on miscarriage (Savitz et al. 2012a; Stein et al. 2009; Darrow et al. 2014; Jensen et al. 2015);
- two studies on stillbirth (Savitz et al. 2012a; Savitz et al. 2012b);
- one study on pregnancy loss (all definitions) (Buck Louis et al. (2016);
- one study on mode of delivery (Arbuckle et al. 2013);
- one study on delivery complications (Nolan et al. 2010); and
- one study on gender outcomes (Bae et al. 2015).

Under 'Maternal outcomes', Kirk et al. reviewed:

- three studies on preeclampsia (Savitz et al. 2012a; Stein et al. 2009; Starling et al. 2014);
- one study on eclampsia (Nolan et al. 2010);
- two studies on pregnancy-induced hypertension (Darrow et al. 2013; Nolan et al, 2010); and

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- two studies on gravidity and parity (Arbuckle et al. 2013; Lee et al. 2013).

Under 'Neonatal and infant diagnoses', Kirk et al. reviewed:

- four studies on congenital outcomes (Savitz et al. 2012a; Stein et al. 2009; Nolan et al. 2010; Stein et al. 2014a); and
- one study on cerebral palsy (Liew et al. 2014).

Under 'Growth during infancy', Kirk et al. reviewed:

- two studies on weight, body mass index and height (Andersen et al. 2012; Maisonet et al. 2012);

The majority of these studies were reviewed by other key national and international reports and systematic reviews, except Govarts et al. (2016) and Kwon et al. (2016). Summaries for the studies reviewed by other key reports and reviews are provided earlier in this section. Summaries of Govarts et al. (2016) and Kwon et al. (2016) are provided in the 'Birthweight' sub-section in 'Summary of studies reviewed' below.

Where Kirk et al. provided additional information on studies previously reported by other key reports and systematic reviews, this information has been included in the relevant subsections below.

Considerations and conclusions

In the 'Executive Summary', Kirk et al. stated that: "*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..., which did not change our conclusions about the inconsistency of findings from published papers.*"

Kirk et al. reported 'Associations at a glance' for each of the subsections under 'Neonatal, infant and maternal outcomes': 'Measurements at birth'; 'Delivery outcomes'; 'Maternal outcomes'; and 'Diagnoses at birth and during infancy'. These 'Associations at a glance' tables were reproduced from Kirk et al. and are reported below to show the overview of reported associations and Kirk et al.'s evaluation of the evidence for the studies they reviewed in each subsection, by respective PFAS.

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[40]
Birth weight	Maternal; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA	Inadequate evidence
Small for gestational age	Umbilical cord; PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
Small for gestational age	Maternal; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFDoA	Inadequate evidence

[40] *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.

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Health outcome	PFAS exposure	Evaluation of evidence
Large for gestational	Maternal; PFOA, PFOS	Inadequate evidence
Placental weight	Maternal; PFOA, PFOS	Inadequate evidence
Birth length	Umbilical cord; PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA	Inadequate evidence
Birth length	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
Ponderal index	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
Head circumference at birth	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

Kirk et al. 2018, pp. 28-29.

Delivery outcomes

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence
Preterm birth	Umbilical cord; PFOA, PFOS, PFNA, PFUdA	Inadequate evidence
Preterm birth	Maternal; PFOA, PFOS, PFHxS	Inadequate evidence
Gestational age	Umbilical cord; PFOA, PFOS	Inadequate evidence
Gestational age	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

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Health outcome	PFAS exposure	Evaluation of evidence
Gender outcomes of pregnancy (male compared to female)	Maternal; PFOA, PFOS, PFNA, PFDA, PFOSA, Et-PFOS-AcOH, Me-PFOS-AcOH	Inadequate evidence

Kirk et al. 2018, pp 41.

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

Kirk et al. 2018, pp. 46.

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

Kirk et al. 2018, pp 48.

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

Kirk et al. 2018, pp 50.

MEASUREMENTS AT BIRTH

Birth weight

Studies reviewed

Kirk et al. evaluated 29 studies on birth weight measurements (Anderson et al. 2010; Apelberg et al. 2007; Arbuckle et al. 2013; Bach et al. 2016; Chen et al. 2012; Darrow et al. 2013; De Cock et al. 2016; Fei et al. 2007; Govarts et al. 2016; Grice et al. 2007; Hamm et al. 2010; Kim et al. 2011; Kishi et al. 2015; Kwon et al. 2016; Lee et all., 2013; Lee et al. 2016; Lenters et al. 2016; Maisonet et al. 2012;

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Nolan et al. 2009; Robledo et al. 2015; Savitz et al. 2012a; Savitz et al. 2012b; Shi et al. 2017; Stein et al. 2009; Wang et al. 2016; Washino et al. 2009; Whitworth et al. 2012b; Wu et al. 2012; Monroy et al. 2008).

The two studies (Kwon et al. 2016; Govarts et al. 2016) that were not reviewed by other key reports or systematic reviews are summarised below in the section 'Summaries of studies reviewed'.

Considerations and conclusions

Kirk et al. made the overall statement about these 28 studies on PFAS exposure and birthweight measurements at the start of the section. "*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.*"

At the end of the section on birthweight, Kirk et al. provided an 'Evaluation' and stated that: "*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 [Andersen et al. 2010; Kishi et al. 2015; Lee et al. 2013; Lenters et al. 2016; Maisonet et al. 2012; Stein et al. 2009; Washino et al. 2009; Wu et al. 2012]. 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."

Summaries of studies reviewed

Kirk et al. reported the study by Kwon et al. (2016) as: "*Kwon et al. [2016] 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) ....*"

Kirk et al. did not comment specifically on the study by Govarts et al. (2016) in the section on birth weight. The study was reported in the Appendix as a cohort study in Flanders, Belgium of: "*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.*" The study was reported to have found "*No significant association between PFOA and PFOS and birth weight.*"

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Birth weight meta-analyses

Kirk et al. undertook a birth weight meta-analysis of studies on PFOA and PFOS.

PFOA birthweight meta-analysis

For the PFOA meta-analysis, Kirk et al. included 11 studies (Bach et al. 2016; Darrow et al. 2013; De Cock et al. 2016; Hamm et al. 2010; Maisonet et al. 2012; Apelberg et al. 2007; Chen et al. 2012; Kwon et al. 2016; Lee et al. 2016; Lenters et al. 2016; Wu et al. 2012) and conducted 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. Kirk et al. reported the following results for the PFOA meta-analysis: "*We conducted a meta-analysis for all five studies with categorised PFOA exposure [Bach et al. 2016; Darrow et al. 2013; De Cock et al. 2016; Hamm et al. 2010; Maisonet et al. 2012] and for all six studies with log transformed PFOA exposure [Apelberg et al. 2007; Chen et al. 2012; Kwon et al. 2016; Lee et al. 2016; Lenters et al. 2016; Wu et al. 2012], combining results from PFOA exposure assessment for both maternal and umbilical cord blood.*"

Kirk et al. reported the results of the PFOA meta-analysis as: "*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)... 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. [2012] 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)... which provides no overall evidence for any trend in birth weight with increasing exposure to PFOA. The strong null result of Lee et al. [2016] 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."

Kirk et al. cautioned about the meta-analysis: "*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.*"

PFOS birthweight meta-analysis

For PFOS, Kirk et al. undertook a meta-analysis on six sets of results from five studies (Bach et al. 2016; Darrow et al. 2013; Hamm et al. 2010; Kishi et al. (2015- female infants); Kishi et al. (2015- male infants); Maisonet et al. 2012).

Kirk et al. reported the results of the PFOS meta-analysis as: "*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;*

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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."

Small for gestational age

Kirk et al. reviewed six studies on 'Small for gestational age' (Chen et al. 2012; Fei et al. 2007; Hamm et al. 2010; Savitz et al. 2012b; Wang et al. 2016; Whitworth et al. 2012b) and reported on these studies as: "*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 [Chen et al. 2012; Fei et al. 2007; Hamm et al. 2010; Savitz et al. 2012b; Wang et al. 2016; Whitworth et al. 2012b] 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. [2012] 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. [2016] 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. [2012] although during a different time frame. The results reported by Wang et al. [2016] were specific to female neonates however, with no statistically signifcant relationship between PFAS and SGA in males. No other studies investigated the association between these three specific exposures and SGA.*

Chen et al. [2012] 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. [2007] Hamm et al. [2010] Savitz et al. [2012b] Wang et al. [2016] and Whitworth et al. [2012b] reported no significant relationship between maternal concentrations of PFOA, PFOS, PFHxS, PFNA and PFDoA and SGA."

Kirk et al. provided the following overall comment on the studies they reviewed: "*The results from the six studies largely conclude that prenatal exposure to PFAS is not significantly associated with SGA. Although Chen et al. [2012] and Wang et al. [2016] 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

Kirk et al. reported on the one paper (Whitworth et al. 2012b) they reviewed on PFAS exposure and large for gestation age as: "*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. [2012b] 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."*

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Placental weight

Of the one study reviewed on placental weight, Kirk et al. reported that: "*Fei et al. [2008a] 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

Kirk et al. reported on 11 studies they reviewed on birth length (Apelberg et al. 2010; Bach et al. 2016; Chen et al. 2012; Lee et al. 2013; Maisonet et al. 2012; Robledo et al. 2015; Shi et al. 2017; Wang et al. 2016; Washino et al. 2009; Wu et al. 2012; Fei et al. 2008a). They made the following comment: "*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.*"

Kirk et al. reported the following as their 'Evaluation' of the 11 studies they reviewed on birth length: "*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.*"

Birth length meta-analysis

Kirk et al. undertook a meta-analysis of the effects of PFOA on birth length for five datasets (Apelberg et al. 2007; Chen et al. 2012; Wang et al. 2016 -female infants; Wang et al. 2016- male infants; Wu et al. 2012) and reported that: "*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). 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).*"

Kirk et al. cautioned: "*These results should be interpreted with caution, due to the between-study heterogeneity and because results were combined for cord and maternal blood.*"

Kirk et al. noted that there was an inadequate number of papers with comparable outcome and PFOS exposure measures for inclusion in a meta-analysis.

Neonatal head circumference

Kirk et al. reviewed eight studies on prenatal exposure to PFAS and measurements of neonatal head circumference (Apelberg et al. 2007; Bach et al. 2016; Chen et al. 2012; Lee et al. 2013; Robledo et al. 2015; Wang et al. 2016; Wahino et al. 2009; Fei et al. 2008a). They made the following two comments on the body of evidence they reviewed:

- "*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.*"
- "*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,*

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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. [2016] 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. [2007] and Wang et al. [2016] were evaluated to have a high risk of bias. The results by Chen et al. [2012] were associated with a moderate risk of bias, and therefore are considered to be more reliable than those reported by Apelberg et al. [2007] for umbilical cord exposure levels."

Abdominal circumference

Of the one study reviewed on abdominal circumference, (Fei et al. 2008a), Kirk et al. reported that: "*The authors found no significant association between the concentration of PFOA and PFOS in maternal serum and abdominal circumference at birth.*"

Chest circumference

For the one study Kirk et al. reviewed on chest circumference (Washino et al. (2009), the findings were reported: "*The study reported no significant association between the concentration of PFOA and PFOS in maternal serum and chest circumference at birth.*"

APGAR score

Kirk et al. reviewed one study on APGAR score[41] (Wu et al. 2012) and reported that: "*Wu et al. [2012] 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)).*"

Kirk et al. commented that: "*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

Preterm birth

Kirk et al. reviewed 10 studies that investigated the association between PFAS exposure and preterm birth, (Arbuckle et al. 2013; Chen at al., 2012; Darrow et al. 2013; Fei et al. 2007; Hamm et al. 2010; Nolan et al. 2009; Savitz et al. 2012a; Savitz et al. 2012b; Stein et al. 2009; Whitworth et al. 2012b). They noted that: "*For all studies, preterm birth was defined as a mother who gave birth to their child before 37 weeks gestation, Savitz et al. [2012b] also investigated whether prenatal exposure to PFAS resulted in birth before 32 weeks gestation.*"

Kirk et al. made two overall comments about the studies they reviewed:

- "*Elevated PFAS levels in maternal serum were not consistently associated with preterm birth; however, the relationship between umbilical cord measurements and preterm birth provide*

[41] 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. (Source: Kirk et al. 2018, page 41).

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statistically significant evidence for a significant positive association between the exposure and health outcome."

- *"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. [2013] and Chen et al. [2012] suggest a positive association between PFOS exposure levels and preterm birth, these results are opposed by the association reported by Whitworth et al. [2012b]. 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."*

Gestational age[42]

Kirk et al. evaluated five studies (Apelberg et al. 2007; Hamm et al. 2010; Maisonet et al. 2012; Nolan et al. 2009; Wu et al. 2012) and made two comments:

- *"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."*
- *"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

Kirk et al. evaluated four studies that reported the association between prenatal exposure to PFAS and the occurrence of miscarriage among pregnant women (Savitz et al. 2012a; Stein et al. 2009; Darrow et al. 2014; Jensen et al. 2015) and made the following statement about these studies: *"Three papers concluded that there were no significant associations between maternal concentrations of PFAS during pregnancy and miscarriage. Jensen et al. [2015] 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. [2015] 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."*

[42] 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. Source: Kirk et al. 2018, page 43

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Stillbirth

Kirk et al. reviewed two studies on stillbirth (Savitz et al. 2012a; Savitz et al. 2012b) and commented that: "*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')

Kirk et al. evaluated the study by Buck Louis et al. (2016), which was reviewed by Priestly (2016). Kirk et al. reported the summary of this study as: "*Buck Louis et al. [2016] 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

Kirk et al. reviewed one study on mode of delivery (Arbuckle et al. 2013), and reported of this study: "*In the cohort study of just over 100 Canadian women, Arbuckle et al. [2013] 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.*"

Kirk et al. made the following comment about the evidence base on mode of delivery: "*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

Kirk et al. reviewed the study by Nolan et al. (2010) and reported that: "*Nolan et al. [2010] 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. [2010] 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. [2010] reported findings associated with estimated maternal exposure to PFAS, rather than blood serum measurements.*"

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Gender outcomes

One study on gender outcomes (Bae et al. 2015) was reviewed by Kirk et al. with the following being reported: "*In a cohort study of 223 women enrolled in the Longitudinal Investigation of Fertility and the Environment (LIFE) study, Bae et al. [2015] 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. [2015] concluded that these significant associations may have been due to chance and the study was evaluated as having a high risk of bias.*"

Kirk et al. made the following comment on the evidence base on gender outcomes: "*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

Kirk et al. evaluated six studies under 'Maternal outcomes' (Arbuckle et al. 2013; Darrow et al. 2013; Nolan et al. 2010; Savitz et al. 2012a; Starling et al. 2014; Stein et al. 2009).

Preeclampsia[43]

Kirk et al. evaluated three studies on preeclampsia (Savitz et al. 2012a; Stein et al. 2009; Starling et al. 2014) and commented that: "*The studies evaluated suggest that elevated levels of PFAS in pregnant women are not associated with preeclampsia.*"

Eclampsia[44]

Kirk et al. reviewed the study by Nolan et al. (2010) and reported: "*Nolan et al. [2010] studied the effect of maternal exposure to PFAS and eclampsia in pregnant women and found no significant association.*"

Pregnancy-induced hypertension[45]

Kirk et al. reviewed the studies by Darrow et al. (2013) and Nolan et al. (2010) and reported that: "*The studies reported no significant association between maternal concentrations of PFOA and pregnancy induced hypertension in women, and Darrow et al. [2013] further reported no significant results for maternal PFOS exposure.*"

Gravidity and parity[46]

Kirk et al. reviewed two studies on gravidity and parity (Arbuckle et al. 2013; Lee et al. 2013), and reported that: "*In a cohort study of approximately 100 deliveries by Canadian women, Arbuckle et al.*

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[43] Preeclampsia is a complication of pregnancy; which usually occurs after 20 weeks of pregnancy. It is characterised by high blood pressure and signs of damage to other organs; such as the kidneys and the liver. Source: Kirk et al. page 47

[44] 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. Source: Kirk et al. page 47

[45] 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.

[46] 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. Source: Kirk et al. page 47)

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[2013] 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. [2013] found no significant association between umbilical cord measurements of PFOA and PFNA and gravidity. Lee et al. [2013] 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. [2013] and Lee et al. [2013] investigated different pregnancy outcomes making it difficult to interpret overall findings."

Kirk et al. commented about the evidence base on gravidity and parity: "*As Arbuckle et al. [2013] 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

Kirk et al. reviewed five studies on neonatal and infant diagnoses (Savitz et al. 2012a; Stein et al. 2009; Nolan et al. 2010; Stein et al. 2014b; Liew et al. 2014).

Congenital outcomes

Kirk et al. reviewed four studies that investigated the association between prenatal exposure to PFAS and congenital anomalies in neonates (Savitz et al. 2012a; Stein et al. 2009; Nolan et al. 2010; Stein et al. 2014b). Kirk et al. noted that: "*All studies investigated maternal exposure levels of PFAS on the health outcome, with Nolan et al. [2010], Savitz et al. [2012a] and Stein et al. [2009] using estimated PFAS exposure and not concentrations of PFAS in maternal serum.*"

Kirk et al. made two comments about these studies:

- "*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 [Stein et al. 2014b].*"
- "*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.*"

The study by Stein et al. (2009) was reviewed by the ATSDR, with a summary provided above. The ATSDR used the term 'birth defect'. Kirk et al. provided more detail about this study: "*Savitz et al. [2012a] and Stein et al. [2009] 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. [2009] further concluded no association for PFOS. In a cohort of 10,105 mother-infant pairs from the C8 Health Project, Stein et al. [2014b] 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.*"

Kirk et al. provided the following detail about the study findings and the congenital abnormalities investigated in the study by Nolan et al. (2010): "*Nolan et al. [2010] determined the relationship between estimated maternal exposure to PFOA and 12 congenital anomalies and reported no*

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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. [2010]."

Cerebral palsy

The study by Liew et al. (2014) was reviewed by the US EPA, under PFOA and PFOS with summaries provided in those respective sections above. Information from Kirk et al. is also included because they provided findings on other PFAS. Kirk et al. reported that: *"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. [2014] found no relationship between maternal concentrations of PFAS and cerebral palsy in females."*

Kirk et al. commented: *"As the study by Liew et al.[2014] 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

Kirk et al. reviewed two studies that investigated weight, height and body mass index in infancy (Anderson et al. 2010 and Maisonet et al. 2012). The study by Andersen et al. (2010) is also reported on in the 'Obesity, overweight and BMI' section of this report under child overweight and obesity.

Weight

Of the study by Andersen et al. (2010), Kirk et al. reported that: *"In a randomly selected sample of 1,400 mother-infant pairs from the Aarhus Birth Cohort, Andersen et al. [2010] 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."*

Of the study by Maisonet et al. (2012), Kirk et al. reported that: *"In the ALSPAC cohort study, Maisonet et al. [2012] 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."*

Kirk et al. made the following comment about these two studies and consistency of evidence: *"It is difficult to compare the findings of Andersen et al. [2010] and Maisonet et al. [2012] 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."*

Body Mass Index

Kirk et al. reported the findings of Andersen et al. (2010) as: *"no significant relationship between maternal levels of PFOA and PFOS and BMI calculations in infants at 5 months, though reported a*

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significant negative association between PFOS and BMI at 12 months (z Score (CI); -0.007 (-0.011– -0.002))", and made the following comment: "*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."*

Height

The study by Andersen et al. (2010) also reported on height in infancy and as reported by Kirk et al. to show: "*no significant relationship between concentrations of PFOA and PFOS in maternal serum and the height of infants at 5 and 12 months old."*

6.6.4. Differences in conclusions

Johnson et al. (2104) discussed that their conclusion differed from that of the C8 Science Panel and the reasons for the difference: "*The panel concluded that PFOA was probably not linked to low birth weight and that the evidence of small reductions in average birth weight in relation to PFOA exposure was inconsistent. Our review occurred at a later date and therefore included more recent publications. These later publications (Chen et al. 2012; Maisonet et al. 2012; Whitworth et al. 2012b) were included in our meta-analysis, showing consistent results and an overall reduction in birth weight associated with PFOA exposure."*

Bach et al. (2015) also discussed differences in conclusions of other systematic reviews on the topic, commenting:

"*The review by Olsen et al. (2009) provided no firm conclusions on the association between exposure to PFOA and PFOS and human fetal growth. A recent systematic review and metaanalysis by Johnson et al. (2014) applied the Navigation Guide systematic review methodology to investigate the association between exposure to PFOA and human fetal growth. Their inclusion criteria were less restrictive compared to ours; for instance, they included studies with estimated PFOA concentrations and other outcomes in addition to birth weight (birth length, head circumference, and ponderal index). Therefore, they included more studies than us (n=18). However, since they conducted literature searches in the spring of 2012, they did not include the two most recent studies (Darrow et al. 2013 and Lee et al. 2013). Another difference between the review by Johnson et al. (2014) and our work is the approach to the risk of bias in individual studies. Johnson et al. (2014) decided that maternal age and gestational age were the most important confounders and concluded that studies were at low risk of confounding if they accounted for both in their design or analysis, or if they reported that neither of these influenced the associations between PFOA and fetal growth outcomes. These authors were more successful than us in retrieving raw data or comparable estimates from the authors of original articles. Therefore, they were able to perform a meta-analysis of 9 studies on PFOA concentrations and birth weight. They found an overall estimate of - 18.9 (95% CI: - 29.8, - 7.9) grams of birth weight per ng/mL increase in serum or plasma PFOA, and concluded that there is sufficient evidence for an association between PFOA exposure and reduced fetal growth."*

Kirk et al. noted in the 'Discussion' that some of their findings differed from those of previous systematic reviews, stating: "*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. [2014] and Johnson et al. [2014] 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. [2014] and Johnson et al. [2014], a more*

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recent systematic review by Bach et al. [2015] found, like this review, the results to be inadequate evidence of an association."

6.6.5. Summary of key national and international reports and systematic reviews

Recent key national and international reports:

ATSDR concluded that higher maternal blood levels of PFOA, PFOS, and PFHxS are associated with lower birth weight in some studies and that decreases are small. ATSDR also reported on indications for a higher risk of pregnancy-induced hypertension and preeclampsia with higher PFOA or PFOS concentrations in blood.

The US EPA concluded that high maternal or cord blood concentrations of PFOA were associated with lower birth weight; likewise associations were found between PFOA exposure and increased risk of pregnancy-induced hypertension or preeclampsia. Furthermore, US EPA concluded that higher maternal PFOS levels are correlated with decreased body weights in offspring and other measures of postnatal growth, as well as pregnancy-induced hypertension.

RIVM evaluated PFOA alone and concluded that evidence is clearest for a relationship between PFOA exposure and lower birth weight; there are also indications for higher risk of pregnancy-induced hypertension and preeclampsia.

FSANZ concluded that there is evidence of an association between PFAS exposure and lower birth weight; while causality can't be established either for PFOS or for PFOA. FSANZ also noted indications for a higher risk of pregnancy-induced hypertension and preeclampsia with PFOA exposure.

Systematic reviews:

Johnson et al. and Lam et al. evaluated PFOA and fetal growth measurements; they concluded there is sufficient evidence of an association between increased PFOA exposure and decreased fetal growth measures.

Bach et al. concluded that high PFOS and PFOA exposure were associated with lower birth weight in most studies, although not all results were statistically significant.

Priestly concluded that associations between increased PFOS/PFOA exposure and decreased foetal development/birth characteristics are suggestive, but not conclusive.

Negri et al. concluded that maternal blood concentrations of PFOA are moderately associated with lower birth weight, while the association is less strong for maternal PFOS concentrations.

Kirk et al., who evaluated a large number of pregnancy, prenatal and birth outcomes in relation to PFAS exposure, found the evidence for all (health) outcomes, including birth weight, pregnancy-induced hypertension and preeclampsia to be *'inadequate'*. Kirk et al. also evaluated two studies on infant (5-20 months of age) growth measurements (body weight, height, and body mass index) in relation to PFAS-exposure, and concluded the evidence was *'inadequate'*.

The systematic reviews and key reports highlighted that:

The reduction in birth weight reported to be associated with PFAS exposure is considered small in size, and may not be biologically relevant, in particular on the individual level.

Blood concentrations of PFAS in the human studies are about 1000-fold lower than animal studies that show an effect on birth weight. This information need to be understood in the light of the well

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known differences in PFAS kinetic behaviour among humans and experimental models, as well as among individual PFAS-compounds.

Currently available human studies on PFAS health effects are of small size, with cross-sectional study design and low exposure contrasts, which limits evidence for causality and dose-response relationships.

6.6.6. Expert Health Panel synthesis to support advice to the Minister

- There are several studies on PFAS exposure associated with pregnancy, prenatal and birth outcomes as well as infant growth; these studies are mainly cross-sectional and based on small-to-intermediate population sizes in just a few study populations.
- From the limited evidence available, current data on pregnancy, prenatal and birth outcomes, and infant growth suggests that significant associations with increased PFAS exposure relate to small changes in end points such as pregnancy-induced hypertension and pre-eclampsia, weight and length at birth, as well as infant growth.
- The evidence is very limited. One major limitation is the lack of mechanistic data explaining if/how PFAS might impact on pregnancy, prenatal development and infant growth processes;
- Lack of mechanistic data explaining if/how PFAS might impact on pregnancy, prenatal development and infant growth processes represents an additional and major limitation for efficient prevention and assessment activities (related to PFAS exposure among public health professionals and regulators).
- Further, existing mechanistic evidence is mainly based on experimental data from cell and animal models. There is minimal human evidence linking pregnancy and/or developmental outcomes associated with PFAS-exposure to demonstrable effects of PFAS on human cell biology and physiology.

6.6.7. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and pregnancy, prenatal and birth outcomes:

- Current evidence does not support PFAS being a major cause of pregnancy-induced hypertension/ pre-eclampsia or other complications.
- PFAS exposure in fetal life was often associated with lower weight and length at birth in general population studies. These decreases in birth weight and length were mostly small and within the normal range. There was also an association with slightly slower infant growth.
- The major concern about PFOA/PFOS exposure in pregnancy would be these effects at general population exposures. However, there are many other PFAS and environmental pollutants that warrant surveillance in the general population.
- A strategy to provide PFAS research that also supports ongoing human biomonitoring of early life exposures would be the most useful way to contribute to prevention and assessment activities by public health researchers and regulators.

To further investigate the association between PFAS exposure and pregnancy, prenatal and birth outcomes in an Australian setting, the Panel suggests the following research priorities:

- Pregnancy, prenatal and birth outcomes, and infant growth measurements associated with PFAS exposure were of high concern to those who responded in the public

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consultation, who generally expressed strong support for "*research into the potential health effects of PFAS exposure on vulnerable populations such as pregnant women, babies, young children and the elderly*".

- Large longitudinal studies are required to provide better data on associations between PFAS and pregnancy, prenatal, birth, and infant outcomes. Access to existing birth cohorts would be the most efficient way to undertake such studies.
- Studies need to be adequately powered and ideally supported by quantitative exposure data (e.g. blood concentrations) as well as relevant effect biomarkers. Access to disease registers, as well as registers, which monitor weight/growth/length-parameters at birth, during childhood and into young adult age, can form the basis for well-designed studies.
- It is most likely that if PFAS exposure causes pregnancy, prenatal, birth, and infant outcomes, this would be due to altered endocrine function and/or metabolic changes rather than direct effects on all cells. Therefore, this research should include analyses of hormones relevant to reproductive and developmental/growth processes.
- As all individuals are exposed to multiple other chemicals, it would be best value to include PFAS measurement in studies that include assessment of other persistent chemicals and other environmental factors affecting normal pregnancy (e.g. smoking, alcohol).

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6.7. Reproductive outcomes and PFAS exposure

This section covers a broad range of effects on sex hormones, sexual maturation, fertility and menopause that have been studied. There is a very large normal or background variation in all these factors. This is extreme for female sex hormones like estradiol, but also nearly tenfold for male measurements like sperm count and testosterone levels. Even more 'clinical' end points like age at menarche or normal time to get pregnant vary by years within the normal population. Roughly 15% of the Australian population of reproductive age are 'sub-fertile' (time to pregnancy > 12 months) and 4% of pregnancies are with the aid of assisted reproductive technology. There are many lifestyle factors that contribute to this variation. For example, the changing typical weight range in the population is believed to have led to large changes in nearly all these factors over the last century, with the age at menarche dropping several years.

With this background, the aim of the studies to look for evidence of PFAS leading to 'endocrine disruption' is a major challenge. The outcomes reported are often difficult to interpret; it is often unclear when there are reported differences if these lead to more individuals outside the normal range or have any other clinically relevant consequences.

Endocrine disruption might feasibly be associated with a range of other health effects that have hormone-related risk factors, but these are covered elsewhere in this review: e.g. diabetes mellitus, breast cancer, heart disease, bone disease [90–93][47]. Several of the key international authority reports and systematic reviews have evaluated the human evidence on exposure to PFAS and reproductive effects, in studies conducted in the general population, highly-exposed communities and in occupationally exposed workers.

6.7.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of the following three key international authority/intergovernmental/governmental reports published between 2015 and 2016 and four systematic reviews from 2013 onwards that reported on exposure to PFAS and reproductive effects.

Key national and international reports

- **US Agency for Toxic Substances and Disease Registry (ATSDR 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **United States Environmental Protection Agency (US EPA 2016a).** Health effects support document for Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA 2016b).** Health effects support document for Perfluorooctane Sulphonate (PFOS).

Systematic reviews

- **Saikat et al. (2013).** The impact of PFOS on health in the general population: a review;
- **Priestley (2016)** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS), (Monash University);
- **Rappazzo et al. (2017)** Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature;
- **Kirk et al. (2018)** (Australian National University). The PFAS Health Study. Systematic Literature Review.

[47] Rappazzo et al.

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The DWQI (2016) reported (page 88) that reproductive and developmental outcomes (with the exception of lower birth weight and birth size of neonates), including decreased sperm count, longer time to pregnancy, birth defects, miscarriage and stillbirth, and overweight and obesity measured by BMI and waist circumference in offspring, were not evaluated in their 'Health-based maximum contaminant level support document: Perfluorooctanoic Acid (PFOA)' Public Review draft. For this reason, the DWQI (2016) report is not considered further in this section.

While the Panel acknowledges that FSANZ (2017) made statements about fertility, FSANZ did not review epidemiological studies in their 'Hazard assessment report (PFOS, PFOA, PFHxS)', instead referring to the reviews of studies by the ATSDR (2015) and US EPA (2016a, b). For this reason, the FSANZ 'Hazard assessment report (PFOS, PFOA, PFHxS)' is not considered further in this section. FSANZ did make one statement about PFOA, based on Bull et al. (2014), that: "*There is no consistent evidence of negative effects of PFOA on sperm quality, sperm DNA integrity or other factors of male fertility. Some studies have reported an association between maternal PFOA exposure and increased time to pregnancy, but other studies have not found this association, and one study found that primipara were not affected whereas multipara were affected (based on human data on reproductive outcomes presented in Bull et al. 2014).*"

6.7.2. Key national and international reports

Agency for Toxic Substances and Disease Registry (ATSDR, 2015)

Studies reviewed

The ATSDR reviewed the following studies on the effect of perfluoroalkyls and human reproduction:

- two studies on male reproductive hormones (inhalation route of exposure) (Olsen et al. 1998b; Sakr et al. 2007b);
- twelve studies on oral route of exposure (Knox et al. 2011b; Buck Louis et al. 2012; Fei et al. 2009; Fei et al. 2012; Joensen et al. 2009; Joensen et al. 2013; Kvist et al. 2012); Raymer et al. 2012; Specht et al. 2012; Toft et al. 2012; Vestergaard et al. 2012; Whitworth et al. 2012a), including:
  - four studies on male reproductive hormones (Raymer et al. 2012; Joensen et al. 2009; Joensen et al. 2013; Specht et al. 2012);
  - five studies on sperm (Toft et al. 2012; Joensen et al. 2009; Joensen et al. 2013; Raymer et al. 2012; Kvist et al. 2012);
  - two studies on endometriosis and menopause (Buck Louis et al. 2012; Knox et al. 2011b);
  - four studies on fertility (Fei et al. 2009; Fei et al. 2012; Whitworth et al. 2012a; Vestergaard et al. 2012).

The ATSDR also reviewed three studies on onset of puberty under 'Oral exposure – developmental effects' (Lopez-Espinosa et al. 2011; Chistensen et al. 2011; Vested et al. 2013).

Considerations and conclusions

The ATSDR did not make any statements about reproductive effects in the 'Public health statement for perfluoroalkyls' or 'Relevance to public health' sections of the profile.

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Under 'Reproductive toxicity', the ATSDR stated that: "*The only relevant information regarding reproductive effects in humans following exposure to perfluoroalkyl compounds is that of a positive association between PFOA levels in serum and levels of estradiol and testosterone in serum from male workers (Sakr et al. 2007b). In another occupational study, serum estradiol but not other sex hormones was elevated in a small group of male workers who had the highest serum PFOA levels (Olsen et al. 1998b). Studies in the general population have not consistently found associations between PFOA or PFOS serum levels and alterations in reproductive hormone levels (Joensen et al. 2009, 2013; Raymer et al. 2012; Specht et al. 2012). Conflicting results have also been found in general population studies examining an association with sperm parameters (Joensen et al. 2009, 2013; Raymer et al. 2012; Toft et al. 2012) and impaired fertility (Fei et al. 2009, 2012; Vestergaard et al. 2012; Whitworth et al. 2012a). A study of highly exposed residents found an association between serum PFOA and PFOS levels and earlier onset of menopause (Knox et al. 2011b). Further studies of workers, highly exposed populations, and members of the general population environmentally exposed to perfluoroalkyl compounds could evaluate end points related to fertility such as sperm characteristic and time to pregnancy.*"

Summary of studies reviewed

INHALATION ROUTE-OCCUPATIONAL EXPOSURE

Reproductive hormones (male)

The ATSDR noted that only two studies provided relevant information regarding reproductive effects in humans, with these studies investigating serum levels of sex hormones in male workers (Olsen et al. (1998b) and Sakr et al. (2007b).

Of these two studies, the ATSDR reported that: "*Assays for dehydroepiandrosterone sulfate, estradiol, FSH, 17α-hydroxyprogesterone, free testosterone, total testosterone, LH, prolactin, and sex hormone-binding globulin provided no evidence for associations between PFOA exposure and hormone levels, but workers with the highest serum PFOA levels had mean estradiol levels 10% greater than workers in other groups (Olsen et al. 1998b). Sakr et al. (2007b) also reported a significant association between serum PFOA and serum estradiol levels in workers; additionally, testosterone levels were significantly associated with serum PFOA in linear regression models.*"

Oral exposure route

The ATSDR reviewed 12 studies under the 'Oral exposure route – reproductive effects' section.

Reproductive hormones (male)

The ATSDR made the following introductory statement and conclusion about the four studies reviewed on male reproductive hormones: "*Reproductive toxicity of perfluoroalkyls has been examined in several studies in the general population and in communities living near a PFOA facility. The possible associations between serum perfluoroalkyl levels and alterations in reproductive hormone levels in men have been examined in four general population studies. Overall, these data do not suggest that background levels of perfluoroalkyls alter reproductive hormone levels in men; some studies have found significant associations, but they are not consistent across studies and most studies have not found significant associations.*"

The ATSDR noted excerpts from these four studies as described below:

- Raymer et al. (2012): "*A cross-sectional study of men living in Durham, North Carolina found significant positive correlations between plasma PFOA levels and free testosterone and LH levels, but not with other reproductive hormones. No associations between serum PFOS levels and reproductive hormones were found.*"

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- Joensen et al. (2013): "*In contrast, significant negative associations between PFOS and testosterone, free testosterone, and free androgen index levels were found in a study of Danish young men; no significant associations between reproductive hormone levels and serum PFOA, PFHxS, or PFHpS were found.*"
- Joensen et al. (2009): "*An earlier study of young Danish men with high or low testosterone levels did not find any associations between serum PFOA, PFOS, or PFHxS levels and reproductive hormone levels.*"
- Specht et al. (2012): "*A study of male partners of pregnant women living in Greenland, Poland, or the Ukraine did not find significant associations between serum PFOA, PFOS, PFHxS, or PFNA levels and reproductive hormone levels. The study did find a significant association between serum PFOA levels and sex-hormone binding globulin levels in the Polish men, but the association was no longer significant after adjustment for potential confounds such as age, BMI, smoking, abstinence time, genital infections, or testicular disorders.*"

Sperm

The ATSDR made the following comment on the five studies reviewed on sperm: "*Examination of sperm parameters in the same groups of men has also resulted in conflicting results.*" The ATSDR also highlighted the following comments from the five studies:

- Toft et al. (2012): "*Toft et al. (2012) reported 22 and 35% decreases in the proportion of normal sperm in male partners of pregnant women living in Greenland, Poland, and the Ukraine with the serum PFOS levels in the second (12–27.3 ng/mL) or third (≥27.3 ng/mL) tertiles, as compared to men in the first tertile. Multiple regression analysis was suggestive of a dose-response relationship (p=0.06) between continuous PFOS exposure and the proportion of normal sperm. Similarly, a 35% lower proportion of normal sperm was observed in men with PFHxS levels in the third tertile (>1.5 ng/mL), as compared to the first tertile. A nonsignificant decrease in the proportion of normal sperm was also observed at higher PFNA concentration and no association between the proportion of normal sperm and PFOA exposure was found. A significant increase in the proportion of motile sperm was found for men with PFOA concentrations in the third tertile (>3.8 ng/mL); this was primarily due to men living in Greenland.*"
- Joensen et al. (2009): "*also found decreases in the proportion of normal sperm in young men with combined PFOA and PFOS serum levels in the highest quartile, as compared to men in the first quartile.*"
- Joensen et al. (2013): "*studied a similar group of young men and did not find a significant association between perfluoroalkyl exposure and the proportion of morphologically normal sperm; the study only analyzed perfluoroalkyl exposure as a continuous variable and did not have a combined PFOA and PFOS group.*"
- Raymer et al. (2012): "*also found no significant associations between sperm parameters and PFOA or PFOS levels.*"
- Kvist et al. (2012): "*Another study of the Greenland, Poland, and Ukraine cohort reported a significant positive association between serum PFOS levels and sperm Y:X chromosome ratios in the entire cohort; when the cohort was divided by country, a significant negative association between serum PFOS levels and Y:X chromosome ratio was found in the Greenland subcohort.*"

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Menstruation, endometriosis and menopause

The ATSDR reviewed two studies on endometriosis and menopause. Of the study by Buck Louis et al. (2012), the ATSDR reported that the authors: "*examined the possible association between the occurrence of endometriosis and serum perfluoroalkyl exposure among 373 women living in Salt Lake City, Utah or San Francisco, California scheduled for laparoscopic or laparotomy surgery. Significant associations between endometriosis diagnosis and serum PFOA and PFNA levels were found; the ORs, after adjustment for age and BMI, were 1.89 (95% CI 1.17–3.06) and 2.20 (95% CI 1.02–4.75). Significant associations were also found for PFOS and PFDeA levels but only in unadjusted models. The likelihood of moderate/severe endometriosis was also significantly associated with age and BMI adjusted serum PFOS levels (OR 1.86, 95% CI 1.05–3.30) and PFOA levels (OR 2.58, 95% CI 1.18–5.64). However, when the serum perfluoroalkyl levels were adjusted for parity, the associations were no longer statistically significant.*"

The study by Knox et al. (2011b) was reported by the ATSDR as: "*A study of women participating in the C8 Health Project examined the possible association between serum PFOA and PFOS levels and the onset of menopause (Knox et al. 2011b). Among women 52– 65 years of age, there was a monotonic increase in the odds of experiencing menopause after adjusting for smoking, age, BMI, alcohol consumption, and participation in a regular exercise program in the four highest quintiles of serum PFOS levels (≥11.9 ng/mL), as compared to the first quintile. An increase in the odds of experiencing menopause was also found in women aged 43–51 years with serum PFOS levels in the third, fourth, and fifth quintiles (≥17.1 ng/mL), but it was not monotonic. No associations between menopause onset and serum PFOS levels were found in the youngest group of women (18–42 years). Similarly, PFOA levels ≥11.3 ng/mL were associated with an increased odds of experiencing menopause among the women in the two oldest groups, but not in the youngest group of women; however, the increased risk was not monotonic. Knox et al. (2011b) also found that PFOS levels were negatively associated with serum estradiol levels in the two oldest groups of women; no significant associations between estradiol and serum PFOA levels were found.*"

Fertility/fecundity

The ATSDR reviewed four general population studies that examined the possible association between serum perfluoroalkyl levels and fertility.

Of the study by Fei et al. (2009), the ATSDR reported that: "*Serum PFOA and PFOS levels (collected during the first trimester of pregnancy) were significantly higher in women enrolled in the Danish National Birth Cohort with longer time to pregnancy, as compared to women who got pregnant in the first 6 months. The odds of infertility, defined as a time to pregnancy of >12 months, was also significantly higher in women with serum PFOA or PFOS levels in the second, third, or fourth quartiles (PFOA ≥3.91 ng/mL, PFOS ≥26.1 ng/mL), as compared to women in the first quartile. The fecundity ORs, which measure the odds of a successful pregnancy (odds nulliparous women with PFOS levels in the third or fourth quartile, as compared to the first quartile. Among parous women, significantly elevated odds of infertility were only observed in the second and third PFOS quartile groups. For PFOA, no significant associations were found among nulliparous women, but were found for parous women in the second, third, and fourth quartiles. The fecundability odds were significantly decreased in nulliparous women with serum PFOS levels in the third and fourth quartiles and parous women with serum PFOA levels in the second, third, and fourth quartiles. Among nulligravid women, a decrease in the fecundity ORs were found in the third and fourth quartiles of PFOS (ORs 0.55 [95% CI 0.36–0.85] and 0.51 [95% CI 0.32–0.79]) and PFOA (ORs 0.51 [95% CI 0.27–0.98] and 0.36 [95% CI 0.19–0.68]).*"

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Of the study by Whitworth et al. (2012a), the ATSDR stated that the authors: "*found a significant increase in the odds of subfecundity (time to pregnancy >12 months) in pregnant women participating in the Norwegian Mother and Child Cohort Study with serum PFOA levels in the second, third, and fourth quartiles (≥1.66 ng/mL) or serum PFOS levels in the third or fourth quartiles (≥13.10 ng/mL). Stratifying the women based on parity resulted in no significant association in nulliparous women; increased ORs were noted in parous women with serum PFOA levels in the third and fourth quartiles and serum PFOS levels in the fourth quartile.*

The findings in the nulliparous women are in contrast to the Fei et al. (2012) study, which found significant associations between the odds of infertility (equivalent to the subfecundity index in the Whitworth et al. 2012a study) with serum PFOA and PFOS. The serum levels of PFOA and PFOS were much lower in the Whitworth et al. (2012a) study; 91 and 96% of the women had PFOA and PFOS serum levels, respectively, which would have fallen in the first quartile (referent group) for the Fei et al. (2009, 2012) study."

The findings of the study by Vestergaard et al. (2012) were reported by the ATSDR as: "*Another study of Danish women (Vestergaard et al. 2012) did not find a significant association between the odds of becoming pregnant within the first six menstrual cycles after discontinuing birth control among nulliparous women with PFOA or PFOS serum concentrations above the median. Additionally, no associations were found between time to pregnancy and serum levels and serum PFHxS, PFNA, PFDeA, Et-PFOSA-AcOH, MePFOSA-AcOH, or PFOSA levels.*"

The ATSDR made the following comment about these studies: "*Although the median PFOS and PFOA serum levels were similar in the Vestergaard et al. (2012) and Fei et al. (2009, 2012) studies, differences in the study design particularly the shorter follow-up period (6 versus >12 months) to evaluate time to pregnancy in the Vestergaard study and the different populations (pregnant women versus non-pregnant women) make it difficult to directly compare the study results of the Vestergaard et al. (2012) study with the Fei et al. (2009, 2012) and Whitworth et al. (2012a) studies.*"

Onset of puberty

Under 'Developmental effects – oral exposure', the ATSDR reviewed and reported on three studies that examined the possible association between perfluoroalkyl exposure and development of the reproductive system.

The ATSDR reported the study by Lopez-Espinosa et al. (2011) as: "*In a study of over 3,000 boys and 2,900 girls aged 8–18 years participating in the C8 Health Project and C8 Science Panel studies, Lopez-Espinosa et al. (2011) found ... significant associations between serum PFOS levels and the age of puberty in boys (as assessed by total testosterone levels) and girls (as assessed by self-reported age of menarche); the differences in the age of puberty in boys and girls with serum PFOS levels in the highest quartile (geometric means of 36.0 and 35.2 ng/mL in boys and girls) compared to those in the lowest quartile (geometric means of 10.2 and 9.8 ng/mL) were 190 and 139 days, respectively. In girls, serum PFOA was also significantly ... associated with age of puberty; the differences between the highest (geometric mean of 151.0 ng/mL) and lowest quartile (geometric mean of 7.7 ng/mL) was 130 days. The biological significance of this 4– 5-month delay in sexual maturation is not known.*"

Of the study by Chistensen et al. (2011), the ATSDR reported that the authors: "*did not find any association between maternal perfluoroalkyl levels and age of menarche in 448 girls participating in the Avon Longitudinal Study of Parents and Children in Great Britain; the median maternal serum levels of PFOA, PFOS, and PFHxS were 3.7, 19.8, and 1.6 ng/mL, respectively.*"

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For the study by Vested et al. (2013), the ATSDR reported that: "*A third study of 169 males aged 19–21 years whose mothers participated in a pregnancy cohort study in Denmark found significant inverse associations between maternal serum PFOS levels and sperm concentration and total sperm count and between maternal serum PFOA levels and percentage of progressive spermatozoa. A positive trend between maternal serum PFOA levels and FSH and LH levels in men were found, but there was no association with testosterone or estradiol levels.*"

The ATSDR reported: "*No studies were located regarding reproductive effects in humans following dermal exposure to perfluoroalkyl compounds.*"

United States Environmental Protection Agency (US EPA. 2016a).

Studies reviewed

The US EPA reviewed the following studies on PFOA and reproductive effects:

- two studies on fertility/ fecundity (Fei et al. 2009; Vélez et al. 2015);
- five studies on male reproductive hormones, sperm count and semen quality (Buck Louis et al. 2015; Joensen et al. 2009, Joensen et al. 2013; Vested et al. 2013);
- three studies on onset of puberty (Christensen et al. 2011; Kristensen et al. 2013; Lopez-Espinosa et al. 2011);
- one study on menopause (Knox et al. 2011b); and
- three studies on male reproductive hormones in occupationally exposed workers (Olsen et al. 1998; Sakr et al. 2007; Costa et al. 2009).

For PFOS, the US EPA reviewed:

- one study on menopause (Knox et al. 2011b);
- Three studies on onset of puberty (Lopez-Espinosa et al. 2011; Christensen et al. 2011; Kristensen et al. 2013);
- five studies on fertility/fecundity (Vélez et al. 2015; Jørgensen et al. 2014; Vestergaard et al. 2012; Fei et al. 2009; Bach et al. 2015c);
- eleven studies on male reproductive hormones, sperm count and semen quality (Lopez-Espinosa et al.2011; Kristensen et al. 2013; Joensen et al. 2009; Joensen et al. 2013; Buck Louis et al. 2015; Raymer et al. 2012; Toft et al. 2012; Ding et al. 2013; Joensen et al. 2013; Specht et al. 2012; Vested et al. 2012).

Considerations and conclusions

In the 'Executive Summary' of the 'Health effects support document (PFOA')', the US EPA made the following statement about reproductive effects: "*Developmental outcomes including delayed puberty onset in girls also have been reported; however, in the two studies examining PFOA exposure in relation to menarche, conflicting results were observed: either no association or a possible indication of an earlier menarche seen with higher maternal PFOA levels in one study and a later menarche seen with higher maternal PFOA levels in the other study.*"

In the 'Summary of health effects – fertility, pregnancy and birth outcomes' section, the US EPA reported that: "*Two studies examined development of puberty in females in relation to prenatal exposure to PFOA as measured through maternal or cord blood samples in follow-up of pregnancy cohorts conducted in England (Christensen et al. 2011) and in Denmark (Kristensen et al. 2013). The results of these two studies are conflicting, with no association (or possible indication of an earlier menarche seen with higher PFOA) in Christensen et al. (2011), and a later menarche seen with higher PFOA in Kristensen et al. (2013). Another study examined PFOA exposure measured concurrently with*

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the assessment of pubertal status (Lopez-Espinosa et al. 2011). An association between later age at menarche and higher PFOA levels was observed, but the interpretation of this finding is complicated by the potential effect of puberty on the exposure biomarker levels (i.e., reverse causality)."

In the 'Executive Summary' of the 'Health effects support document (PFOS)', the US EPA reported that: *"Numerous epidemiology studies have examined occupational populations at large-scale PFOS production plants in the United States and a residential population living near a PFOA production facility in an attempt to determine the relationship between serum PFOS concentration and various health outcomes. Epidemiology data report associations between PFOS exposure and reproductive and developmental parameters. Data also suggest a correlation between higher PFOS levels and decreases in female fecundity and fertility...."*

In the 'Summary and conclusions of the human epidemiology studies – fertility, pregnancy and birth outcomes' section, the US EPA reported: *"Although there was some suggestion of an association between PFOS exposures and semen quality parameters in a few studies (Joensen et al. 2009; Toft et al. 2012), most studies were largely null (Buck Louis et al. 2015; Ding et al. 2013; Joensen et al. 2013; Raymer et al. 2012; Specht et al. 2012; Vested et al. 2013). For example, morphologically abnormal sperm associated with PFOS were detected in three (Buck Louis et al. 2015; Joensen et al. 2009; Toft et al. 2012) out of nine (Buck Louis et al. 2015; Ding et al. 2013; Joensen et al. 2013; Raymer et al. 2012; Specht et al. 2012; Vested et al. 2013) studies. Small increased odds of infertility was found for PFOS exposures in studies by Jørgensen et al. (2014) [OR = 1.39; 95% CI: 0.93–2.07] and Vélez et al. (2015) [OR = 1.14; 95% CI: 0.98– 1.34]. Although one study was null (Vestergaard et al. 2012), PFOS exposures were associated with decreased fecundability ratios (FRs), indicative of longer time to pregnancy, in studies by Fei et al. (2009) [FR = 0.74 (95% CI: 0.58–0.93) and in studies by Jørgensen et al. (2014) [FR = 0.90; 95% CI: 0.76–1.07]. Whitworth et al. (2012) data suggested that reverse causality may explain their observation of subfecundity odds of 2.1 (95% CI: 1.2–3.8) for the highest PFOS quartile among parous females, but a reduced odds among nulliparous females (OR = 0.7; 95% CI: 0.4–1.3). A recent analysis of the pooled DNBC study samples found limited evidence of reverse causality with an overall FR of 0.83 (95% CI: 0.72–0.97) for PFOS exposures, as well as comparable ratios for parous (0.86; 95% CI: 0.70–1.06) and nulliparous (0.78; 95% CI: 0.63– 0.97) females (Bach et al. 2015). The same authors reported an increased infertility OR of 1.75 (95% CI: 1.21–2.53) and OR for parous (OR = 1.51; 95% CI: 0.86–2.65) and nulliparous (OR = 1.83; 95% CI: 1.10–3.04) females. Although there remains some concern over the possibility of reverse causation explaining some previous study results, these collective findings indicate a consistent association with fertility and fecundity measures and PFOS exposures."*

Summaries of studies reviewed

Fertility/fecundity – PFOA / PFOS

The US EPA reviewed two studies (Fei et al. 2009; Valez et al. 2015) on fecundity/fertility, which the ATSDR also reviewed. Summaries of these studies are provided above. The US EPA did not review two studies the ATSDR reviewed (Fei et al. 2012; Whitworth et al. 2012b). However, the US EPA reviewed the 2015 studies by Vélez et al. and Bach et al. which the ATSDR did not review.

Of the study by Vélez et al. (2015), the US EPA reported in the 'Health effects support document for PFOA': *"Participants enrolled in the Maternal-Infant Research on Environmental Chemicals Study, a Canadian pregnancy and birth cohort, were evaluated for an association between serum PFOA levels (as well as PFOS and PFHxS) and TTP (Vélez et al. 2015). A total of 1,743 females, enrolled between 2008 and 2011 and having a blood sample collected during the first trimester were included. Infertility was defined as having a TTP of >12 months or requiring infertility treatment for the current pregnancy.*

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The geometric mean plasma PFOA level was 1.66 ng/mL. The crude fecundity OR per one SD increase in log-transformed serum concentration was significantly lower for PFOA (OR=0.91, 95% CI 0.86, 0.96) (and for PFHxS). In fully adjusted models, PFOA (and PFHxS) was associated with an 11% reduction in fecundability per one SD increase in log-transformed serum concentration (OR=0.89; 95% CI 0.83, 0.94). The adjusted odds of infertility increased by 31% per one SD increase of PFOA (OR=1.31; 95% CI 1.11–1.53) (and of PFHxS). No significant associations were observed for PFOS." For this study, the US EPA reported in the Health effects support document for PFOS: *"The geometric mean plasma PFOS level was 4.59 ng/mL. No statistically-significant associations with fecundity were observed, although an increased risk was observed for infertility (OR = 1.14; 95% CI: 0.98–1.34) per one SD increased in PFOS. In contrast, statistically-significant associations were detected for infertility and reduced fecundity and both PFOA and PFHxS."*

While a summary of the study by Fei et al. (2009) is provided above in the ATSDR section, the US EPA commented about this study: *"Although the results of the study suggest that plasma PFOA concentration could reduce fecundity, the authors noted that selection bias, the unknown quality of the sperm, unknown frequency and timing of intercourse, and abnormal hormone levels might have an impact on the results and fecundity."*

Findings on studies regarding PFOS are reported above under 'Summary and conclusions of human epidemiology studies (PFOS)'.

Onset of puberty – PFOA/PFOS

The US EPA and ATSDR reviewed two of the same studies (Lopez-Espinosa et al. 2011; Christensen et al. 2011). Summaries for these two studies are reported above under the ATSDR. Additional details about the studies by Lopez-Espinosa et al. (2011) and Christensen et al. (2011) were reported by the US EPA in the 'Health Effects support document for PFOA and PFOS' and these details are included below. The US EPA did not review the study by Vested et al. (2013) that the ATSDR reviewed. However, the US EPA reviewed the study by Kristensen et al. 2013 which the ATSDR did not review.

Of the study by Kristensen et al. (2013), the US EPA reported in the 'Health effects support document for PFOA': *"Effects of prenatal exposure to PFOA (and PFOS) on female and male reproductive function was evaluated in 343 females and 169 males whose mothers participated in a cohort in 1988– 1989 (Kristensen et al. 2013; Vested et al. 2013). Maternal blood samples were collected during week 30 of gestation. Follow-up was initiated in 2008 when the offspring were ~20 years old. Median serum PFOA level was 3.6 ng/mL for the mothers with daughters evaluated. In adjusted regression analysis, daughters from mothers in the highest PFOA tertile had a 5.3-month later age at menarche (95% CI 1.3, 9.3) than those in the lowest tertile. No association was found between prenatal exposure to PFOS and age of menarche. No statistically significant relationships were found between PFOA (or PFOS) exposure and cycle length, reproductive hormone levels, or number of follicles assessed by ultrasound (Kristensen et al. 2013)."*

For PFOS, the US EPA reported in the 'Health effect support document': *"Median serum PFOS level was 21.1 ng/mL for the mothers with daughters evaluated. Potential confounders adjusted for included maternal smoking during pregnancy, social class, and daughter's BMI. No statistically-significant association was found between prenatal exposure to PFOS and age of menarche. In adjusted regression analysis, daughters from mothers in the highest PFOA tertile had a later age at menarche compared with those in the lowest tertile. No statistically-significant relationships were found between PFOS (or PFOA) exposure and cycle length, reproductive hormone levels, and number of follicles assessed by*

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ultrasound (Kristensen et al. 2013). Study limitations included retrospective collection of some health outcome data, such as age of the menarche, which was queried 2–10 years afterward."

For PFOS, the US EPA reported in the 'Health effects support document for PFOS', on the study by Lopez-Espinosa et al. (2011), including: "*The median serum PFOS level was 18 ng/mL among these female participants, and exposures were examined continuous and categorical (quartiles) variables. Pubertal development was based on hormone levels (total > 50 ng/dL and free > 5 pg/mL testosterone in boys and estradiol > 20 pg/mL in girls) or onset of menarche. although participant age at survey and time of day of blood sampling were the only confounders that were identified and adjusted for, other covariates considered as potential confounders included BMI z-score, height annual household family income, ethnicity, ever smoking, and ever alcohol consumption. A reduced odds of having reached puberty was found with increasing PFOS levels, with girls having a difference of 138 days between the highest and lowest PFOS quartile. A reduced odds of postmenarche was found for both PFOS (138 days of delay) and PFOA (130 days of delay)."*

Regarding the study by Christensen et al. (2011), the US EPA reported in the 'Health Effects support document for PFOA' that the authors: "*used data from a prospective cohort study in the United Kingdom to perform a nested case-control study examining the association between age at menarche and gestational exposure to perfluorinated chemicals, including PFOA and PFOS. The study population from the Avon Longitudinal Study of Parents and Children included single-birth female subjects who had completed at least two puberty staging questionnaires between the ages of 8 and 13 years and whose mothers provided at least one analyzable prenatal serum sample. If more than one serum sample was available, the earliest sample provided was used for analysis. The study does not provide information as to when samples were collected. The females were divided into two groups: those who experienced menarche prior to age 11.5 years (n = 218) and a random sample of those who experienced menarche after age 11.5 (n = 230). Confounders such as the mother's prepregnancy BMI, age at delivery, age at menarche, educational level, and the child's birth order and ethnic background were included in linear and logistic regression models used to analyze the data. The median maternal serum PFOA concentrations were 3.9 and 3.6 ng/mL for the early menarche and nonearly menarche groups, respectively. The authors noted a modest nonsignificant association between the odds of earlier menarche and prenatal serum PFOA concentrations above the median. For all models, the CIs included the null value of 1.0. Similar results were obtained for PFOS."*

For this study, regarding PFOS, the US EPA reported that: "*median maternal serum PFOS concentrations were 19 and 20 ng/mL for the early menarche and non-early menarche groups, respectively. Although not statistically-significant, decreased adjusted odds ratios for earlier age at menarche were found for the prenatal PFOS examined as a continuous [OR = 0.68; 95% CI: 0.40–1.13] and the categorical [OR = 0.83; 95% CI: 0.56–1.23] exposure dichotomized as the median value (19.8 ng/mL). Results were null for the continuous PFOA exposure measure and slightly elevated for the categorical exposure [OR = 1.29; 95% CI: 0.86–1.93] above the median value of 3.7 ng/mL. The limitations of the study included having a small sample size, using a single maternal gestational serum sample for perfluorinated chemical measurement, and the self-reported nature of some covariates including menarche status and age at menarche."*

Male reproductive hormones and sperm – PFOA/PFOS

The ATSDR and US EPA both reviewed studies by Joensen et al. (2009), Joensen et al. (2013), Vested et al. (2013), Lopez-Espinosa et al. (2011), Toft et al. (2012) and Raymer et al. (2012). Summaries of these studies are provided under the ATSDR section above. The US EPA provided more detail about

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these studies, and this is included below. Additionally, the US EPA also reviewed the studies by Buck Louis et al. (2015) and Kristensen et al. (2013).

Of the study by Joensen et al. (2009), the US EPA reported for PFOA that the authors: "*examined the association between PFASs, including PFOA, and testicular function in 105 Danish males who provided semen and blood samples as part of reporting for the military draft in 2003. The males chosen for the study had the highest testosterone concentrations (ranging from 30.1 to 34.8 nmol/L; n = 53; 18.2–24.6 years) and lowest testosterone concentrations (ranging from 10.5 to 15.5 nmol/L; n = 52; 18.2–25.2 years). Regression models were used to analyze associations between PFOA and testicular function. Median serum PFOA concentration was 4.4, 5.0, 4.9 ng/mL in the high testosterone, low testosterone, and combined groups, respectively. A nonsignificant negative association was observed between serum PFOA concentration and semen volume, sperm concentration, sperm count, sperm motility, or sperm morphology. No association was observed between serum PFOA concentration and testosterone, estradiol, sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and inhibin B. However, significantly fewer (p <0.05) morphologically normal sperm were seen in males with high combined levels of PFOA/PFOS (6.2 million spermatozoa) than in males with low PFOA/PFOS levels (15.5 million).*"

Details provided by the US EPA for the study by Joensen et al. (2013) were: "*In a slightly expanded study, Joensen et al. (2013) investigated the associations between PFASs, including serum PFOA concentration, and reproductive hormones and semen quality in healthy young Danish males (mean age 19.6 years). Serum samples were analyzed for PFOA as well as total testosterone (T), estradiol, SHBG, LH, FSH, and inhibin-B. The mean PFOA level was 3.5 ng/mL. No associations were found between PFOA levels (or 12 other PFAS) and any hormone level or semen quality parameters. PFOS levels were negatively associated with testosterone.*"

For PFOS, the US EPA reported in the 'Health effects support document (PFOS)': "*To address previous study limitations and expand the generalizability of the findings, a later study by Joensen et al. (2013) was conducted to investigate the associations between serum PFOS concentration and reproductive hormones and semen quality. Study participants included a random sample of 247 healthy young Danish males (mean age 19.6 years) recruited in 2008– 2009 from the same study population. Serum samples were analyzed for PFOS, as well as total testosterone (T), estradiol (E), sex hormone-binding globulin (SHBG), LH, FSH, and inhibin-B. Semen samples were collected the same morning as the blood samples, and self-administered questionnaires were also completed by the study participants. Confounders adjusted for in the various regression models included time to semen analysis, abstinence time, BMI, and smoking. The mean PFOS level was 8.5 ng/mL. Inverse associations were detected for PFOS and various outcomes including T, calculated free T (FT), free androgen index (FAI), and ratios of T/LH, FT/LH, and FAI/LH (all p-values ≤ 0.05). PFOS was also inversely associated with estradiol, T/E ratio, and inhibin-B/FSH ratio, and positively associated with SHBG, LH, FSH, and inhibin-B, although statistical significance was not attained. No associations were detected between PFOS levels and any semen quality parameters. Study strengths included improved generalizability due to the random selection of subjects from the general population and a higher participation rate was (30%) compared to other population-based semen quality studies.*"

Of the study by Buck Louis et al. (2012), the US EPA reported that: "*An association between serum levels of seven PFASs and 35 semen quality parameters was evaluated in 462 males enrolled in the LIFE study cohort (Buck Louis et al. 2015). The males were from Michigan and Texas with a mean age of 31.8 years and mean PFOA levels 4.29– 5.09 ng/mL. PFOA was significantly associated with a lower percentage of sperm with coiled tails, an increased curvilinear velocity, and a slightly larger acrosome area of the head. In total, six PFASs (including PFOA) were associated with changes in 17 semen quality*

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end points." For PFOS, the US EPA reported *"The study participants had a mean age of 31.8 years and mean PFOS levels were 17 ng/mL for Michigan residents and 21 ng/mL for Texas residents. Statistically-significant associations were detected between PFOS exposures and for a lower percentage of sperm with coiled tails; no associations were found for any other end point. In total, six PFAS (including PFOS) were associated with changes in 17 semen quality end points. Study strengths included improved generalizability, since participants were from the general population and had a higher participation rate (42%) compared to other population-based semen quality studies. A key study limitation of this and many of these types of epidemiology studies is the uncertainty related to the critical exposure window(s) relative to timing of the collected samples and the multiple comparisons (n = 245) that were examined."*

The study by Vested et al. (2012) was reviewed by the ATSDR with a summary provided under the 'Oral exposure – developmental effects' section above. The US EPA provided some additional detail in the 'Health effects support document for PFOA', including: *"Median serum PFOA level was 3.8 ng/mL for mothers with sons evaluated. Multivariable regression models showed significant negative trends for sperm concentration and total sperm count in association with in utero exposure to PFOA. A 34% reduction in sperm concentration (95% CI 5 to 58%) and a 34% reduction in total count (95% CI 12 to 62%) were estimated for the highest exposure tertile compared with the lowest tertile. Maternal PFOA level also was positively associated with higher FSH and LH levels in the sons. No associations were found between PFOA level and percentage of progressive sperm, sperm morphology, semen volume, or testicular volume. PFOS was not associated with any outcome (Vested et al. 2013)."*

The studies by Kristensen et al. (2013) and Vested et al. (2013) were reviewed in the 'Health effects support document for PFOS', with the summaries reported as: *"Reproductive function and other reproductive end points also were evaluated in the sons of the mothers who participated in the Aarhus, Denmark cohort (Kristensen et al. 2013). The median (25th–75th percentile) serum PFOA level was 21.2 ng/mL (0.017.4–0.026.5 ng/mL) for the mothers with sons who were evaluated. PFOS was not associated with any outcome of reproductive function analyzed with multivariable regression models. No associations were found between PFOS (and PFOA) levels and percentage of progressive sperm, sperm morphology, semen volume, or testicular volume. Monotonic exposure-response relationships were detected for in utero PFOA exposure and sperm concentration, total sperm count, and percentage of progressive spermatozoa (based on the computer-assisted semen analysis), and positive associations for follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were associated with PFOA (Vested et al. 2013)."*

The study by Raymer et al. (2012) was also reviewed by the ATSDR. Additional details about the study were reported by the US EPA as: *"Raymer et al. (2012) conducted a cross-sectional study of the relationships between PFAS and semen quality and reproductive hormones. The study population included 256 males recruited between 2002 and 2005 from Duke University Medical Center's IVF Clinic. Reproductive health questionnaires were administered to participants. Blood and semen samples were used to detect PFAS and were both collected at the time of evaluation. Linear and logistic regression models were used to calculate effect estimates and were adjusted for age, period of abstinence, and tobacco use. The average PFOS levels in plasma were 37.4 ng/mL and 0.8 ng/mL in semen. The strongest correlations detected between PFAS and hormones were between plasma PFOS and LH (r = 0.12), plasma PFOA and LH (r = 0.16), plasma PFOS and triiodothyronine (r = 0.14), as well as semen PFOS and FSH (r = 0.13). No statistically significant associations were detected between PFOS and PFOA concentrations and reproductive hormones or different semen quality outcomes. The older population (mean age = 42 years) may limit comparability with previous studies and generalizability of study findings."*

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Of the study by Toft et al. 2012, the US EPA commented that: "*The variable participation rates across study sites and potential for participation bias (i.e., if participation was related to fertility status and exposure levels) complicate interpretation of these results. The cross-sectional nature of this study also limits the ability to draw causal inference from these types of studies, especially since temporality could not be established some of the study population based on the timing of the blood and semen samples (e.g., nearly 60% of the Greenland samples were collected approximately a year before the semen samples).*"

Information about studies reviewed on PFOS and sperm is also available above in the 'Considerations and conclusions' section.

Menstruation, endometriosis and menopause – PFOA/PFOS

The US EPA reviewed the same study as the ATSDR (Knox et al. 2011b), but did not review the study by Buck Louis et al. (2012), which the ATSDR reviewed.

In the 'Health support document for PFOA', the US EPA provided additional detail to the ATSDR profile for the study by Knox et al. (2011b) conducted in a high-exposure community: "*Knox et al. (2011b) examined the endocrine disrupting effects of perfluorocarbons in females from the C8 Health Project by analyzing the relationship between serum PFOA, serum estradiol concentration, and menopause onset. The population included females over age 18 years (n = 25,957). Serum PFOA and estradiol concentrations were determined from blood samples. Females who were pregnant; had had full hysterectomies; and were taking any prescription hormones, selective estrogen receptor modulators, and/or fertility agents were excluded from estradiol analysis. Serum PFOA concentrations were grouped into quintiles (natural log-transformation)—Q1 = 0.25–11.2; Q2 = 11.3–19.8; Q3 = 19.9–36.7; Q4 = 36.8–84.9; and Q5 = 850–22412 ng/mL. Estradiol analysis was calculated by age group—18–42 years, >42 ≤ 51 years, and >51 ≤ 65 years. Menopause was determined by questionnaire. Menopause analysis was calculated by age group—30–42 years, >42 ≤ 51 years, and >51 ≤ 65 years—and excluded those who reported having had hysterectomies. Logistic regression models were adjusted for smoking, age, BMI, alcohol consumption, and regular exercise. PFOA concentration in females who had had hysterectomies was significantly higher than in females who had not had hysterectomies. Serum PFOA and estradiol concentrations were not associated, while PFOS levels were negatively associated with estradiol. The odds of attaining menopause analysis in the oldest group of females, showed that all quintiles were significantly higher for all quintiles than the lowest, and in females between the ages of 42 and 51 years, Q3, Q4, and Q5 were significantly higher than the lowest. PFOS also was associated with increased odds of attaining menopause in women 42–51 years and >51 years. Data interpretation was limited by the cross-sectional study design and survey-reported menopause without age or independent confirmation.*"

In the 'Health effects support document for PFOS', the US EPA reported the following about the study by Knox et al. (2011b) that: "*These data were cross-sectional, with a one-time serum measurement collected for participants. The mean PFOS level of all the females was 18 ng/mL. The analyses of menopause excluded participants who reported undergoing a hysterectomy. Logistic regression models were adjusted for age, smoking, alcohol consumption, BMI, and exercise. The analysis for menopause was determined upon three groups of females: childbearing (aged 30– 42), perimenopausal (aged > 42–51) and menopausal (aged > 51– ≤ 65). These same groups were used for the estradiol concentrations except the childbearing group was extended to include those > 18 years; exclusions for this analyses included pregnant females, females with a full hysterectomy, or females taking hormones, fertility drugs, or selective estrogen receptor modulators. Among females aged 51–65, statistically-significant ORs for menopause were detected across PFOS quintiles, including a monotonic*

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dose-response relationship. Similar results were found with PFOA quintiles (OR range: 1.5–1.7). Although dose-response relationships were not evident, consistent ORs for menopause were detected among the perimenopausal age group, as well for both PFOS and PFOA exposures (OR range: 1.2–1.4). Inverse associations were detected between estradiol concentrations and PFOS in the perimenopausal group (β = −3.65; p < 0.0001) and menopausal group (β = −0.83; p < 0.007). Serum PFOA and estradiol concentrations were not associated. Despite the contaminated water supplies, the PFOS exposure levels were comparable to those from NHANES and likely represented general population levels. A study limitation was the one-time serum measurement and cross-sectional study design; thus, exposure misclassification is likely despite long half-lives reported for PFAS. The level of PFOS was significantly higher in the set of females that had undergone a hysterectomy. Menopause and having undergone a hysterectomy, therefore, may be associated with increased serum PFAS due to the loss of menstruation as a route for removing PFOS with the associated menstrual blood loss. Thus, reverse causation cannot be ruled out as an alternative explanation for the study findings."

Occupational exposure (male reproductive hormones)

The US EPA reviewed three studies of occupationally exposed workers (Olsen et al. 1998; Sakr et al. 2007; Costa et al. 2009). The ATSDR also reviewed the studies by Olsen et al. (1998) and Sakr et al. (2007), with summaries provided above.

The US EPA provided greater detail about the study by Olsen et al. (1998) and this is included below: "*Olsen et al. (1998) examined several hormones, including cortisol, estradiol, FSH, dehydroepiandrosterone sulfate, 17 gamma-hydroxyprogesterone (a testosterone precursor), free testosterone, T, LH, prolactin, and SHBG in male workers at the Cottage Grove, Minnesota, production plant for 1993 and 1995. This was the same population used for the thyroid hormone study described above for 111 workers in 1993 and 80 in 1995. Employees were placed into four exposure categories based on their serum PFOA levels: 0–1000 ng/mL , 1000– < 10000 ng/mL , 10000– < 30000 ng/mL , and >30000 ng/mL . Statistical methods used to compare PFOA levels and hormone values included multivariable regression analysis, ANOVA, and Pearson correlation coefficients. No association between serum PFOA and any hormone was observed, but some trends were observed. When the mean measures of the various hormones were compared by exposure categories, there was a statistically significant elevation in prolactin (p = 0.01) in 1993 only for the 10 workers whose serum PFOA levels were between 10000 and 30000 ng/mL compared to the lower two exposure categories.*

Estradiol levels in the >30000 ng/mL PFOA group in both years were 10% higher than in the other PFOA groups, but the difference was not statistically significant. These results were confounded by estradiol being correlated with BMI (r = 0.41, p<0.001 in 1993, and r=0.30, P<0.01 in 1995).The authors postulated that the study might not have been sensitive enough to detect an association between PFOA and estradiol because measured serum PFOA levels were likely below the observable effect levels suggested in animal studies (e.g., 55000 ng/mL PFOA in the CD rat). Only three employees in this study had PFOA serum levels that high. They suggest that the higher estradiol levels in the highest exposure category could suggest a threshold relationship between PFOA and estradiol."

Of the study by Costa et al. (2009), the US EPA reported that the authors: "*...found no association between serum PFOA concentration and estradiol or testosterone in 53 male workers at a PFOA production plant in Italy based on medical surveillance data collected between 2000 and 2007.*"

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6.7.3. Systematic reviews

Saikat et al. (2013)

Saikat et al. (2013) reviewed the epidemiological evidence on the impact of PFOS on the fertility of the general population.

Studies reviewed

Saikat et al. reviewed two studies on fertility:

one study on female fertility (Fei et al. 2009); and

one study on male fertility and sperm (Joensen et al. 2009).

The two studies reviewed by Saikat et al. were also reviewed by the ATSDR, US EPA and Priestly, with summaries provided above.

Considerations and conclusions

Saikat et al. made the following statement in the 'Abstract': "*Small but statistically significant associations have been reported with PFOS and infertility.*"

Saikat et al. made the following comment about the study by Fei et al. (2009) in the 'Limitations' section: "*The second cohort study, Fei et al. [2009] demonstrating a significant association between blood serum PFOS and self-reported female infertility was limited by selection bias. They chose a population of women with a successful pregnancy to study the risk of infertility. Therefore it is possible that the detected association is underestimated and may actually be higher. This study was further limited by the self-reported outcome measurement which has the potential to introduce recall bias.*"

In the 'Coherence with evidence' section, Saikat et al. commented about the study by Joensen et al. (2009): "*Male fertility (testicular function) was only investigated in one small study*[19] *(n=546) that did not demonstrate an association.*"

Priestly (2016)

Priestly reviewed the human literature on fecundity (difficulty in getting pregnant, time to achieve pregnancy or menstrual cycle problems that could impinge on fecundity) and some studies on events in the later life of off spring (delayed menarche), under the section 'Altered foetal development and effects on pregnancy'. Priestly also reviewed the literature on 'Altered sperm levels and function'.

Studies reviewed

Priestly reviewed the following studies:

- six studies on fecundity (Fei et al. 2009; Bach et al. 2015c; Vestergaard et al. 2012; Whitworth et al. 2012; Jørgensen et al. 2014; Valez et al. 2015);
- two studies on menstruation and menopause (Lyngsø et al. 2014; Taylor et al. 2014);
- three studies on onset of puberty (Wu et al. 2015; Kristensen et al. 2013; Christensen et al. 2011);
- one study on ovarian hormone concentrations (Barrett et al. 2015);
- one study on polycystic ovary syndrome (Vagi et al. 2014);

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- nine studies on altered sperm levels and function (Joensen et al. 2009; Joensen et al. 2013; Louis et al. 2015[48]; Toft et al. 2016; Vested et al. 2013; Jensen et al. 2014; Tsai et al. 2015; Toft et al. 2012; Leter et al. 2014).

Considerations and conclusions

Priestly, in his 'Executive Summary', stated that: "*The epidemiological studies are suggesting, but not yet proving, a possible link between PFOS/PFOA and ... altered sperm function.*"

Under the section 'Altered sperm levels and function', Priestly made the following comment on the studies he reviewed: "*Interpretation of chemical factors that lead to reduced sperm count is always difficult. The studies reported so far have been relatively inconsistent with regard to sperm quality, but there is greater consistency with regard to alterations of testosterone levels. Most of the authors conceded their findings need to be corroborated with larger studies.*"

Summaries of studies reviewed

Fecundity/fertility

The studies by Fei et al. (2009), Verstergaard et al. 2012; Whitworth et al. 2012; and Valez et al. (2015) were reviewed by the ATSDR and/or US EPA, with summaries provided above. Priestly provided the following information about the studies by Bach et al. (2015b) and Jørgensen et al. (2014).

Of the study by Bach et al. (2015c), Priestly provided the following information in Table 5 (pg 37): "*Update on the Danish National Birth Cohort; n=550 in sample 1 (new); n=1400 sample 2 (previously reported); Serum PFOS & PFOA from 1st trimester; TTP by questionnaire;No change on TTP or infertility for PFOS in sample 1; trend to longer TTP for PFOA only in parous women; findings of lower fecundibility in sample 2 (previously reported in 2009) confirmed in both parous and nulliparous women 13-22% lower in 3 higher quartiles for PFOS and PFOA.*"

Priestly reported the study by Jørgensen et al. (2014) as: "*INUENDO cohort of 1710 pregnant women from Greenland, Poland & Ukraine; 938 sub-group with PFAS serum levels; 2001-04; Serum PFOA, PFOS, PFHxS, PFNA measured at ante-natal care visits; reported TTP; Median (mg/mL[49]) serum levels PFOA 0.92 -2.67 across 3 regions; PFOS 4.93 – 20.32; PFNA 0.6 – 0.7 Higher PFNA levels were associated with longer TTP in the pooled sample (log-scale FR = 0.80; 95% CI 0.69-0.94) and specifically in women from Greenland (log-scale FR = 0.72; 95% CI 0.58-0.89). ORs for infertility were also increased in the pooled sample (log-scale OR = 1.53; 95% CI 1.08-2.15) and in women from Greenland (log-scale OR = 1.97; 95% CI 1.22-3.19). However, in a sensitivity analysis of primiparous women these associations could not be replicated. Associations with PFNA were weaker for women from Poland and Ukraine. PFOS, PFOA and PFHxS were not consistently associated with TTP.*"

Menstruation and menopause

Priestly reviewed the studies by Lyngsø et al. (2014) and Taylor et al. (2014) which were not reviewed by the ATSDR or US EPA. Priestly reported for the study by Lyngsø et al. (2014): "*INUENDO cohort of 3833 pregnant women from Greenland, Poland & Ukraine; 1743 sub-group with menstrual cycle data; 2002-04; Serum PFOA, PFOS, measured at ante-natal care visits; menstrual cycle questionnaire; Higher exposure to PFOA associated with longer menstrual cycle on polled estimated from 3 countries; OR for long cycles 1.8 (95% CI 1.0, 1.33) in highest tertile; no effects seen for*

[48] Priestly references Louis et al. 2015, whereas other studies have referenced this as Buck Louis et al. 2015. Please refer to Buck Louis et al. 2015 in the reference list.
[49] The Panel believes this may be incorrect and it may be meant to be ng/mL.

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PFOS, although tendency for more irregular cycles OR 1.7 (0.8, 3.5); authors noted variability across countries in participation rates and lacking data on possible confounders (stress, disease and gynaecological disorders)."

Of the study by Taylor et al. (2014), Priestly reported that the authors: *"described a positive association between PFAS serum concentrations and early menopause in women (n = 2,732) using the NHANES dataset of PFAS measurements from 1999-2000 to 2009-2010. After adjusting for age at interview, education, smoking status and parity, women whose PFAS were in the higher two tertiles had consistently higher rates of early menopause than those in the lowest tertile. The relationship with PFOA, PFNA and PFHxS was monotonic, but with PFOS, the adjusted odds ratio was higher in tertile 2 than in tertile 3. There was a positive dose-response for all four PFAS with hysterectomy. However, they cautioned that the apparent relationship between PFAS and menopause may reflect reverse causation, since the PFAS can accumulate with time after hysterectomy and/or menopause due to reduced clearance via the menstrual flow."*

Onset of puberty

Priestly reviewed three studies on onset of puberty. Two of these studies (Kristensen et al. 2013; Christensen et al. 2011) were reviewed by the ATSDR and/or US EPA.

Priestly also reviewed the study by Wu et al. (2015). This study was not reviewed by the ATSDR or US EPA. Priestly provided the following information on this study: *"A similar conclusion about the influence of changing PFAS pharmacokinetics in women explaining the apparent delay in menarche is proposed by Wu et al. (2015) from the same research group. They used a Monte Carlo simulation of a PBPK model to investigate the time-course of changes in PFAS blood level in women during growth phases and menarche. They were able to match model-predicted PFAS blood levels with changing physiological parameter inputs into the models, suggesting that the relationship between PFAS and delayed menarche could be explained, at least partly, on the pharmacokinetic changes in women."*

Ovarian hormone concentrations

Priestly reviewed one study on PFAS exposure and ovarian hormone concentrations (Barrett et al. 2015). This paper was not reviewed by the ATSDR or US EPA. Of the study by Barrett et al. (2015), Priestly reported that: *"It seems that any interaction between PFAS and sex hormone status is complex. Barrett et al. (2015) found a weak negative association between serum PFOS levels and ovarian hormone concentrations (E2 and progesterone), but only in nulliparous women, and not in parous women. There were no effects of other PFAS in either parous or nulliparous women."*

Polycystic ovary syndrome

Priestly reviewed one paper on polycystic ovary syndrome (Vagi et al. 2014). This paper was not reviewed by the ATSDR or US EPA.

Priestly reported the following about this paper: *"In a study attempting to link the incidence of polycystic ovary syndrome with exposure to endocrine disrupting chemicals, Vagi et al. (2014) found that only PFOA and PFOS, among the 29 POPs and EDCs measured, had significantly higher serum concentrations in cases compared to controls (PFOA 4.1 vs 2.3 ng/mL; PFOS 8.2 vs 4.9 ng/mL). Lower urinary concentration of monobenzyl phthalate was the only other finding of note. The authors did not draw any causal inferences, but indicated that these preliminary findings need further investigation."*

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Sperm levels and function

Priestly reviewed nine studies on altered sperm levels and function. The studies by Joensen et al. (2009), Joensen et al. (2013), Vested et al. (2013); Tsai et al. (2015); Toft et al. (2012) were also reviewed by the ATSDR and/or US EPA, with summaries of these studies provided above.

Priestly reviewed four studies in addition to those reviewed by the ATSDR and/or US EPA (Louis et al. 2015; Toft et al. 2016; Leter et al. 2014; Jensen et al. 2014).

Of the study by Louis et al. (2015) Priestly reported that: "*In a U.S cohort from two regions (Michigan and Texas), there were some small, but inconsistent effects of PFAS on sperm quality across 17 end points measured. PFOSA was possibly associated with smaller sperm head/perimeter, a lower percentage of DNA stainability and a higher percentage of bicephalic and immature sperm. PFDeA, PFNA, PFOA and PFOS were possibly associated with a lower percentage of sperm with coiled tails (Louis et al. 2015). The divergent results provide no substantive evidence of an effect on sperm quality, consistent with some of the above studies.*"

The study by Toft et al. (2016) was reported by Priestly as: "*In another study on young Danish men, Toft et al. (2016) found that the highest tertile of PFOS concentration (>1.4 ng/mL) in stored amniotic fluid from their mothers was associated with a 40% lower insulin-like Factor 3 and an 18% higher testosterone concentration in amniotic fluid, compared to the lowest tertile PFOS (<0.8 ng/mL). There was no association between PFOS levels and the incidence of cryptorchidism or hypospadias in these young men. An earlier study from the same group (Vested et al. 2013) found that in utero exposure to PFOA, but not PFOS, was associated with lower sperm counts and higher levels of LH in men 19-21 years of age. Jensen et al. (2014), using a nested case-control methodology, also found no association between cord blood PFOS or PFOA with congenital cryptorchidism in boys from Denmark and Finland.*"

Of the study by Leter et al. (2014), Priestly reported that: "*In a companion study on 262 male partners from Greenland, Poland and Ukraine, no consistent PFAS effects were noted on sperm DNA global methylation.*"

For Jensen et al. (2014), Priestly reported this study as: "*Jensen et al. (2014), using a nested case-control methodology, also found no association between cord blood PFOS or PFOA with congenital cryptorchidism in boys from Denmark and Finland.*"

Rappazzo et al. (2017)

Rappazzo et al. reviewed the literature on exposure to perfluorinated alkyl substances and onset of puberty in children.

Studies reviewed

Rappazzo et al. reviewed six studies of pubertal onset indicators (Kristensen et al. 2013; Lopez-Espinosa et al. 2011; Lopez-Espinosa et al. 2016; Christensen et al. 2011; Maisonet et al. 2015b; Tsai et al. 2015).

Considerations and conclusions

In the 'Abstract', Rappazzo et al. concluded that: "*While there are a limited number of studies for any one particular health outcome, there is evidence for positive associations between PFAS and ...age at menarche.*"

At the end of the section 'Pubertal onset indicators', Rappazzo et al. stated: "*The six studies of pubertal onset indicators have generally mixed results and varied study design. The most consistent*

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evidence is for later age at menarche associated with either PFOA or PFOS exposure or both. This is supported by toxicological evidence from mouse models in which female offspring had delayed mammary gland development [White et al. 2011] and vaginal opening [Yang et al. 2009] with in utero and peri-pubertal exposure to PFOA, respectively."

In the 'Conclusion' section of the paper, Rappazzo et al. commented that: "*Within the published literature, there is an incomplete assessment of pubertal onset in girls. Epidemiologic publications for pubertal onset in girls across PFAS concentrations look at age at menarche, but lack information on thelarche or the onset of female breast development. Breast development has been shown to be sensitive to PFAS exposure in laboratory animals and the dearth of information on this end point in developing human populations is an area that could be expanded to allow for better cross-species comparison. Existing cohorts of US girls with data on these pubertal end points could be mined to understand these associations."*

Summaries of studies reviewed

Onset of puberty

The studies by Kristensen et al. (2013), Lopez-Espinosa et al. (2011), Christensen et al. (2011) were also reviewed by the ATSDR and/or US EPA with summaries provided above. Rappazzo et al. reviewed three studies (Lopez-Espinosa et al. 2016; Maisonet et al. 2015b; Tsai et al. 2015) that neither the ATSDR nor US EPA reviewed, and summaries of these studies are provided below.

Of the study by Lopez-Espinosa et al. (2016), Rappazzo et al. reported that: "*In a further examination of the C8 cohort, [Lopez-Espinosa et al. 2016] found insulin-like growth factor, a marker of pubertal onset, to be negatively associated with serum PFOA (in girls), PFOS and PFNA (both boys and girls)."* Rappazzo et al. then provided context "*It is important to note that menstrual blood loss is a potential route of PFAS excretion, thus in cross-sectional studies of girls with later pubertal onset may have higher PFAS levels than girls with earlier pubertal onset."*

Rappazzo et al. reported the study by Maisonet et al. (2015b) as: "*In a subset of the same population as Christensen, Maisonet, Rubin, Holmes, Calafat, Kato, Flanders, Heron, McGeehinand Marcus [2011], higher levels of PFOA, PFOS and PFHxS were associated with higher levels of total testosterone in girls, while no associations were observed with sex hormone-binding globulin concentrations [Maisonet et al. 2015b]."*

For the study by Tsai et al. (2015), Rappazzo et al. reported that: "*A cross-sectional study in Taiwan examined follicle stimulating hormone levels in association with serum PFAS in 12–17 year olds, finding decreased FSH associated with increasing PFOS in boys and PFU in girls; there was no evidence of associations for PFOA and PFNA [Tsai et al. 2015]."*

Kirk et al. (2018)

For 'Reproductive effects', Kirk et al. (2018) reviewed the most studies of all of the key international authority reports and systematic reviews and reported the outcomes comprehensively.

Studies reviewed

Kirk et al. evaluated 34 papers that investigated the effect of PFAS on reproductive outcomes in children and adults, including:

- Under reproductive hormone levels:

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- eleven studies on testosterone (Joensen et al. 2009; Itoh et al. 2016; Lopez-Espinosa et al. 2016; Maisonet et al. 2015b; Olsen et al. 1998b; Raymer et al. 2012; Toft et al. 2016; Tsai et al. 2015; Vested et al. 2013; Zhou et al. 2016; Joensen et al. 2013);
- ten studies on oestradiol (Joensen et al. 2009; Itoh et al. 2016; Lopez-Espinosa et al. 2016; Olsen et al. 1998b; Raymer et al. 2012; Vested et al. 2013; Zhou et al. 2016; Joensen et al. 2013; Barrett et al. 2015; Knox et al. 2011b);
- seven studies on Luteinizing hormone (Joensen et al. 2009; Itoh et al. 2016; Olsen et al. 1998b; Raymer et al. 2012; Tsai et al. 2015; Vested et al. 2013; Joensen et al. 2013);
- seven studies on Follicle -Stimulating Hormone (Joensen et al. 2009; Itoh et al. 2016; Olsen et al. 1998b; Raymer et al. 2012; Tsai et al. 2015; Vested et al. 2013; Joensen et al. 2013);
- seven studies on Sex hormone-binding globulin ((Joensen et al. 2009; Itoh et al. 2016; Maisonet et al. 2015b; Olsen et al. 1998b; Tsai et al. 2015; Vested et al. 2013; Joensen et al. 2013);
- nine studies on other reproductive hormones (Joensen et al. 2009; Itoh et al. 2016; Lopez-Espinosa et al. 2016; Olsen et al. 1998b; Raymer et al. 2012; Toft et al. 2016; Vested et al. 2013; Joensen et al. 2013; Barrett et al. 2015);

- nine studies on time to pregnancy (TTP), fecundity and fertility (Fei et al. 2009; Valez et al. 2015; Bach et al. 2015b; Buck Louis et al. 2013; Jorgensen et al. 2014; Lum et al. 2017; Vetergaard et al. 2012; Whitworth et al. 2012b; Whitworth et al. 2016);
- six studies on sperm characteristics (Buck Louis et al. 2015; Joensen et al. 2009; Joensen et al. 2013; Raymer et al. 2012; Toft et al. 2012; Vested et al. 2013);
- seven studies on menstruation and menopause (Lum et al. 2017; Lyngsø et al. 2014; Buck Louis et al. 2012; Campbell et al. 2016; Dhingra et al. 2016; Knox et al. 2011b; Taylor et al. 2014);
- three studies on onset of puberty (Lopez-Espinosa et al. 2011; Christensen et al. 2011; Kristensen et al. 2013);
- two studies on congenital cryptorchidism and hypospadias (Jensen et al. 2014; Toft et al. 2016).

Considerations and conclusions

Kirk et al. did not make any statements specifically about exposure to PFAS and reproductive effects in the 'Executive Summary'.

In the 'Discussion' section of the systematic review, Kirk et al. commented: *"Similarly, we found inadequate evidence for a positive association of PFAS exposure with ... late menarche. Rappazzo et al. (48) reported ...a consistent positive association with asthma but not with age at menarche."*

The reported associations determined by Kirk et al. for each of the reproductive health outcomes are provided below in the 'Associations at a glance' tables. The comments, summaries and conclusions made by Kirk et al. about each reproductive health outcome are provided in the 'Summaries of studies reviewed' below.

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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

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

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

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

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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

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

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

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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

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

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

Congenital cryptorchidism and hypospadias

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence

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

Summaries of studies reviewed

REPRODUCTIVE HORMONE LEVELS

Testosterone (T)

Kirk et al. reviewed 11 studies that investigated the effect of elevated PFAS exposure levels on concentrations of T in males and females (Joensen et al. 2009; Itoh et al. 2016; Lopez-Espinosa et al. 2016; Maisonet et al. 2015b; Olsen et al. 1998b; Raymer et al. 2012; Toft et al. 2016; Tsai et al. 2015; Vested et al. 2013; Zhou et al. 2016; Joensen et al. 2013).

Kirk et al. made two overall comments about the studies they reviewed on testosterone:

- *"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."*
- *"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)

For Oestradiol (e2), Kirk et al. reviewed 10 studies that investigated the effect of PFAS exposure on e2 levels in males and females (Joensen et al. 2009; Itoh et al. 2016; Lopez-Espinosa et al. 2016; Olsen et al. 1998b; Raymer et al. 2012; Vested et al. 2013; Zhou et al. 2016; Joensen et al. 2013; Barrett et al. 2015; Knox et al. 2011b).

Kirk et al. made the following comment on the studies they reviewed: *"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."*

One of the studies Kirk et al. reviewed on child and adolescent exposure to PFAS and oestradiol in children and adolescents was not reviewed by other key international reports or systematic reviews (Itoh et al. 2016). Kirk et al. also reported on the study by Lopez-Espinosa et al. (2016) that Rappazzo et al. also reviewed. Of these two studies, Kirk et al. reported the following summaries and comment: *"Lopez-Espinosa et al. [2016] 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. [2016] 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.*

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[2013] 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)

Kirk et al. reviewed seven studies that investigated the association between increased PFAS exposure levels and LH serum concentrations in men and women (Joensen et al. 2009; Itoh et al. 2016; Olsen et al. 1998b; Raymer et al. 2012; Tsai et al. 2015; Vested et al. 2013; Joensen et al. 2013). Please see 'Associations at a glance -LH' above for reported associations by PFAS.

Of the seven studies, Kirk et al. concluded about the studies in adults: "*In summary, the literature does not suggest a significant association between PFAS exposures and changes in LH in adults, although Raymer et al. [2012] did report positive associations with PFOA and similar results for PFOS of borderline statistical significance.*", and about the studies in adolescents: "*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)

Kirk et al. reviewed seven studies that investigated the effect of PFAS exposure on serum FSH concentrations (Joensen et al. 2009; Itoh et al. 2016; Olsen et al. 1998b; Raymer et al. 2012; Tsai et al. 2015; Vested et al. 2013; Joensen et al. 2013).

Kirk et al. made the following comments:

- "*The literature does not support an association between PFAS and FSH levels in adults.*"
- "*In contrast, results are conflicting for the effect of PFAS exposure on FSH in adolescents.... In summary, Tsai et al. [2015] and Vested et al. [2013] 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)

Kirk et al. evaluated seven studies that investigated the association between PFAS exposure levels and SHBG in males and females (Joensen et al. 2009; Itoh et al. 2016; Maisonet et al. 2015b; Olsen et al. 1998b; Tsai et al. 2015; Vested et al. 2013; Joensen et al. 2013).

Kirk et al. made the following conclusions and comments about the studies they reviewed:

- "*Conclusively, the results did not support a significant effect related to increased PFAS exposure and SHBG levels in males of all ages.*"
- "*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. [2015].*"

Other reproductive hormones

Kirk et al. evaluated nine studies that investigated the effect of PFAS on additional reproductive hormones in men and women (Joensen et al. 2009; Itoh et al. 2016; Lopez-Espinosa et al. 2016; Olsen et al. 1998b; Raymer et al. 2012; Toft et al. 2016; Vested et al. 2013; Joensen et al. 2013; Barrett et al. 2015).

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Of these nine studies, Kirk et al. provided the following comments: "*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, FECUNDITY AND INFERTILITY

Time to pregnancy and fecundity

Kirk et al. reviewed nine studies on elevated levels of PFAS and time to pregnancy (TTP) in women (Fei et al. 2009; Valez et al. 2015; Bach et al. 2015b; Buck Louis et al. 2013; Jorgensen et al. 2014; Lum et al. 2017; Vetergaard et al. 2012; Whitworth et al. 2012b; Whitworth et al. 2016).

All of the studies except Jorgensen et al. (2014) and Whitworth et al. (2016) were reviewed previously in this section.

Of the study by Jorgensen et al. (2014), Kirk et al. reported that: "*Jørgensen et al. [2014] stated no association between PFOA, PFOS, PFHxS and PFNA and TTP.*" For Whitworth et al. (2012b and 2016), the study was reported as: "*Two studies by Whitworth et al. [2012b, 2016] observed no associations between PFAS and TTP among women.*"

Kirk et al. made two comments about the studies they reviewed for TTP:

- "*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.*"
- "*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. [2009] and Velez et al. [2015].*"

Infertility

For infertility, Kirk et al. reviewed four studies (Fei et al.2009; Valez et al. 2015; Jorgensen et al. 2014; Bach et al. 2015b). Kirk et al. made two comments: "*In contrast to the findings for TTP, 3 of the 4 evaluated studies found elevated PFAS exposure levels are associated with infertility in women*", and "*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.*"

Kirk et al. also undertook meta-analyses on TTP, fecundity and infertility.

For the meta-analyses on fecundity, Kirk et al. reported the results as: "*There was substantial heterogeneity in study effects regarding fecundity for both PFOA (I^2=77.30%; Q=17.65; df=4; p=0.001) and PFOS (I^2=50.6%; Q=8.10; df=4; p=0.088) 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).*"

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Kirk et al. cautioned about the results: "*Due to the substantial heterogeneity associated with this outcome, these results should be interpreted with caution.*"

For the meta-analyses on infertility, Kirk et al. reported the following results and caution: "*There was substantial heterogeneity in study effects for infertility for PFOA (I^2=71.10%; Q=13.86; df=4; p=0.008) and PFOS (I^2 = 62.50%; Q=10.68; df=4; p=0.030)). 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.*"

Kirk et al. provided the following 'Evaluation' of the nine studies they reviewed for TTP, fecundity and infertility: "*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. [2015] 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

Kirk et al. reviewed six studies on sperm quality in males of reproductive age (Buck Louis et al. 2015; Joensen et al.2009; Joensen et al. 2013; Raymer et al. 2012; Toft et al. 2012; Vested et al. 2013).

All of these studies have been previously reviewed by ATSDR, US EPA, Saikat et al. or Priestly with summaries provided above.

Kirk et al. made two overall comments on these six studies:

- "*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.*"

"*Across the six studies, the association between PFAS exposure levels and adverse sperm characteristics is unclear. Buck Louis et al. [2015] 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. [2015] 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. [2015] 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. [2012] for elevated PFHxS levels and a reduced proportion of normal sperm in a semen sample provides limited evidence. However, the studies*

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conducted by Buck Louis et al. [2015] and Toft et al. [2012] were associated with a low and moderate risk of bias assessment, respectively."

Menstruation, menopause and endometriosis

Kirk et al. reviewed two studies on menstrual cycle length (Lum et al. 2017; Lyngsø et al. 2014). The study by Lyngsø et al. (2014) was also reviewed by Priestly (2016), above. Of the study by Lum et al. (2017), Kirk et al. reported that: "*Lum et al. [2017] 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. [2017] did not find an association between PFOS, PFNA, PFOSA, Me-PFOSA-AcOH and Et-PFOSA-AcOH and changes in menstrual cycle length."*

Kirk et al. made the following comment about the two studies: "*As stated by Lum et al. [2017], Lyngso et al. [2014] 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."*

Kirk et al. reviewed two studies on endometriosis (Buck Louis et al.2012; Campbell et al. 2016). The study by Buck Louis et al. (2012) was reported earlier in this section. Of the study by Campbell et al. (2016), Kirk et al. reported that: "*Campbell et al. [2016] identified a significant positive association between PFOA (adjusted OR Q1 vs Q4 (95% CI); 2.86 (0.63, 12.91)) and PFOS (adjusted OR Q1 vs Q4 (95% CI); 3.48 (1.00, 12.00)) and endometriosis in women aged 20–50-years old enrolled in the NHANES study, along with a positive association for PFNA (adjusted OR Q1 vs Q4 (95% CI); 3.24 (0.81, 12.91)). In agreement with Buck Louis et al. [2012], Campbell et al. [2016] found no association between PFHxS and endometriosis."*

Kirk et al. made the following comment about these two studies: "*These studies suggest an association between elevated PFOA and PFOS exposures and increased rates of endometriosis in women, however, evidence is limited. While the study conducted by Buck Louis et al. [2012] was only associated with a moderate risk of bias, the study by Campbell et al. [2016] was evaluated to have a high risk of bias."*

For onset of menopause, Kirk et al. reviewed three studies (Dhingra et al. 2016; Knox et al. 2011b; Taylor et al. 2014). Priestly reviewed the study by Taylor et al. (2014) with a summary reported above. A summary of the study by Knox et al. (2011b) is available under ATSDR above. Of the study by Dhingra et al. (2016), Kirk et al. reported: "*Conversely, Dhingra et al. [2016] reported that earlier age at menopause was not associated with PFOA exposure."* Kirk et al.'s comments on these three studies were: "*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. [2014] 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."*

Kirk et al. reviewed the study by Taylor et al. (2014) on hysterectomy rates, reporting that the authors: "*investigated the effects of PFAS exposure on rates of hysterectomy in women. The study*

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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."

Kirk et al. made the following comment about this study and the evidence base: "*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

Kirk et al. reviewed three studies on onset of puberty (Lopez-Espinosa et al. 2011; Christensen et al. 2011; Kristensen et al. 2013). These studies were reviewed by the ATSDR, US EPA, Rappazzo et al. and Priestly, with summaries above. Kirk et al. made the following comment about these three studies: "*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.*"

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6.7.4. Summary of considerations and conclusions from key national and international reports and systematic reviews

Recent key national and international reports:

- The ATSDR concluded studies in the general population have not found consistent associations with reproductive hormone levels and results with sperm parameters and fertility are conflicting; the ATSDR noted one study in a highly exposed community found an association with earlier onset of menopause and one occupational study found a positive association between PFOA and oestradiol and testosterone in male workers.
- The US EPA concluded, for PFOA, the reported associations with age at menarche are conflicting; for PFOS, epidemiology studies report associations between higher levels of PFOS and reproductive and developmental parameters and decreases in female fecundity and fertility.

Systematic reviews:

- Saikat made no specific conclusions about the two studies reviewed.
- Priestly commented epidemiological studies are suggesting, but not yet proving, a possible link between PFOS/PFOA and altered sperm function.
- Rappazzo et al. concluded there is evidence of a positive association between PFAS and a later age at menarche, based on a limited number of studies.
- Kirk et al. concluded there was inadequate evidence to permit a conclusion regarding the presence or absence of a causal association between PFAS and all reproductive health outcomes.
- While PFOS serum values are presented in studies, actual estimates of PFOS exposure (doses/duration) are currently not available.

6.7.5. Expert Health Panel synthesis to support advice to the Minister

- There is very little animal evidence referred to by the reviews to support that PFAS may alter endocrine function at concentrations found in humans with environmental and occupational exposures.
- There are many human studies on PFAS and reproductive effects, with most studies examining multiple biomarkers and clinical end points and multiple chemical exposures, often with a post-hoc analysis of observed associations. There is thus a substantial risk that many findings are due to bias or chance. This is reflected in the lack of consistency in the findings of studies. The reviews are not generally in direct conflict, although often highlighting different measures that might be an issue worth pursuing further.
- There is a strong potential for 'reverse causation' in associations with late menarche and early menopause, as menstrual blood loss and female sex hormones might both increase elimination of PFAS (thus the absence of these would be associated with higher levels).
- There is strong potential for confounding by other persistent organic pollutants with endocrine effects in studies in the general population (which is where many of these studies have found associations). There is also potential for confounding by many other factors e.g. BMI and age.
- Overall, the human evidence is weak for a link between PFAS and clinically important reproductive effects; the reviews conclude the strongest evidence of an association is for delayed puberty or poor sperm but these are of unclear significance and quite likely confounded.

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- The human dose-response threshold for these potential effects is very poorly characterised; the majority of studies have been with background population levels rather than highly exposed individuals.

6.7.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and reproductive health outcomes:

- It is feasible that PFAS have effects on human reproduction and reproductive hormones, however, despite several studies and reviews, the rationale and evidence is deficient in most respects. Studies have generally compared average values or out-of-range values in those with higher or lower measured PFAS. While this approach works for some outcomes where it is clear what is 'normal' and desirable, studies of human reproductive function are more difficult to do well. This is an extremely complex and variable area of human biology and people's reproductive capacity is expected to vary greatly over time due to many other factors (e.g. age, diet, alcohol consumption, contraceptive use and obesity). Further, interpretation of laboratory results often requires both knowledge of the reproductive stage of the individual and simultaneous interpretation of several tests, to determine what is abnormal and important and what might be contributing to them. This applies in research as well as for individuals seeking specialist medical treatment.
- Fertility issues were highlighted by a small number of respondents to the public consultation.

To further investigate the association between PFAS exposure and reproductive health outcomes in an Australian setting, the Panel suggests the following research priorities:

- Studies of the effects of PFAS on reproductive health seem likely to provide useful information only if done on existing well-characterised longitudinal cohorts that are examining clinical outcomes (e.g. measuring PFAS in stored samples and whether these affected later fertility). The need for a specific reproductive cohort is that there are many potentially important factors and confounders that are unlikely to be recorded well even in general health records (e.g BMI, smoking, contraceptive use, sexual history, etc) and interpretation of laboratory tests often requires clinical analysis. The best value would come from adding this to an existing cohort, because setting a study up from scratch would take a long time and be very expensive, and the evidence to date implicating PFAS is not compelling.
- Cross-sectional studies of multiple reproductive biomarkers have been done many times and further studies are likely to be largely unhelpful, unless they are combined with a method of rapidly eliminating PFAS so that a before-after design can be used to provide evidence for causal mechanisms.

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6.8. Immunological effects and PFAS exposure

The major function of the immune system is to protect the host from environmental agents such as microbes or chemicals, thereby preserving the integrity of the body. Immunity depends on an intricate homeostatic system aimed at maintaining a delicate balance between health and disease. Its function is maintained by a series of complex, highly regulated, multi-cellular, physiologic mechanisms designed to accomplish a singular goal: to differentiate self from non-self.[50]

All of the key international reports and several of the systematic reviews reviewed the epidemiological evidence on exposure to PFAS and effects on the immune system.

6.8.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of seven published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and four systematic reviews since 2013 that analysed the human epidemiological evidence regarding exposure to PFAS and immune function.

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **National Toxicology Program (NTP 2016).** NTP Monograph on Immunotoxicity Associated with exposure to perflurooctanoic Acid (PFOA) or perfluorooctance Sulfonate (PFOS);
- **United States Environmental Protection Agency (US EPA 2016a).** Health effects support document for Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA 2016b).** Health effects support document for Perfluorooctane Sulphonate (PFOS);
- **New Jersey Drinking Water Quality Institute (DWQI Public Review draft 2016).** Health-based maximum contaminant level support document: Perfluorooctanoic Acid (PFOA);
- **Dutch National Institute for Public Health and the Environment (RIVM 2017).** PFOA exposure and health: A review of scientific literature;
- **Food Standards Australia New Zealand (FSANZ 2017)**. Hazard Assessment report (PFOS, PFOA, PFHxS).

Systematic reviews and reviews

- **Chang et al. (2016).** A critical review of perfluorooctanoate and perfluorooctanesulphonate exposure and immunological health conditions in humans;
- **Priestly (2016)**. Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS). Monash University;
- **Rappazzo et al. (2017).** Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature;
- **Kirk et al. (2018).** The PFAS Health Study: systematic literature review. Australian National University.

No other key national or international reports or systematic reviews considered by the Panel reviewed epidemiological studies on immunological effects.

[50] Sources: https://www.ncbi.nlm.nih.gov/pubmed/3685787; http://www.who.int/ceh/capacity/immune_diseases.pdf

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6.8.2. Key national and international reports

Agency for Toxic Substances and Disease Registry (ATSDR)

In 2015, the ATSDR in its draft toxicological profile for perfluoroalkyls considered the human evidence for immunological and lymphoreticular effects and immunotoxicity.

Studies reviewed

The ATSDR reviewed five studies on immunological effects:

- one study on autoimmune diseases (Steenland et al. 2013); and
- four studies on vaccine response (Grandjean et al. 2012; Grandjean and Budtz-Jørgensen 2013; Granum et al. 2013; Looker et al. 2014;). Two of the studies also investigated infectious diseases (Looker et al. 2014; Granum et al. 2013).

Considerations and conclusions

The ATSDR did not make any specific conclusions about immunological effects. In the 'Relevance to public health, summary of health effects' section, the ATSDR indicated that they considered the evidence base for immunological effects to be *'inconsistent'*.

Summaries of studies reviewed

Autoimmune diseases

Of the study on autoimmune diseases (Steenland et al. 2013), the ATSDR reported in the section 'Immunological and lymphoreticular effects – oral exposure': "*The possible association between elevated serum PFOA levels and the occurrence of autoimmune diseases were examined in a cohort of 28,541 adults living or working in a community with elevated PFOA levels in the water (C8 Health Project participants) and 3,713 past and current workers at a nearby DuPont facility (Steenland et al. 2013). A significant association between the likelihood of ulcerative colitis and serum PFOA levels was found; ORs were significantly higher for subjects with estimated annual serum PFOA levels in the three highest quartiles, as compared to the first quartile. No other significant associations between serum PFOA levels and autoimmune disease (e.g., Crohn's disease, rheumatoid arthritis, type-1 diabetes, lupus, and multiple sclerosis) were found.*"

Under the section 'Health effects – immunotoxicity', the ATSDR commented again about Steenland et al. (2013): "*The study did not establish whether the ulcerative colitis was due to immunotoxicity.*"

Vaccine response

The ATSDR reviewed four studies on vaccine response (Looker et al. 2014; Grandjean et al. 2012; Grandjean and Budtz-Jørgensen 2013; Granum et al. 2013).

Of the study by Looker et al. (2014), the ATSDR reported: "*Another study of the C8 Health Project participants examined 411 adults who received an influenza vaccination; the geometric mean serum PFOA and PFOS concentrations were 33.74 and 8.32 ng/mL, respectively (Looker et al. 2014). A reduced antibody response to one of the three flu strain (A/H3N2) influenza vaccinations was found at higher serum PFOA concentrations; the altered response could result in an increased risk of not attaining the antibody threshold considered to offer long-term protection from this virus strain. There were no consistent alterations for the other virus strains (A/H1N1 or flu B). The study also found no associations between serum PFOA or PFOS levels and self-reported frequency of colds or flu.*"

Of the study by Grandjean et al. (2012), the ATSDR reported: "*Grandjean and associates examined the possible association between serum antibody concentrations of tetanus and diphtheria toxoids in*

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children living in the Faroe Islands (measured at 5 and 7 years of age) and serum perfluoroalkyl concentrations (measured at 5 years of age). Negative associations between antibody concentrations and PFOS, PFOA, PFHxS, PFNA, and PFDeA levels were found; the strongest associations were with PFOA and PFOS (Grandjean et al. 2012). Multiple regression analysis predicted that a 2-fold increase in serum PFOA levels could result in 36 and 25% decreases in tetanus and diphtheria antibody levels, respectively, at 7 years of age. Similarly, a 2-fold increase in serum PFOS could result in 24 and 28% decreases in tetanus and diphtheria antibody levels, respectively (Grandjean et al. 2012)."

Of the study by Grandjean and Budtz-Jørgensen (2013), the ATSDR reported that: "*A subsequent paper examined the dose-response relationship using benchmark dose (BMD) analysis (Grandjean and Budtz-Jørgensen 2013). Although the investigators reported BMDL values of 1.3 ng/mL for serum PFOS and 0.3 ng/mL for serum PFOA, they did not provide sufficient information regarding model fit, and although they noted that a benchmark response of 5% was used, they did not indicate that a control group was used.*"

The study by Granum et al. (2013) was reported by the ATSDR as: "*Similarly, Granum et al. (2013) examined possible associations between pediatric vaccine antibody levels in children 3 years of age and maternal serum PFOA, PFOS, PFNA, and PFHxS levels. Of the four vaccine antibody levels examined (rubella, measles, haemophilus influenza type b, and tetanus), the only statistically significant associations were with rubella. Negative associations were found for rubella vaccine antibody levels and maternal serum PFOA, PFOS, PFNA, and PFHxS levels.*"

Infectious diseases – colds and influenza

The ATSDR reported that Looker et al. (2014) in their study of C8 Health Project participants: "*also found no associations between serum PFOA or PFOS levels and self-reported frequency of colds or flu.*"

Of the study by Granum et al. (2013), the ATSDR noted that: "*The study also examined whether there was an association between maternal perfluoroalkyl levels and the incidence of infectious disease in the children (Granum et al. 2013). Positive associations were found between serum PFOA and PFNA levels and the number of episodes of common colds in children aged 1–3 years, but when the data over the 3 years were dichotomized, there were no significant associations.*"

National Toxicology Program (NTP)

In 2016, the NTP published its Monograph on immunotoxicity associated with exposure to PFOA and PFOS. The NTP reported it conducted a systematic review to evaluate the evidence on exposure to PFOS or PFOA and immune-related health effects to determine whether exposure to either chemical is associated with immunotoxicity.

Studies reviewed by NTP

The NTP reviewed 20 human studies[51] and sorted those for PFOA and PFOS into three main categories of immune response: immunosuppression, hypersensitivity-related outcomes, and autoimmunity. These studies included:

- six studies on vaccine (antibody) response for both PFOA and PFOS[52] (Granum et al. 2013; Grandjean et al. 2012; Mogensen et al. 2015; Kielsen et al. 2016; Looker et al. 2014; Stein et al. 2016);

[51] Note that some studies are included in several categories.
[52] Taken from Table 10, page 23 of the NTP report.

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- four studies on infectious diseases[53] (Fei et al. 2010; Granum et al. 2013; Okada et al. 2012; Looker et al. 2014);
- thirteen studies on hypersensitivity, asthma, and allergic diseases[54] (Ashley-Martin 2015; Granum et al. 2013; Okada et al. 2012; Okada et al. 2014; Smit et al. 2015; Wang et al. 2011; Buser and Scinicariello 2016; Dong et al. 2013; Zhu et al. 2016; Humblet et al. 2014; Stein et al. 2016; Anderson-Mahoney et al. 2008; Steenland et al. 2015); and
- three studies on autoimmune diseases[55] (Steenland et al. 2013; Steenland et al. 2015; Osuna et al. 2014).

Considerations and conclusions for PFOA

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

Under the 'Main findings' section for PFOA, the NTP gave a brief description of the basis for the confidence ratings for the human studies[56]: "*<u>There is moderate confidence</u> that exposure to PFOA is associated with suppression of the antibody response in humans based on the available studies. The results present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFOA were associated with suppression in at least one measure of the anti-vaccine antibody response to common vaccines across multiple studies. There were no changes in the confidence rating for the human body of evidence after considering factors that may increase or decrease confidence. Heterogeneity in the findings may be explained by variation between studies in the different vaccinations tested, time between vaccination and measurement of the antibody response, and analyses or ways to measure the antibody response.*"

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

- *Human body of evidence: Moderate Confidence = Moderate Level of Evidence*
- *Animal body of evidence: High Confidence = High Level of Evidence*

[53] Taken from Table 13, page 38 of the NTP report.
[54] Taken from Table 15, page 45 of the NTP report.
[55] Taken from Table 18, page 57 of the NTP report.
[56] The text on animal studies has been excluded.

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- *Initial hazard conclusion (Moderate x High) = Presumed to be an Immune Hazard to Humans*
- *Final hazard conclusion (after consideration of biological plausibility) = Presumed to be an Immune Hazard to Humans."*

Considerations and conclusions for PFOS

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

Under the 'Main findings' PFOS section, the NTP gave a brief description of the basis for the confidence ratings for the human studies[57]: "*There is moderate confidence that exposure to PFOS is associated with suppression of the antibody response in humans based on the available studies. The results present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFOS were associated with suppression in at least one measure of the anti-vaccine antibody response to common vaccines across multiple studies. There were no changes in the confidence rating for the human body of evidence after considering factors that may increase or decrease confidence. Heterogeneity in the findings may be explained by variation between studies in the different vaccinations tested, time between vaccination and measurement of the antibody response, and analyses or ways to measure the antibody response."*

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

- *Human body of evidence: Moderate Confidence = Moderate Level of Evidence*
- *Animal body of evidence: High Confidence = High Level of Evidence*
- *Initial hazard conclusion (Moderate x High) = Presumed to be an Immune Hazard to Humans Systematic Review of Immunotoxicity Associated with Exposure to PFOA or PFOS 20*
- *Final hazard conclusion (after consideration of biological plausibility) = Presumed to be an Immune Hazard to Humans."*

[57] The text on animal studies has been excluded.

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Summaries of studies reviewed

Vaccine response – PFOA and PFOS

The NTP reviewed six studies (Granum et al. 2013; Grandjean et al. 2012; Mogensen et al. 2015; Kielsen et al. 2016; Looker et al. 2014; Stein et al. 2016) on antibody response in humans. In their 'Summary' of the human antibody response data for PFOA, they reported: "*There is moderate confidence that exposure to PFOA is associated with suppression of the antibody response based on the available human studies. The results show consistent PFOA- associated suppression in at least one measure of the anti-vaccine antibody response across multiple studies with evidence from developmental, childhood, and adult exposures (see Table 10 for list of studies). There were no changes in confidence rating for the body of evidence after considering factors that may increase or decrease confidence (see Table 12 for confidence ratings summaries for the body of Systematic Review of Immunotoxicity Associated with Exposure to PFOA or PFOS evidence). Heterogeneity in the findings may be explained by variation between studies in the different vaccinations tested, time between vaccination and measurement of the antibody response, and analyses or ways to measure the antibody response. The confidence rating for the human antibody data is the same for PFOA and PFOS.*"

The NTP's 'Summary' of the human antibody response data for PFOS was the same as for PFOA (as above). In addition, the NTP noted: "*The human body of evidence for PFOA and PFOS on the antibody response is based on the same six epidemiological studies with very similar results and findings for both chemicals. The confidence ratings for the human data are the same for PFOA and PFOS.*"

Infectious diseases – PFOA and PFOS

The NTP reviewed four studies (Fei et al. 2010; Granum et al. 2013; Okada et al. 2012; Looker et al. 2014) on disease resistance and infectious disease outcomes.

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

The NTP's 'Summary' of human infectious disease data for PFOS noted: "*There is low confidence that exposure to PFOS is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease). One of three prospective studies (Fei et al. 2010) that examined the relationship between maternal PFOS exposure and disease outcomes in offspring reported some evidence of PFOS-associated increase in infectious disease and no association was found in the single adult cross-sectional study (Looker et al. 2014). Confidence in the body of evidence for the three*

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prospective studies (Fei et al. 2010, Okada et al. 2012, Granum et al. 2013) was decreased for a lack of consistency across studies and within the Fei et al. (2010) study by sex (PFOS was associated with increased hospitalization for infectious disease in girls, but not in boys) and age group analyzed (PFOS was associated with increased hospitalization in analyses combining ages 0- 10, but not for individual age groups), to support a final rating of low confidence...As discussed below, the fact that few specific infectious disease end points have been examined (e.g., data are restricted to colds, influenza, gastroenteritis and otitis media) contributes to the low confidence for drawing a conclusion on infectious disease in general. In contrast, the findings by Fei et al. (2010) of an association between maternal PFOS and what is likely to be a less sensitive measure of disease (i.e., hospitalization for any infectious disease, which would only capture the most severe outcomes and could miss potential associations with individual diseases) contributes to the confidence in the association."

Hypersensitivity, asthma and allergic diseases – PFOA and PFOS

In the 'Hypersensitivity-related effects and outcomes' section, the NTP reviewed six prospective maternal exposure studies (Ashley-Martin 2015; Granum et al. 2013; Okada et al. 2012; Okada et al. 2014; Smit et al. 2015; Wang et al. 2011), five cross-sectional child exposure studies (Buser and Scinicariello, 2016; Dong et al. 2013; Zhu et al. 2016; Humblet et al. 2014; Stein et al. 2016), and two adult exposure studies: one ecological (Anderson-Mahoney et al. 2008) and one retrospective cohort study (Steenland et al. 2015).

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

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

Autoimmune diseases – PFOA and PFOS

The NTP reviewed three studies on autoimmunity in humans: two retrospective cohort studies (Steenland et al. 2013; Steenland et al. 2015) and one prospective pilot study (Osuna et al. 2014). The ATSDR reviewed the study by Steenland et al. (2013) with a summary of that study provided above.

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Of the study by Steenland et al. (2015), the NTP reported: "*In a follow-up study of workers (n = 3713) exposed to PFOA that were a subset of the original analysis, there was a significant trend (p ≤ 0.05) for ulcerative colitis (28 validated cases) with increasing PFOA exposure level based on unlagged or 10-year lagged exposure at much higher exposure levels (mean serum PFOA in 2005-2006 was 325 ng/ml for workers versus 87 ng/ml in the combined cohort) (Steenland et al. 2015).*"

Steenland et al. (2015) also investigated rheumatoid arthritis. The NTP reported the following summary of the study: "*In contrast to the community study, there is some evidence that rheumatoid arthritis (28 cases) is associated with PFOA exposure in the workers. There was a positive trend for rheumatoid arthritis by quartiles of PFOA exposure; however, only the trend test using midpoint of the quartiles was statistically significant (p ≤0.05), whereas analyses using continuous log transformed cumulative exposure were not significant (p = 0.54 and p = 0.75 for 10-year lag or no lag exposure) (Steenland et al. 2015).*"

Of the study by Osuna et al. (2014), the NTP reported that: "*The only other autoimmune study located is a pilot study that reported prenatal concentrations of PFOA were not associated with autoantibodies to several neural or non-neural antigens in 7 year old children (n = 38) from the Faroe Island birth cohort (Osuna et al. 2014). Although the study did not find an association with PFOA exposure, autoantibody data without support from other related end points (e.g., for the neural antigens studied) is not considered to provide clear evidence for or against an effect on autoimmunity (WHO 2012).*"

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

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

United States Environmental Protection Agency (US EPA)

Also in 2016, the US EPA published 'Health effects support documents for PFOA and PFOS'. These documents were used by the US EPA to establish health advisories on PFOA and PFOS for drinking water officials.

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Studies reviewed

Under 'Hazard identification – immunotoxicity' (PFOA), the US EPA reviewed eight studies, including:

- four studies on infectious disease (Fei et al. 2010; Okada et al. 2012; Granum et al. 2013; Looker et al. 2014);
- three studies on vaccine response (Looker et al. 2014; Grandjean et al. 2012; Granum et al. 2013);
- two studies on asthma (Dong et al. 2013; Humblet et al. 2014); and
- one study on autoimmune diseases (Steenland et al. 2015).

Under 'Hazard identification – immunotoxicity' (PFOS), the US EPA reviewed eight studies, including:

- four studies on infectious diseases (Fei et al. 2010; Okada et al. 2012; Granum et al. 2013; Looker et al. 2014);
- five studies on vaccine response (Okada et al. 2012; Granum et al. 2013; Looker et al. 2014; Grandjean et al. 2012; Stein et al. 2015[58]);
- two studies on asthma (Dong et al. 2013; Humblet et al. 2014).

Considerations and conclusions: PFOA

For PFOA, the US EPA stated in the 'Executive Summary', firstly: "*Human epidemiology data report associations between PFOA exposure and decreased vaccination response [and several other health effects]*", then secondly: "*Associations between PFOA exposure and risk of infectious diseases (as a marker of immune suppression) were not identified, but a decreased response to vaccines in relation to PFOA exposure was reported in studies in adults in the high-exposure community population and in studies of children in the general population.*"

Under 'Hazard identification – summary and conclusions from the human epidemiology studies', the US EPA reported for 'Immune function': "*Associations between prenatal, childhood, or adult PFOA exposure and risk of infectious diseases (as a marker of immune suppression) have not been consistently seen, although there was some indication of effect modification by gender (i.e., associations seen in female children but not in male children) (Fei et al. 2010a; Granum et al. 2013; Looker et al. 2014; Okada et al. 2012). Three studies have examined associations between maternal and/or child serum PFOA levels and vaccine response (measured by antibody levels) in children (Grandjean et al. 2012; Granum et al. 2013) and in adults (Looker et al. 2014). The study in adults was part of the high-exposure community C8 Health Project. A reduced antibody response to one of the three influenza strains tested after subjects received the flu vaccine was seen with increasing levels of serum PFOA; these results were not seen with PFOS. The studies in children were conducted in general populations in Norway and in the Faroe Islands. Decreased vaccine response in relation to PFOA levels was seen in these studies, but similar results also were seen with correlated PFASs (e.g., PFOS).*"

Considerations and conclusions: PFOS

The US EPA did not mention immunological effects in the 'Executive Summary' of the 'Health effects support document for PFOS'. Under 'Hazard identification – summary and conclusions from the human epidemiology studies', the US EPA reported the following: "*A few studies have evaluated associations with measures indicating immunosuppression. Two studies reported decreases in response*

[58] Reported as Stein et al. (2015) in the US EPA Bibliography. Please refer to Stein et al. (2016) in this Bibliography, as it is the same study.

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to one or more vaccines in children aged 3, 5, and 7 years (e.g., measured by antibody titer) in relation to increasing prenatal serum PFOS levels or at 5 years of age (Grandjean et al. 2012; Granum et al. 2013). Decreased rubella and mumps antibody concentrations in relation to serum PFOS concentration were found among 12–19 year old children in the NHANES, particularly among seropositive children (Stein et al. 2015[59]). A third study of adults found no associations with antibody response to influenza vaccine (Looker et al. 2014). In the three studies examining exposures in the background range among children (i.e., general population exposures, geometric means < 20 ng/ml), the associations with PFOS were also seen with other correlated PFASs, complicating conclusions specifically for PFOS.

No clear associations were reported between prenatal PFOS exposure and incidence of infectious disease among children (Fei et al. 2010b; Okada et al. 2012), although an elevation in risk of hospitalizations for an infectious disease was found among girls suggesting an effect at the higher maternal serum levels measured in the Danish population (mean maternal plasma levels were 35.3 ng/mL). With regard to other immune dysfunction, serum PFOS levels were not associated with risk of ever having had asthma among children in the NHANES with median levels of 17 ng/mL (Humblet et al. 2014). A study among children in Taiwan with higher serum PFOS concentrations (median with and without asthma 33.9 ng/mL and 28.9 ng/mL, respectively) found higher odds ratios for physician-diagnosed asthma with increasing serum PFOS quartile (Dong et al. 2013). Associations also were found for other PFASs. Among asthmatics, serum PFOS was also associated with higher severity scores, serum total IgE, absolute eosinophil counts and eosinophilic cationic protein levels."

Vaccine response

The US EPA reviewed two studies on vaccine response in relation to higher exposure to PFOA in children (Grandjean et al. 2012; Granum et al. 2013) and one study on adults (Looker et al. (2014). These studies were reviewed by the ATSDR (2015) and NTP (2016), with information on these studies in those sections above.

The US EPA made the following summary statement about these three studies: "*In summary, three studies have reported decreases in response to one or more vaccines (e.g., measured by antibody titer) in relation to higher exposure to PFOA in children (Grandjean et al. 2012; Granum et al. 2013) and adults (Looker et al. 2014). In the two studies examining exposures in the background range (i.e., general population exposures, < 10 ng/ml), the associations with PFOA also were seen with other correlated PFASs. This limitation was not present in the study in adults in the high-exposure C8 community population. Serum PFOA levels in this study population were approximately 14–90 ng/mL.*"

For PFOS and vaccine response, the US EPA also reviewed and provided summaries of the findings for Grandjean et al. (2012), Granum et al. (2013 and Looker et al. (2014). The US EPA did not provide any conclusive statement in this section.

Infectious diseases

The US EPA reviewed the studies by Fei et al. (2010), Okada et al. (2012), Granum et al. (2013) and Looker et al. (2014). These studies were reviewed by the ATSDR (2015) and NTP (2016) with details of the studies in the sections above.

For PFOS, the US EPA reviewed the same four studies they reviewed for PFOA (Okada et al. 2012; Fei et al. 2010; Granum et al. 2013; Looker et al. 2014). In addition, they reviewed the study by Grandjean et al. (2012). The findings of this study are mentioned in the US EPA's 'Hazard identification – summary and conclusions from the human epidemiology studies' for PFOS, above.

[59] All other sources (except Priestly) reference this as Stein et al. 2016. The Panel presumes that the US-EPA and Priestly used the e-pub version.

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Asthma

The US EPA reviewed two studies on asthma (Dong et al. 2013; Humblet et al. 2014) and provided summaries of the studies. These studies were also reviewed by the NTP (2016) with details provided above.

For PFOS and asthma, the US EPA's comment about these studies is mentioned above in the 'Hazard identification – summary and conclusions from the human epidemiology studies'.

Autoimmune diseases

The US EPA reviewed the study by Steenland et al. (2015) and provided a summary of the study and findings. This study was also reviewed by the NTP and ATSDR.

New Jersey Drinking Water Quality Institute (DWQI)

In 2016, the DWQI published its 'Health-based maximum contaminant level support document for PFOA'.

Studies reviewed

The DWQI only evaluated studies on antibody concentrations following vaccination for their report, and reviewed five studies (Grandjean et al. 2012, Granum et al. 2013, Kielsen et al. 2015, Looker et al. 2014 and Stein et al. 2016).

In the report the DWQI stated that they did not review studies on: "*immune function (with the exception of immune response following vaccination which was reviewed above) including asthma and other allergies, autoimmune disease including osteoarthritis, lupus, juvenile diabetes, rheumatoid arthritis, multiple sclerosis, and Crohn's disease*."

Considerations and conclusions

In the 'Executive Summary', the DWQI stated: "*For some other end points that were comprehensively reviewed, limited evidence of an association with PFOA was found. Although there is consistent evidence of decreased antibody concentrations following vaccination, most of the vaccine types were evaluated in only one or two studies and there is limited evidence of exposure-response*."

Also, in the 'Executive Summary', under 'Mode of action – immune system effects', the DWQI reported that: "*PFOA suppresses the immune system in both non-human primates and mice. As noted above, decreased response to vaccinations has been associated with PFOA in human epidemiological studies. Data from mouse studies indicate that these effects on the immune system occur through both PPAR-alpha dependent and independent modes of action. Both PPAR-alpha dependent and independent effects on the immune system are considered relevant to humans for the purposes of risk assessment*."

Summaries of studies reviewed

Vaccination response

The studies by Grandjean et al. (2012), Granum et al. (2013), Looker et al. (2014), and Stein et al. (2016) were also reviewed by the ATSDR, NTP and/or US EPA. In addition to these four studies, the DWQI reviewed one other study (Kielsen et al. 2015).

The Panel notes that the study by Kielsen et al. (2015) was very small (n=12), and considers the study too small to be meaningful.

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The DWQI also made the following comments and observations about the studies they reviewed: "*Associations between decreased antibody concentration and increasing PFOA concentration may be related to a threshold such that limited evidence of associations was found among the two studies with median serum PFOA concentrations below 2 ng/ml (Granum et al, 2013 and Kielsen et al. 2015). Both of these studies also had small sample sizes which may have restricted the power of the study to detect a statistically significant decrease.*

Specificity of the observed association may also be difficult to interpret since responses to many different vaccines were evaluated, with each type of vaccine included only in a few (and often in only one or two) studies. Unlike many of the other outcomes evaluated in studies of the human health effects of PFOA, four of the studies that assessed associations with antibody concentrations following vaccination had a prospective study design, allowing temporality assessment. Since the exposures and outcomes were followed over time, it can be concluded that exposures preceded the outcome. There was limited evidence or exploration of exposure-response relationships.

Data from other human studies and toxicology studies provide support for biological plausibility of decreased immune system response to vaccines in humans. As discussed in the Toxicology and Mode of Action sections, PFOA suppressed the immune system in studies of both nonhuman primates and rodents. Fletcher et al. (2009) reported several statistically significant associations between several markers of immune function (decreased IgA; decreased IgE in females only; increased anti-nuclear antibody; decreased C-reactive protein) and serum PFOA levels in communities with drinking water exposure to PFOA in a C8 Science Panel status report (Fletcher et al. 2009). As yet, only the information on C-reactive protein has been published (Genser et al. 2015). Genser et al. (2015) found consistent and significant associations of serum PFOA with this effect, both within each of the six water districts included in the study and on an aggregated basis. They concluded that these within- and between-district associations strengthen the evidence of causality for this effect.

Review of epidemiologic studies provides evidence of consistent findings among studies of decreased antibody concentrations following vaccination and PFOA. However, there are a limited number of comparisons across the same vaccination types, making consistency/specificity difficult to evaluate. While there is epidemiologic evidence of temporality, evidence of an exposure-response is limited."

The DWQI also noted the findings of the NTP Monograph on PFOA and PFOS, published in 2016: "*Additionally, the National Toxicology Program (NTP) recently completed a draft systematic review of immunotoxicity of PFOA, based on consideration of human and animal studies, along with mechanistic data (NTP, 2016). The draft NTP assessment concluded that PFOA is presumed to be an immune hazard to humans based on (1) a high level of evidence from animal studies and a moderate level of evidence from human studies that PFOA suppresses antibody response, and (2) a high level of evidence from animal studies and a low level of evidence from human studies that PFOA increases hypersensitivity-related outcomes. NTP also considered additional, although weaker, evidence primarily from epidemiological studies that PFOA reduced infectious disease resistance and increased autoimmune disease. NTP states that the evidence for effects on multiple aspects of the immune system supports the overall conclusion that PFOA alters immune function in humans.*"

Dutch National Institute for Public Health and the Environment (RIVM, 2017)

In 2017 the RIVM published its review of the scientific literature in the report 'PFOA exposure and health'.

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Studies reviewed

The RIVM only reviewed studies on vaccination response and ulcerative colitis. They reviewed six epidemiological studies on vaccination response (Looker et al. 2014; Grandjean et al. 2012; Granum et al. 2013; Kielsen et al. 2016; Mogensen et al. 2015; Stein et al. 2016) and two studies on ulcerative colitis (Steenland et al. 2013; Steenland et al. 2015).

In addition to reviewing the epidemiological studies above, the RIVM also considered the findings of reports by international authorities in coming to its conclusions. RIVM considered the reports of the C8 Science Panel, ATSDR (2015), DWQI (2016), NTP (2016) and US EPA (2016a) for vaccination response and ulcerative colitis. Additionally, for ulcerative colitis, RIVM considered the reports by ECHA-RAC (2015)[60].

Considerations and conclusions

In the 'Synopsis' , the RIVM reported there was the clearest evidence for a number of health effects – immunological effects were not included in this list. The RIVM then went on to state that: "*For all other examined associations, the evidence is less clear...Indications have also been found for a higher risk of chronic inflammation of the bowel (ulcerative colitis)... Furthermore, associations have been found between exposure to PFOA and decreased vaccination response....*"

Autoimmune diseases

In the 'Discussion and conclusions' section, the RIVM provided some detail about exposure to PFOA in the two studies (Steenland et al. 2013; Steenland et al. 2015) they reviewed on ulcerative colitis, stating: "*Both published studies found an association and a dose-response relationship. One was performed in the combined high-exposed community and occupational study population, and one in the occupational population of the C8 Health Project. Although no studies were conducted in the general population, individuals with low serum PFOA concentrations were also examined in those studies. In the combined study population, in the 2nd – 4th quartiles of PFOA concentrations (>158 ng/mL), an increased risk of ulcerative colitis (RR were 1.76-2.86) was observed (Steenland et al. 2013). In workers, the RR ranged from 3.0 to 6.57 in the 2nd to 4th quartiles (covering PFOA concentrations of 800 to over 7,000 ng/mL) (Steenland et al. 2015). The evidence base is limited and should be extended to other study populations.*"

Vaccine response

The RIVM reviewed and provided summaries of six studies on vaccination response (Looker et al. 2014; Grandjean et al. 2012; Granum et al. 2013; Kielsen et al. 2016; Mogensen et al. 2015; Stein et al. 2016). Summaries of these studies are provided under the ATSDR, NTP and DWQI sections above. The RIVM did make the observation about the findings of the study by Kielsen et al. (2016) which showed: "*no significant association was observed in one study that examined adults in the general population in Denmark (Kielsen et al. 2016) ...Only 12 individuals were examined and the analyses had a low statistical power, which may explain why no association was observed.*"

In the 'Discussion and conclusions' section, the RIVM commented on the international organisation's conclusions including: "*However, as discussed by the DWQI (2016), most of the specific vaccine types were evaluated in only one or two studies. More research is therefore needed to confirm these findings. For this reason, most organizations have concluded that there is not enough evidence available yet to*

[60] ECHA-RAC (2015) Committee for Risk Assessment (RAC), Committee for Socio-economic analysis (SEAC). The Expert Health Panel did not review the reports by ECHA-RAC.

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determine whether an association between blood levels of PFOA and decreased vaccination response exists."

The RIVM then commented about the studies they reviewed, noting that: *"Associations with inadequate seroprotection have also been reported. For example, in a study conducted by Grandjean et al. (2012), the odds ratios of antibody concentrations falling below the protective level for diphtheria and tetanus in children at age seven, associated with a doubling of PFOA concentrations at age five, were 3.27 (95% CI, 1.43 to 7.51) and 4.20 (95% CI, 1.54 to 11.44), respectively."*

Food Standards Australia New Zealand (FSANZ, 2017)

In 2017, Food Standards Australia New Zealand published its 'Hazard assessment report on PFOA, PFOS and PFHxS' to provide advice on appropriate health-based guidance values (HBGV) for these three PFAS.

Studies reviewed

FSANZ commissioned a review of PFAS and immunomodulation: (Drew and Hagen, 2016).

Considerations and conclusions of FSANZ

In the 'Executive Summary', FSANZ noted that the US NTP concluded that both PFOS and PFOA are presumed to be an immune hazard to humans. FSANZ stated: *"A literature review commissioned by FSANZ concluded that there are both positive and negative studies showing associations for increasing PFOS and PFOA concentrations to compromise antibody production in humans. However, to date there is no convincing evidence for increased incidence of infective disease associated with PFOS or PFOA effects on human immune function."*

Under the section 'Human data – immune function (PFOA)', FSANZ reported that: *"There is some evidence of an association between serum PFOA levels and failure of adequate antibody response in children to vaccinations against diphtheria and against tetanus (Grandjean et al. 2012 as reported in Bull et al. 2014, reviewed by US EPA 2016). However, the data are not sufficient to establish a causal relationship between PFOA exposure and clinical relevant impairment of vaccine response (Drew and Hagen 2016)."*

Under 'Human data – immune function (PFOS)', FSANZ noted three studies on vaccination and one study on asthma in children (authors names not provided) that had been reviewed by the US EPA (2016b) and then reported the following detail on the literature review they commissioned: *"FSANZ commissioned a review of the potential of PFASs to modulate the immune system (Drew and Hagen 2016). The review noted that that there are both positive and negative epidemiology studies on associations between serum PFOS concentrations and compromised antibody production. The report concluded that while PFOS may present an immune hazard to humans, the epidemiology data available do not provide compelling evidence for increased incidence of disease associated with PFOS effects on immune function. A number of limitations with the available data were noted. These included comparisons of 'low' and 'high' exposure groups where the differences are over a very low and narrow serum concentration range (0.002 – 0.05 mg/L), and potential co-exposures to other environmental chemicals that are known to have immunomodulating effects. It was noted that many of the associations are weak and the effects are small and of questionable clinical significance."*

In the 'Discussion and conclusions' section, FSANZ also noted the following comments about the NTP (2016) report and the literature review by Drew and Hagen (2016), commissioned by FSANZ:

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Considerations and conclusions: PFOA

"The NTP (2016) has concluded that PFOA and PFOS are presumed to be immune hazards in humans. Mouse studies indicate that PFOA may cause atrophy and changed cellularity in immune system organs of mice, and at lower doses may suppress humoral responses to antigens. Data from animal studies are not sufficiently robust for use in quantitative human risk assessment. Furthermore, currently available epidemiology data are insufficient to establish a cause and effect relationship between PFOA exposure and clinically relevant immunomodulatory effects in humans (Drew and Hagen 2016)."

Considerations and conclusions: PFOS

"The NTP report is focused on hazard identification and does not identify a level of exposure at which immune function in humans is likely to be compromised. A literature review commissioned by FSANZ concluded that the weight of evidence from the available animal studies indicates that PFOS can adversely modulate immune system responsiveness (Drew and Hagan 2016). However, there are significant uncertainties regarding species sensitivity, strain sensitivity and the influence of route of administration on immune system modulation by PFOS that have yet to be resolved. As a result, it is not possible to determine a reliable NOAEL or LOAEL for adverse effects on immune function for use in a quantitative risk assessment of PFOS at this time. Drew and Hagan (2016) concluded that the epidemiology data available do not provide compelling evidence for increased incidence of disease associated with PFOS effects on immune function."

6.8.3. Systematic reviews

Chang et al. (2016)

Chang et al. systematically and critically reviewed the epidemiological evidence on the association between PFOS and PFOA and various immune-related health conditions in humans. The manuscript of this review was supported by the 3M company, a manufacturer of PFAS[61].

Studies reviewed

Chang et al. reviewed the following studies that had reported associations between PFOA and /or PFOS with immune-related health conditions:

- ten studies of immune biomarker levels or gene expression patterns (Olsen et al. 2003; Emmett et al. 2006; Costa et al. 2009; Lin et al. 2011; Wang et al. 2011; Okada et al. 2012; Dong et al. 2013; Garum et al. 2013; Ashley-Martin et al. 2015; Pennings et al. 2015);
- ten studies of atopic or allergic disorders (Anderson-Mahoney et al. 2008; Leonard et al. 2008; Wang et al. 2011; Okada et al. 2012; Dong et al. 2013; Granum et al. 2013; Humblet et al. 2014; Okada et al. 2014; Smit et al. 2015; Steenland et al. 2015);
- five studies of infectious diseases (Leonard et al. 2008; Fei et al. 2010; Okada et al. 2012; Granum et al. 2013; Looker et al. 2014);
- four studies of vaccine responses (Grandjean et al. 2012; Granum et al. 2013; Looker et al. 2014; Kielsen et al. 2015);
- three studies of autoimmune disease (Osuna et al. 2014; Steenland et al. 2013, 2015).

Chang et al. noted that several studies evaluated multiple end points.

[61] The authors stated: *"The authors retained sole control of the manuscript content and the findings, and statemtns in this paper are those of the authors, and not those of the authors' employer or the sponsors."*

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Considerations and conclusions

Chang et al. stated in the 'Abstract': "*With few, often methodologically limited studies of any particular health condition, generally inconsistent results, and an inability to exclude confounding, bias, or chance as an explanation for observed associations, the available epidemiologic evidence is insufficient to reach a conclusion about a causal relationship between exposure to PFOA and PFOS and any immune-related health condition in humans. When interpreting such studies, an immunodeficiency should not be presumed to exist when there is no evidence of a clinical abnormality. Large, prospective studies with repeated exposure assessment in independent populations are needed to confirm some suggestive associations with certain end points.*"

In the 'Conclusions' section, Chang et al. elaborated further on the literature they reviewed: "*Based on a maximum of only seven epidemiologic studies of any particular condition (asthma) and a body of literature with major methodological limitations, an evaluation of the weight of epidemiologic evidence according to the Bradford Hill viewpoints reveals generally weak associations, no specific end points with consistent findings across all relevant studies, uncertainty about any critical duration of exposure and window(s) of susceptibility, mixed exposure-response trends, and a dearth of supportive animal and mechanistic data. Thus, the available evidence is insufficient to conclude that a causal relationship has been established between PFOA or PFOS exposure and any immune condition in humans. Most existing studies were cross-sectional or retrospective in design, evaluated PFOA and/or PFOS exposure at a single point in time, and relied upon self-reported health outcomes.*"

Autoimmune diseases

Chang et al. reviewed three studies on osteoarthritis (Innes et al. 2011; Steenland et al. 2015; Uhl et al. 2013) and commented: "*Taken together, the results of these three studies (...) do not demonstrate a consistent association between serum PFOA or PFOS levels and osteoarthritis.*"

Of the two studies by Steenland et al. (2013; 2015), on ulcerative colitis, Chang et al. commented that: "*Overall, the results from these studies do not establish an association between PFOA exposure and risk of any autoimmune disease, and the results for ulcerative colitis require independent confirmation.*"

Of the study by Osuna et al. (2014), Chang et al. provided the following details: "*A pilot prospective birth cohort study of 38 children in the Faroe Islands evaluated whether PFOA and PFOS levels measured in cord blood at birth and in serum at age 7 years were associated with serum concentrations of IgG and IgM autoantibodies against six neural proteins and three non-neural proteins in children at age 7 (Osuna et al. 2014). This study was based on the premise that increased autoantibodies might indicate tissue damage (and the subsequent release of self-antigens) following chemical exposure. Prenatal and age-7 PFOA levels were not significantly associated with any of the 18 autoantibodies measured (2 isotypes each of 9 autoantibodies). For PFOS most associations were also non-significant, except for a single significant inverse association between prenatal PFOS levels and anti-actin IgG levels at age 7 (-22% change in autoantibody concentration per 2-fold increase in cord blood PFOS, $p \leq 0.05$). Given the numerous associations tested, the lack of adjustment for confounders, and the selection of subjects with available data, this single association – which would suggest a protective effect against tissue damage, but which the authors interpreted as potentially indicating an immunosuppressive effect – could well be a spurious finding, and its clinical relevance is unclear.*"

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Vaccine response

Chang et al. reviewed four studies on vaccine response, including two birth cohort studies (Grandjean et al. 2012; Granum et al. 2013) and two cross-sectional studies of adults (Kielsen et al. 2015; Looker et al. 2014). Chang et al. made the following comments on these studies including:

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

"*None of the studies demonstrated a clinically recognizable increased risk of infectious diseases as a consequence of a diminished vaccine response. Overall, although these results are not sufficient to establish a causal effect of PFOA or PFOS exposure on an impaired serological vaccine response, some of the positive associations are striking in magnitude and require replication in independent studies.*"

Infectious diseases

Of the two studies that investigated the common cold (Granum et al. 2013; Looker et al. 2014), Chang et al. commented: "*Overall, these findings provide inconsistent evidence regarding a potential effect of PFOA exposure on the frequency of common cold episodes, and they suggest no significant association with PFOS exposure.*"

Of the two studies reviewed on otitis media (Okada et al. 2012; Granum et al. 2013), Chang et al. commented that: "*Despite their limitations, discussed earlier, these studies provide no solid evidence of an effect of PFOA or PFOS on otitis media in young children.*"

Chang et al. reviewed three studies (Leonard et al. 2008; Fei et al. 2010; Granum et al. 2013) on 'other infections' and commented: "*Collectively, in light of the equivocal findings and the methodological limitations discussed earlier, these studies do not offer consistent evidence to support any effect of PFOA or PFOS on the occurrence of infectious diseases, and the few significant results (in either direction) could have occurred by chance.*"

Hypersensitivity, asthma and allergic diseases

Chang et al. reviewed seven studies on asthma (Anderson-Mahoney et al. 2008; Dong et al. 2013; Granum et al. 2013; Humblet et al. 2014; Leonard et al. 2008; Smit et al. 2015; Steenland et al. 2015). These studies have been reviewed by other key reports and reviews, with summaries provided previously in this section. Chang et al. made the following overall statement about the studies on asthma they reviewed: "*Overall, given the conflicting findings, the temporal ambiguity of exposure and outcome assessment in most studies, potential misclassification of self-reported asthma in several studies, and the greater weight accorded to the Norway and Greenland/Ukraine studies due to their prospective design and direct measurement of prenatal exposures, these studies collectively do not indicate a causal relationship between PFOA or PFOS exposure and asthma risk.*"

Chang et al. reviewed five studies on eczema and wheezing (Granum et al. 2013; Okada et al. 2012, 2014; Smit et al. 2015; Humblet et al. 2014), and made the following statement about these studies: "*Overall, despite being constrained by the lack of repeated exposure assessment and modest case numbers, the four birth cohort studies suggest no significant adverse impact of prenatal PFOA or PFOS*

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exposure on the onset of eczema or wheezing in early childhood, and the cross-sectional study indicates no apparent association between PFOA or PFOS exposure and the prevalence of wheezing in adolescence."

Chang et al. also reviewed two studies that investigated food allergy (Okada et al. 2012; Granum et al. 2013) and commented that: "*Taken together, these studies provide no evidence for a causal relationship between early-life PFOA or PFOS exposure and the development of food allergy in childhood."*

Of the two studies (Okada et al. 2014; Wang et al. 2011) that investigated atopic health conditions (eczema, wheezing, allergic rhino conjunctivitis, atopic dermatitis) Chang et al. made the following comment: "*Overall, despite their reliance on unvalidated parent-reported outcomes and use of a single exposure measurement per subject, these studies suggest no apparent relationship between pre- or perinatal PFOA or PFOS levels and risk of various atopic disorders in early life."*

Immune biomarkers or gene expression profiles

Chang et al. reviewed nine studies on various circulating immune biomarkers (Olsen et al. 2003; Emmett et al. 2006; Costa et al. 2009; Lin et al. 2011; Wang et al. 2011; Okada et al. 2012; Dong et al. 2013; Garum et al. 2013; Ashley-Martin et al. 2015) and one study on gene expression patterns (Pennings et al. 2015).

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

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

Of the two studies on eosinophil count (Emmett et al. 2006; Dong et al. 2013) reviewed by Chang et al., the following summary comment was made: "*Taken together, these two studies suggest no apparent effect of PFOA or PFOS on eosinophil count in non-asthmatic individuals at a single time point. Given the cross-sectional nature of the Taiwan study, the temporal directionality of the observed associations in children with asthma is unclear."*

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For C-reactive protein, two studies were reviewed, with Chang et al. making the following comments: "*Limitations include the cross-sectional study design, the single exposure and outcome measures for a biomarker that fluctuates within individuals, the lack of quantitative exposure assessment in the former study (Costa et al. 2009), and participation rates of 10% and 49% for normotensive and hypertensive subjects, respectively, in the latter study (Lin et al. 2011). Even so, these statistically null results do not suggest any substantial impact of PFOA or PFOS on C-reactive protein levels.*"

Of the study on gene expression by Pennings et al. (2015), Chang et al. commented: "*The authors interpreted these results as providing a mechanistic link between prenatal PFAS exposure and impaired immune function in early childhood. However, the interpretation is not clear-cut, especially given that expression levels of hundreds of immune-related genes were not correlated with the exposure or outcomes. Moreover, the small number of subjects and the large number of comparisons raise concerns about a large number of false-positive findings; thus, independent confirmation and targeted mechanistic studies are needed to substantiate these results.*"

Chang et al. also reviewed two studies on other immune biomarkers (lymphocytes, basophils, neutrophils, monocytes, OgA, IgG, IgM, α1 globulins, α2 globulins, β globulins and ϒ globulins) and commented: "*Given many of the methodological limitations identified above [e.g. cross-sectional design, probable confounding, and selection bias in Emmett et al. (2006b), lack of quantitative exposure assessment in Costa et al. (2009)], these isolated, as-yet unreplicated results do not establish any association of PFOA or PFOS with biomarkers of adverse immune function.*"

Priestly (2016)

Priestly's 2016 literature review and report on the potential health effects of perfluoroalkyl compounds, mainly PFOS, was an update of his 2015 report and included papers mostly published between August-September 2015 and October 2016. Like Chang et al. (2016) above, Priestly also reviewed studies on gene expression.

Studies reviewed

Priestly reviewed 28 studies in the section 'Altered immune functions': Heilmann et al. 2006; De Witt et al. 2009; Heilmann et al. 2010; Fei et al. 2010; Grandjean et al. 2010; Wang et al. 2011; Corsini et al. 2012; Grandjean et al. 2012; Potera 2012; Grandjean and Budz-Jørgensen 2013; Granum et al. 2013; Okada et al. 2013; Dong et al. 2013; Corsini et al. 2014; Looker et al. 2014; Osuna et al. 2014; Ashley-Martin et al. 2015; Hansmeir et al. 2015; Mogensen et al. 2015; Stein et al. 2015; Grandjean et al. 2016; Kielsen et al. 2016; Stein et al. 2016; Dalsager et al. 2016; Buser and Scinicariello 2016; Oulhote et al. 2016a; Jusko et al. 2016; Pennings et al. 2016[62]. Priestly also reviewed one study on asthma in the section 'Miscellaneous end points' (Humblet et al. 2014). Priestly also reviewed two studies on osteoarthritis (Innes et al. 2011; Uhl et al. 2013) which are discussed in the 'Skeletal Effects' section of this report.

Considerations and conclusions

In the 'Executive Summary', Priestly stated: "*The epidemiological studies are suggesting, but not yet proving, a possible link between PFOS/PFOA and ...disturbances in the immune system.*"

In the section 'Altered immune functions', Priestly made the following 'Comment' about the studies he reviewed: "*The associations with immune dysfunction appear to be stronger than for other end*

[62] Note that Priestly references Stein et al. 2015, whereas all other sources (except the US-EPA) use Stein et al. 2016. The Panel presumes that the US-EPA and Priestly used the e-pub version.

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points. There is consistency with some observations in animal studies and the potential for a plausible mode of action to explain the results. However, there remains some ambiguity in that in some studies, only some antibody responses are affected, while others are unaffected. This apparent selectivity is puzzling. Other factors that need to be considered are that other POPs have been implicated in producing the same effects on antibody responses, and there is inconsistency between studies of which specific PFAS are implicated in the response. The extent to which these confounding effects can be discounted is influenced by the robustness of statistical analytical techniques used to discriminate the contribution of selected PFAS."

Priestly reviewed seven studies that were not reviewed by other key reports or systematic reviews (Heilmann et al. 2006; Heilmann et al. 2010; De Witt et al. 2009; Corsini et al. 2012; Corsini et al. 2014; Grandjean et al. 2016; Dalsager et al. 2016; Jusko et al. 2016; Ouholte et al. 2016). Priestly's review and comments about these studies are included below.

Autoimmune diseases

Priestly did not review any studies on autoimmune diseases in this report.

Vaccine response

Of the paper by Potera (2012), Priestly noted: "*Perhaps the most significant epidemiological studies relating to PFAS have come from the group led by Phillipe Grandjean (Grandjean et al. 2012, 2016) with a commentary from Potera (2012) suggesting that the findings clearly focussed a need to better understand the role of PFAS in modifying immune functions.*"

Of the study by Grandjean et al. (2016), Priestly reported: "*In a follow-up study of this cohort at age 13 y (Grandjean et al. 2016), serum PFAS and serum antibodies had generally declined from age 7, although in some 40% of subjects had a higher titre, with only 13% having received a booster injection during that period. Decreased diphtheria Ab levels were statistically significant with higher levels of PFDA at age 7 and PFOA at age 13 (around 25% decrease for each doubling of PFAS concentration). Doubling in PFAS exposure at age 7 was associated with losses in diphtheria antibody concentrations at age 13 of 10-30% for the five PFASs. Fewer associations with PFAS were found for tetanus Ab concentrations.*"

Priestly reported the following about the two studies by Heilmann et al. (2006, 2010): "*In earlier studies of the Faroe Island cohort, Heilmann et al. (2006, 2010) reported that, associated with a doubling of the cumulative PCB exposure, the antibody response to diphtheria was reduced by 24.4% at age 18 months, but not at age 7 years, while the antibody response to tetanus vaccination was reduced by 16.5% at age 7. At age 5 years, the odds ratio of anti-diphtheria antibodies falling below a clinically protective level were 30% higher for a doubling of PCB in maternal post-partum milk and serum PCB at 18 months of age.*"

Regarding the study by Jusko et al. (2016), Priestly provided the following summary: "*The potential for other POPs to confound the analysis is further illustrated by findings that early-life exposures to PCBs and DDE reduced the antibody response to BCG vaccine at 6-months of age in a birth cohort from Eastern Slovakia (Jusko et al. 2016). The reduction of BCG-specific IgG levels was 37% for infants with PCB-153 concentrations at the 75th percentile, compared with those at the 25th percentile. Both POPs appeared to have additive effects on reducing anti-BCG Ab concentrations.*"

Hypersensitivity, asthma, and allergic diseases

Priestly reviewed and summarised the study by Humblet et al. (2014) under the section 'Miscellaneous end points'. Priestly made no specific comment about this study.

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Immune biomarkers or gene expression profiles

Of the study by Oulhote et al. (2016), Priestly reported: "*In a later study of Faroe island children, prenatal methylmercury and other POPs exposures lowered white blood cell counts at age 18 months (n=42) and 5 years (n=56). The effect was variable, with methylmercury mainly reducing lymphocytes, and organochlorines marginally reducing neutrophils. The effect of PFAS was different, in that basophil counts were higher by around 46% (Oulhote et al. 2016a).*"

Priestly reviewed the studies by Pennings et al. (2016), De Witt et al. (2009) and Corsini et al. (2012, 2014) with regard to possible mode of action for reported immunosuppressive effects of PFAS.

The study by Pennings et al. (2016) was referred to previously under the systematic review by Chang et al. (2016).

Of the study by Hansmeir et al. (2015), Priestly reported: "*Hansmeir et al. (2015) indicate that studies of the interaction between PFAS and genes are only just beginning, and that initial analyses of differentially expressed proteins in cord blood from infants exposed to different levels of PFOA/PFOS may shed some light on possible molecular mechanisms of action of the PFAS.*"

Of the studies by De Witt et al. (2009) and Corsini et al. (2012, 2014), Priestly provided the following summaries: "*In a review of the interactions of PFOS and PFOA with the PPARα receptor, De Witt et al. (2009) pointed out that, while activation of this receptor modulates lipid and glucose homeostasis, cell proliferation/differentiation and inflammation, it also has a role in immune responses. Given that human PPARα receptor expression is much less than that in rodents, the authors note that some biological effects of PFOA/S may be independent of this receptor, and that future research needs to consider other possible modes of action for the PFAS. In a later review (Corsini et al. 2014) expanded on the possible mechanisms by which PFAS-induced immunomodulation could occur. These included both stimulatory and inhibitory effects on PPARα receptors as well as interactions with NF-κB activation, transcription or inactivation. Corsini et al. (2014) also compared the serum levels of PFOS & PFOA in human studies (occupational and general populations) with in vitro concentrations they had found to alter immune cell functions (Corsini et al. 2012). They calculated Margin of Exposure (MoE) estimates of 15000 for PFOA and 0.5-10.8 for PFOS for general population exposures, but only 12 and 0.005 for occupationally-exposed groups. The relative in vitro potencies of the PFAS were: fluorotelomer (heptadecafluorodecanol)>PFOSA>PFOS>PFDA>PFBS, with PFOA the least potent. However, the differences in potency were small and variable across the different cellular mechanisms.*"

Rappazzo et al. (2017)

In 2017, Rappazzo et al. published their systematic review of the epidemiologic literature on exposure to perfluorinated alkyl substances and health outcomes in children. They performed a risk of bias analysis on the studies and "*determined the risk of bias across the studies was low to moderate.*"

Studies reviewed

Rappazzo et al. reviewed:

- thirteen studies that investigated outcomes on asthma, infection and immunity in children (Fei et al. 2010; Wang et al. 2011; Grandjean et al. 2012; Okada et al. 2012; Granum et al. 2013; Dong et al. 2013; Humblet et al. 2014; Okada et al. 2014; Ashley-Martin et al. 2015; Smit et al. 2015; Stein et al. 2016; Zhu et al. 2016; Qin et al. 2017);
- two studies on susceptibility to infections or diseases (Fei et al. 2010; Granum et al. 2013);

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- three studies on vaccination response: (Grandjean et al. 2012; Granum et al. 2013; Stein et al. 2016);
- six studies on asthma (Dong et al. 2013; Qin et al. 2017; Humblet et al. 2014; Stein et al. 2016; Zhu et al. 2016; Smit et al. 2015);
- five studies that investigated allergies or similar outcomes (Okada et al. 2014; Okada et al. 2012; Smit et al. 2015; Wang et al. 2011; Stein et al. 2016);
- five studies on immune biomarkers or gene expression profiles (Granum et al. 2013; Ashley-Martin et al. 2015; Wang et al. 2011; Dong et al. 2013; Zhu et al. 2016).

Considerations and conclusions

In the 'Abstract', Rappazzo et al. stated: "*While there are a limited number of studies on any one particular health outcome, there is evidence for positive associations between PFAS and ... immunity (including vaccine response and asthma).*"

At the end of the section 'Immunity, allergic response, infection, and asthma', the authors noted that: "*Studies of individual health outcomes are limited in number, therefore conclusions should be made with caution; current evidence potentially suggests that antibody response to vaccination and asthma may be influenced by PFAS. The studies of vaccine response were well done cohort study designs and despite the small number offer compelling evidence. The asthma studies are less consistent and include a broader range of study designs and quality. There is no evidence for relationships between PFAS and IgE levels, allergy, and infection. In the one study that looked across these outcomes, several positive associations were observed and these in combination may indicate that prenatal PFAS exposure is linked to childhood humoral immunomodulation [Granum et al. 2013], which is supported by animal studies [De Witt et al. 2012].*"

In the 'Conclusions' section, the authors also stated that: "*Similar to this evaluation, the National Toxicology Program Office of Health Assessment and Translation recently performed a systematic review on the immunotoxicology associated with exposure to PFOA or PFOS and concluded that PFOA or PFOS is "presumed to be an immune hazard to humans" (NTP, 2016).*"

Autoimmune diseases

Rappazzo et al. did not review any studies on autoimmune diseases.

Infectious diseases

Rappazzo et al. reviewed and provided summaries of two studies on susceptibility to infections or diseases (Fei et al. 2010; Granum et al. 2013).

Rappazzo et al. commented about these two studies: "*Two studies examined susceptibility to infections or diseases. In the Danish National Birth Cohort, Fei, et al. [2010] saw no associations between prenatal exposure to PFOA or PFOS (serum) and risk of hospitalizations for infectious disease in the first year of life. Granum, Haug, Namork, Stolevik, Thomsen, Aaberge, van Loveren, Lovik and Nygaard [2013] observed positive associations between maternal plasma PFOA and PFNA and common cold incidence in the first 3 years of life and between PFOA and PFHxS and gastroenteritis. However, these associations were unadjusted for potential confounders [Granum et al. 2013].*"

Vaccination response

The authors reviewed three studies on vaccination response (Grandjean et al. 2012; Granum et al. 2013; Stein et al. 2016) and commented that: "*These studies show some effect of PFAS serum concentration on suppression of antibody response to vaccination.*"

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Of these three studies, Rappazzo et al. provided the following summaries. Of the study by Grandjean et al. (2012), Rappazzo et al. reported: "*In a Faroe Islands birth cohort, Grandjean, et al. [2012] examined serum PFAS concentrations prenatally and at age 5 in association with tetanus and diphtheria serum antibody titers at age 5 (prior to vaccination booster) and at age 7 (after booster). Antibody concentrations at age 5 were generally not associated with combined PFAS concentrations, except diphtheria where a doubling of prenatal PFAS concentration was associated with a substantial decrease in antibody concentrations [Grandjean et al. 2012]. PFAS concentrations at age 5, including when adjusting for prenatal PFAS, have strong negative associations with antibody concentrations for both tetanus and diphtheria at age 7 [Grandjean et al. 2012]. For individual PFAS, associations with antibody concentrations at age 7 are congruent with the results for total PFCs [Grandjean et al. 2012]. However, prenatal PFOS showed a strong negative association with diphtheria antibody concentration at age 5; prenatal PFNA and PFDA also had negative associations with diphtheria antibody concentrations at age 5.*

Grandjean, Andersen, Budtz-Jorgensen, Nielsen, Molbak, Weihe and Heilmann [2012] also examined odds of antibody levels falling below a clinically protective level (0.1 IU/mL), observing positive ORs for diphtheria and tetanus at age 7 with a two-fold increase in PFOS at age 5; results were similar for PFOA.

People from the Faroe Islands have higher persistent organic pollutant (i.e., PCB) and methylmercury serum concentrations than those from the general US population [Fangstrom et al. 2005]; in this study, PFAS and PCB concentrations were not correlated with each other, and adjustment for PCBs in this model did not appreciably change the results [Grandjean et al. 2012]."

Of the study by Granum et al. (2013) Rappazzo et al. reported: "*Granum, et al. [2013] also examined antibody concentrations in a subcohort of the Norwegian Mother and Child Cohort Study (MoBa) study. Increases in maternal plasma PFAS concentrations at delivery (PFOA, PFOS, PFNA, PFHxS) were negatively associated with children's anti-rubella antibody titer at three years of age [Granum et al. 2013]. They also observed potential associations between PFOS and PFOA and measles vaccine antibody concentrations, however these associations were unadjusted for potential confounders [Granum et al. 2013].*"

Of the study by Stein et al. (2016), Rappazzo et al. noted: "*In NHANES (1999–2000 and 2003–2004), Stein et al. (2016) found serum PFOA and PFOS to be associated with decreases in rubella and mumps antibodies in children 12–19 years old.*"

Hypersensitivity, asthma and allergic diseases

Rappazzo et al. reviewed six studies on asthma (Dong et al. 2013; Qin et al. 2017; Humblet et al. 2014; Stein et al. 2016; Zhu et al. 2016; Smit et al. 2015).

Rappazzo et al. reported the following about these six studies: "*Six recent studies have examined asthma in association with PFAS. In the Taiwanese Genetics and Biomarkers study for Childhood Asthma, Dong, et al. [2013] found positive ORs and increasing trends for asthma with serum PFOA, PFOS, PFDA, PFHxS, and PFNA. Perfluorobutanesulfonic acid (PFBS) and perfluorododecanoic acid (PFDoA) had positive ORs for asthma, though without a clear trend or only at the highest exposure levels [Dong et al. 2013]. In this study, asthmatic children were recruited from hospitals, while non-asthmatic children were recruited from schools. If the school population was not similar to the population that gave rise to the asthmatic population bias may have been introduced [Dong et al. 2013]. In a subset of the same population, Qin et al. [2017] observed decrements in metrics of lung function (forced expiratory volume, forced expiratory flow 25–75%, and forced vital capacity) with doubling of several PFAS concentrations (PFOA, PFOS, PFHxS, PFNA) in children with asthma but not*

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in children without asthma. In a cross-sectional study using adolescent NHANES participants, Humblet, et al. [2014] observed a positive OR between self-report of ever having asthma and increasing serum PFOA concentration; PFNA also had a positive OR but a wide CI. Generally null associations were observed for other PFAS (PFOS and PFHxS) and ever asthma, or for any PFAS and current asthma or wheeze [Humblet et al. 2014]. In a subset of that population (NHANES 2005–2006) Stein et al. [2016] found similar elevated ORs with wide CIs for PFOA, PFNA and asthma; they also reported elevated ORS with PFOS, but not PFHxS. In another cross-sectional analysis of asthmatic and non-asthmatic children in Taiwan, increasing quartiles of serum PFOA were associated with increasing odds of asthma [Zhu et al. 2016]. PFOS, PFBS, PFDA, PFHxS, and PFNA were also associated with asthma, for some PFAS the associations were divergent by sex (PFOS only associated with asthma in males) or potentially sex divergent (PFBS had stronger effect in males), while the others had similar effects across sexes [Zhu et al. 2016]. In a cohort across Greenland and the Ukraine Smit, et al. [2015] reported generally null associations between asthma or wheeze and a factor representing maternal plasma PFAS concentrations."

Rappazzo et al. reviewed five studies that investigated allergies or similar outcomes (Okada et al. 2014; Okada et al. 2012; Smit et al. 2015; Wang et al. 2011; Stein et al. 2016). Of these studies Rappazo et al. commented: "*Five studies looked at allergies or similar outcomes, generally finding null results.*"

Rappazzo et al. provided the following detail about the studies they reviewed: "*In the two analyses of the Hokkaido Study on Environment and Children's Health, no associations with maternal serum PFOA or PFOS and food allergies or eczema, and null to potentially negative associations with total allergies, were observed [Okada et al. 2014; Okada et al. 2012]. A cohort in Greenland and the Ukraine reported no association between a factor representing maternal plasma PFAS concentrations and current or ever eczema in children [Smit et al. 2015]. Wang et al. (2011) observed no associations with increasing quartiles of cord blood serum PFOA and atopic dermatitis; they did report positive associations with PFOS and PFNA, though both had large confidence limit ratios, indicating low precision, and no trends. In a cross-sectional analysis of NHANES data, Stein et al. [2016], observed associations with increased PFOA, PFOS and rhinitis; there was some evidence for associations with allergy as well, but no PFAS were associated with wheeze.*"

Immune biomarkers or gene expression profiles

For these health immunological outcomes, Rappazzo et al. reviewed and provided summaries of five studies (Granum et al. 2013; Ashley-Martin et al. 2015; Wang et al. 2011; Dong et al. 2013; Zhu et al. 2016).

Rappazzo et al. provided the following details about these studies: "*In addition to vaccination antibodies, Granum, Haug, Namork, Stolevik, Thomsen, Aaberge, van Loveren, Lovik and Nygaard [2013] also examined allergen-specific IgE antibodies, using a test that distinguishes atopic and non-atopic status, reporting no associations between atopic status and concentrations of any PFAS (plasma) in the Norwegian cohort. Newborn immune function markers were examined in a cohort of 10 Canadian cities and generally null associations were observed between immune function and any maternal serum PFAS [Ashley-Martin et al. 2015]. In a Japanese cohort, increasing maternal serum PFOA concentrations were negatively associated with cord blood IgE in 18 months old girls but not boys [Okada et al. 2012]. In the Taiwan Birth Panel cohort study, IgE levels at 2 years of age were not associated with PFOA, PFOS, or PFNA in cord blood serum [Wang et al. 2011]. Wang, Hsieh, Chen, Fletcher, Lien, Chiang, Chiang, Wu and Chen [2011] also observed positive associations between PFOS and PFOA and IgE levels, both measured in cord blood, but only in boys. Another study in Taiwan*

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examined IgE levels in children with and without asthma, reporting statistically significant p-values for trend with increasing concentrations of serum PFAS (PFOA, PFOS, PFDA, PFDoA, PFNA, and PFTA), though linear changes were not reported [Dong et al. 2013]. In a cross-sectional analysis of asthmatic boys, Zhu et al. [2016] observed higher levels of IgE, and Th1 and TH2 cytokines, which might have contributed to asthma development, with higher levels of serum PFOS, PFOA, and PFDA."

Kirk et al. (2018)

In their draft systematic review, Kirk et al. reviewed the human epidemiological evidence on exposure to PFAS and immunological effects in children and adults. The authors reviewed the evidence on vaccine response, infectious disease, asthma and allergic diseases and autoimmune diseases.

Studies reviewed

Kirk et al. reviewed 15 studies in total that investigated the effect of PFAS on the immune system in children and adults. They noted that 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. Kirk et al. undertook a risk of bias assessment of the studies they reviewed and determined that all of the papers on immunological effects they reviewed had "*a moderate to high risk of bias*".

Kirk et al. evaluated:

- two studies on autoimmune diseases (Steenland et al. 2015; Steenland et al.2013);
- seven studies on vaccine response covering diphtheria, tetanus, measles, mumps and rubella (MMR), and influenza vaccines (Grandjean et al. 2012; Mogensen et al. 2015; Grandjean et al 2016; Kielsen et al. 2016; Granum et al. 2013; Stein et al. 2016; Looker et al, 2014);
- four papers on the incidence of infectious diseases in children and adults covering hospitalisations due to infection, middle ear infection, gastroenteritis, and colds and influenza (Fei et al. 2010; Okada et al. 2012; Granum et al. 2013; Looker et al. 2014);
- nine studies on asthma and allergic outcomes, covering asthma, allergies, allergic rhinoconjunctivitis, wheezing and eczema (Dong et al. 2013; Zhu et al. 2016; Humblet et al. 2014; Stein et al. 2016; Steenland et al. 2015; Goudarzi et al. 2016; Okada et al. 2012; Okada et al. 2014; Granum et al. 2013).

Considerations and conclusions

In the Plain Language Summary Kirk et al. stated, "*We found limited evidence in a small number of relevant studies that ...higher levels of PFAS in the blood resulted in lower levels of antibodies than usual following vaccination against some vaccine preventable infections*".

In the Executive Summary, Kirk et al. made two statements about exposure to PFAS and immunological effects, firstly: "*We found limited evidence of an association between two PFAS chemicals and seven health effects, namely ...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*", and: "*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*".

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In the Discussion, Kirk et al. provided further information to support their conclusion, stating: "*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*".

Below is the evaluation of the evidence between specific PFAS and immunological outcomes reported by Kirk et al. following their review of the 15 studies on the effect of PFAS exposure on the immune system in children and adults. The tables have been reproduced from Kirk et al. (pg. 122-131).

Autoimmune diseases

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

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	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

Infectious disease

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

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Asthma and allergic diseases

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

Summaries of studies reviewed

Autoimmune diseases

Both studies on autoimmune diseases (Steenland et al. 2015; Steenland et al. 2013) evaluated by Kirk et al. were reviewed by the ATSDR (2015) NTP (2016), RIVM (2017), with summaries of these studies provided earlier in this section.

Kirk et al. commented at the end of the section on autoimmune diseases that: "*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. [2015] 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. [2013] contributed to a high risk of bias assessment for the study.*"

Vaccination response

Diphtheria vaccine

Kirk et al. reviewed four papers that investigated PFAS and antibody response to diphtheria vaccination (Grandjean et al. 2012; Mogensen et al. 2015; Grandjean et al 2016; Kielsen et al. 2016). These studies have been summarised earlier in this section, with Priestly having reviewed and summarised the study by Grandjean et al. (2016).

Of these four studies, Kirk et al. concluded: "*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.*"

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Tetanus vaccine

Of the five papers that investigated tetanus vaccine response (Grandjean et al. 2012; Mogensen et al. 2015; Grandjean et al 2016; Kielsen et al. 2016; Granum et al. 2013), Kirk et al. made two comments: "*Five papers reported tetanus antibody response, with conflicting results*", and: "*It is important to note that three of the five papers were on the same cohort in the Faroe Islands, making an 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

For the two studies on MMR vaccine (Granum et al. 2013; Stein et al. 2016), Kirk et al. concluded: "*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

For the two studies on influenza vaccine (Stein et al. 2016; Looker et al, 2014), Kirk et al. reported: "*Looker et al. [2014] investigated a cohort of 403 adults who had pre- and post-influenza vaccination titres tested. Looker et al. [2014] 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. [2016] in a study of 78 adults who were vaccinated with FluMistTM—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 diseases

Of the one paper (Fei et al. 2010) on hospitalisation due to infection during early childhood Kirk et al. concluded in the Associations at a glance table (above): "*Inadequate evidence: PFOA, PFOS*".

Kirk et al. concluded in the Associations at a glance table above: "*Inadequate evidence: PFOA, PFOS, PFHxS, PFNA*" for the two papers they reviewed (Okada et al. 2012; Granum et al. 2013) on middle ear infection.

For gastroenteritis, a review of the study by Granum et al. (2013), led Kirk et al. to comment: "*Therefore, Granum et al. [2013] 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*". Kirk et al. assigned: "*Inadequate evidence: PFOA, PFOS, PFHxS, PFNA*" for this study in the 'Associations at a glance' table above.

For colds and influenza, from the two papers (Granum et al. 2013; Looker et al. 2014), Kirk et al. reviewed, they commented: "*Given the limited number of studies that are at high risk of bias, these results should be viewed with caution*", and assigned the following evaluation of evidence in the Associations at a glance table above: "*Inadequate evidence: PFOA, PFOS, PFhxS, PFNA*".

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Asthma and allergic diseases

Kirk et al report they reviewed six studies that investigated the association between PFAS and asthma diagnosis (Dong et al. 2013; Zhu et al. 2016; Humblet et al. 2014; Stein et al. 2016; Granum et al. 2013; Steenland et al. 2015). They made a general comment for the studies they reviewed: "*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.*"

All of the studies except that by Steenland et al. (2015) were on children and asthma. Kirk et al. reported on the study by Steenland et al. (2015), as: "*Steenland et al. [2015] 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).*"

At the end of the section on Asthma, Kirk et al. commented: "*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. [2013] and Zhu et al. [2016] reported differing results, with Zhu et al. [2016] 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. [2014] and Stein et al. [2016], contrast many of the associations found by Dong et al. [2013] and Zhu et al. [2016]. 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. [2013] and Zhu et al. [2016].*"

Of the four studies reviewed on Allergies (Goudarzi et al. 2016; Okada et al. 2012; Okada et al. 2014; Stein et al. 2016), Kirk et al. reported: "*Goudarzi et al. [2016] 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. [2012; 2014] investigated total allergic diseases during early infancy in relations to PFAS exposure using data from the Hokkaido Study. Okada et al. [2014] 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. [2012] 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. [2016] reported on the effect of PFAS exposure and IgE sensitization 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. [2-16] 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. [2016] did not identify any associations between PFOA and PFNA and IgE sensitisation in children."

Of these four studies on allergies, Kirk et al. made the following comment: "*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. [2016] and Okada et al. [2014]*

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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. [2016] regarding IgE sensitization should be the subject of future research."

On allergic rhinoconjunctivitis, Kirk et al. commented: *"Although the findings presented by Goudarzi et al. [2016] show a negative association between PFNA, PFDoA and PFUdA and allergic rhinoconjunctivitis, the findings have not been replicated"*, and assigned their evaluation of the evidence as: *"Inadequate evidence: PFOA, PFOS, PFHxS, PFNA, PFDA, PFDoA, PFTrDA, PFUdA"* in the 'Associations at a glance table' above.

Of the four studies reviewed on wheezing (Granum et al. 2013; Okada et al. 2012; Goudarzi et al. 2016; Humblet et al. 2014), Kirk et al. reported: *"All studies reported no association between the exposure and health outcome."*

For eczema, Kirk et al. reviewed four studies (Granum et al. 2013; Okada et al. 2012; Goudarzi et al. 2016; Okada et al. 2014) and reported these studies as: *"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. [2016] 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. [2014] 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. [2012] reported no significant association between PFOA and PFOS and eczema in children in the first 18-months of life."*

Kirk et al. made the following comment about these four studies on eczema: *"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. [2016] and Okada et al. [2014] 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."*

6.8.4. Summary of considerations and conclusions from key national and international reports and systematic reviews

Recent key national and international reports:

- The ATSDR concluded that the evidence was inconsistent for vaccination response and did not establish whether any human disease was increased directly due to immunotoxicity.
- The NTP concluded PFOA and PFOS were *"presumed to be an immune hazard to humans"*.
- The US EPA concluded that, for PFOA and PFOS, a decreased response to vaccines has been reported but no clear association between PFOA and PFOS and infectious disease has been identified.
- The DWQI concluded the evidence for PFOA and decreased antibody response following vaccination was *'limited'*.
- RIVM concluded that evidence supports a higher risk of ulcerative colitis and associations exist between PFOA and decreased vaccination response but the evidence is not clear.

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- FSANZ concluded there is no convincing evidence that PFOS/PFOA increased incidence of infective disease and that data on vaccine response and PFOA is insufficient to establish causation.

Systematic reviews:

- Chang et al. concluded that with limited evidence and issues with studies, evidence is insufficient to reach a causal relationship between PFOS/PFOA and any immune-related health outcome in humans.
- Priestly concluded the evidence suggested, but did not prove, a link between PFOS/PFOA and disturbances in the immune system.
- Rappazzo et al. concluded there is evidence for positive associations between PFAS and immunity (vaccine response and asthma) in children.
- Kirk et al. concluded there was *'limited evidence'* for immunological effects of PFAS exposure.

6.8.5. Expert Health Panel synthesis to support advice to the Minister

- There is animal evidence that PFAS may alter immune function at concentrations found in humans with environmental and occupational exposures.
- There are few human studies on PFAS and immunological effects, with studies examining multiple immune biomarkers and clinical end points and multiple chemical exposures, often with a post-hoc analysis of observed associations. There is thus a substantial risk that many findings are due to bias or chance. This is reflected in the lack of consistency in the findings of studies, which in turn has led to very diverse conclusions of the reviews summarised above.
- There is strong potential for confounding by other persistent organic pollutants with immune effects in studies in the general population (which is where many of these studies have found associations).
- Inflammatory and immune disease also alter transporter expression, and thus it is feasible that inflammatory disease could cause reduced elimination of PFAS (i.e. reverse causation).
- The strongest evidence for a link between PFAS and clinically important immunological effects is for impaired vaccine response. However, the human dose-response/threshold for potential immune effects is very poorly characterised, and the overall human evidence is weak.

6.8.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and immunological effects:

- PFAS are likely to alter the function of the immune system; however, it is unclear if this occurs at current exposures or has any clinically important consequences. In particular there is no consistent evidence for increased risk of infections or auto-immune disease.
- Impaired vaccine response is the most consistent reported association. Internationally, most studies that have observed decreased antibody levels have not found significant increases in incidence of human disease or associations of higher blood levels of PFAS with infectious disease. However, they were generally very underpowered to detect important differences in disease incidence (given the rarity of many of these diseases).

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To further investigate the association between PFAS exposure and immunological effects in an Australian setting, the Panel suggests the following research priorities:

- Measuring vaccine response is a strong candidate for further studies as it has the advantage of prospective (post-exposure) design and objective outcomes.
- Studies of infections or auto-immune disease would be best nested within a very large study of overall health outcomes (ideally supported by data linkage to avoid recall biases).
- Cross-sectional studies of multiple immune biomarkers have been done many times and further studies are likely to be largely unhelpful, unless they are combined with a method of rapidly eliminating PFAS so that a before-after design can be used to provide evidence for causal mechanisms.

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6.9. Neurodevelopmental and neurophysiological effects

Evidence shows the development of the brain and nervous system is a dynamic process that occurs over various life stages, and with important programming and vulnerability at each stage of development (Rappazzo et al. 2017). Alterations in neurodevelopment may have a life-long impact on quality of life. A small number of international authority reports and several systematic reviews have reviewed the human epidemiological evidence on exposure to PFAS and neurodevelopmental and neurophysiological effects in children and adults.

6.9.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of the following three international authority/intergovernmental/governmental reports published in 2015 and 2016 and five systematic reviews from 2013 onwards that reported on exposure to PFAS and neurodevelopmental and neurophysiological effects:

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **United States Environmental Protection Agency (US EPA 2016a).** Health effects support document for Perfluorooctanoic Acid (PFOA), 2016;
- **United States Environmental Protection Agency (US EPA 2016b).** Health effects support document for Perfluorooctane Sulphonate (PFOS), 2016.

Systematic reviews

- **Saikat et al. (2013).** The impact of PFOS on health in the general population: a review;
- **Roth and Wilks (2014).** Neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals: a systematic review of the epidemiological literature using a quality assessment scheme;
- **Priestly (2016).** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS). Monash University;
- **Rappazzo et al. (2017).** Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature;
- **Kirk et al. (2018).** The PFAS Health Study. Systematic Literature Review. Australian National University.

While the Panel acknowledges the DWQI noted that epidemiological studies had investigated neurological and neurodegenerative disorders including self-reported memory impairment and Parkinson's disease, cognitive and behavioural developmental milestones, performance testing, and attention deficit hyperactivity disorder (ADHD) in children, the DWQI did not review the epidemiological literature on neurodevelopment and is not considered further in this section. While FSANZ (2017) did comment on PFOS and human data on neurodevelopmental and neurophysical effects, FSANZ did not review epidemiological studies in detail; instead they reported on conclusions by other authoritative groups, notably the ATSDR (2015) and US EPA (2016b). For PFOA, in the 'Effects on offspring of PFOA-exposed parents', FSANZ noted four studies which had been previously reviewed by the US EPA (2016a) and EFSA (2008), and under 'Other effects' stated that: "*No consistent associations have been reported between serum PFOA levels and memory loss or senility.*" No other key reports or systematic reviews reported on epidemiological studies on PFAS and neurodevelopment.

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6.9.2. Key national and international reports

Agency for Toxic Substances and Disease Registry (ATSDR, 2015)

Studies reviewed

The ATSDR reviewed:

- two studies on neurophysiological effects in adults (Power et al. 2013; Gallo et al. 2013);
- three studies on neurodevelopmental effects in children (Fei et al. 2008b; Fei and Olsen, 2011; Stein et al. 2013);
- three studies on ADHD in children (Hoffman et al. 2010; Stein and Savitz, 2011; Gump et al. 2011).

The ATSDR noted: "*No information was located regarding neurological effects in humans following inhalation exposure to perfluoroalkyl compounds.*"

Considerations and conclusions

The ATSDR did not make any statements or conclusions about human neurodevelopmental or neurophysiological effects in the 'Public health statement for perfluoroalkyls' or the 'Relevance to public health' sections of the toxicological profile.

Summary of studies reviewed

Neurophysiological effects in adults- oral exposure route

Under the 'Oral exposure – neurological effects' section, the ATSDR reviewed Power et al. (2013) who used NHANES data for adults aged 60–85 years (1999–2000 and 2003–2008 cycles). The ATSDR reported that Power et al. (2013): "*found an inverse association between serum PFOA, PFOS, PFHxS, and PFNA levels and self-reported limitation due to difficulty remembering or periods of confusion; however, the ORs included the null value. When the subjects were categorized by diabetic status and the use of medication for the treatment of diabetes, significant inverse associations between limitations due to difficulty remembering and serum PFOS (OR 0.39, 95% CI 0.19–0.78), PFNA (OR 0.43, 95% CI 0.21–0.87), and PFHxS (0.49, 95% CI 0.29–0.84) were found.*"

Of the study by Gallo et al. (2013), who used the a C8 Science Panel study of 4,462 adults aged ≥50 years, the ATSDR reported that Gallo et al. (2013) found: "*self-reported short-term memory loss impairment was negatively associated with serum PFOS, PFOA, and PFHxS levels. Comparisons with the referent group (subjects with serum perfluoroalkyl levels in the first quintile) were statistically significant for serum PFOS levels of ≥20.5 ng/mL (OR 0.86, 95% CI 0.78–0.96), serum PFOA levels of ≥14.1 ng/mL (OR 0.88, 95% CI 0.79–0.97), and PFHxS levels of ≥5.7 ng/mL (OR 0.89, 95% CI 0.79–0.99).*"

The ATSDR compared the two studies and concluded that in relation to the results found by Gallo et al. (2015): "*Unlike the Power et al. (2013) study, the inverse association between serum perfluoroalkyls was weaker and was not statistically significant in diabetics. In sensitivity analy[s]es, the association between serum PFOA levels and memory impairment was compared within and across water districts. Within a water district, the association between serum PFOA and memory impairment was significant, but there was no association between the geometric mean concentration of PFOA in a district and memory impairment.*"

Under the 'Neurotoxicity' section, the ATSDR commented: "*Neurological examinations were not conducted (or at least it was not explicitly indicated) in the studies of perfluoroalkyl workers (Gilliland*

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and Mandel 1996; Mundt et al. 2007; Olsen and Zobel 2007; Olsen et al. 1998b, 1999, 2003a; Sakr et al. 2007a, 2007b); it is reasonable to assume that no frank clinical signs were detected in the groups examined. A general population study (Power et al. 2013) and a study of highly-exposed residents (Gallo et al. 2013) have examined the possible association between serum perfluoroalkyl levels and memory and found conflicting results; no other epidemiology studies examining neurological effects were identified."

Neurodevelopmental effects in children – oral exposure route

The ATSDR reported on three studies on neurodevelopmental effects in children.

Of the study by Fei et al. (2008b), the ATSDR noted that: "*Neurodevelopment was also evaluated in the children of mothers in the Danish National Birth Cohort study at 6 months, 18 months, and 7 years of age. Maternal serum PFOA and PFOS levels were not associated with alterations in the time to achieve developmental milestones in 6- and 18-month-old children (Fei et al. 2008b)."*

Of the study by Fei and Olsen (2011), the ATSDR reported that: "*At 7 years of age, no significant alterations (after adjustment for potential confounders) in behavioral or social development and maternal serum PFOA and PFOS levels were found (Fei and Olsen 2011)."*

Of the study by Stein et al. (2013), the ATSDR reported that: "*Stein et al. (2013) examined 320 children aged 6–12 who lived in a PFOA-contaminated area of the Mid-Ohio Valley from the time of the mother's pregnancy until C8 Health Project enrollment. Estimated in utero serum PFOA concentrations or the child's serum PFOA level were not significantly associated with an adverse effect on neuropsychological tests, including IQ, reading and math skills, language, memory and learning, visual-spatial processing, and attention."*

The ATSDR reviewed three studies on ADHD in children and commented: "*Several studies (Gump et al. 2011; Hoffman et al. 2010; Stein and Savitz 2011) have found significant associations between exposure to perfluoroalkyls and the likelihood of attention deficit/hyperactivity disorder (ADHD) and impulsivity in children."*

The ATSDR reported on Hoffman et al. (2010) who used NHANES data for serum perfluoroalkyl levels in children aged 12–15 years and reported: "*Hoffman et al. (2010) found a significant dose-response relationship between serum PFOS, PFOA, and PFHxS levels and the likelihood of ADHD diagnosis; no association was found with PFNA levels."*

Of the study by Stein and Savitz (2011), the ATSDR reported that: "*Stein and Savitz (2011) also found an increase in the likelihood of ADHD diagnosis in children aged 5–18 years participating in the C8 Health project with serum PFHxS levels in the second (2.9–<5.2 ng/mL), third (5.2–<10.1 ng/mL) or fourth (10.1–276.4 ng/mL) quartiles; the likelihood of ADHD diagnosis was not significantly associated with serum PFOA, PFOS, or PFNA levels. The study also found an increased likelihood of learning problems in children with serum levels of PFHxS in the fourth quartile."*

For the study by Gump et al. (2011), the ATSDR reported that: "*Gump et al. (2011) evaluated the potential effect of perfluoroalkyl exposure on impulsivity, which is a defining feature of ADHD, using the differential reinforcement of low rates of responding task in 83 children aged 9–11 years living in New York and participating in another study on the effects of low-level lead exposure on cardiovascular responses to acute stress. Significant associations between serum PFOS, PFNA, PFDeA, PFHxS, and PFOSA levels and impulsivity were found; no associations were found for PFOA."*

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Regarding ADHD, the ATSDR concluded in the 'Developmental effects' section that: "*The conflicting results of studies examining an association between perfluoroalkyl exposure and risk of ADHD (Grump et al. 2011; Hoffman et al. 2010; Stein and Savitz 2011) preclude a weight of evidence determination.*"

Neurodevelopment was also included in the 'Developmental toxicity' section, where the ATSDR stated that: "*Although studies of highly exposed residents and the general population have reported alterations in neurodevelopment (Fei et al. 2008b; Gump et al. 2011; Hoffman et al. 2010; Stein and Savitz 2011), [and several other health effects], the effects were not consistently found across studies or were only examined in a single study. The available studies are cross-sectional, account for a limited number of potential confounders, and do not establish causality.*"

United States Environmental Protection Agency (US EPA) 2016a and 2016b

Studies reviewed

For PFOA, the US EPA reviewed six studies relating to PFOA and various neurodevelopmental effects and neurophysiological effects, including:

- one high-exposure community study (Stein et al. 2013);
- five general population studies in children (Fei et al. 2008a; Liew et al. 2014; Fei and Olsen 2011; Høyer et al. 2015a; Hoffman et al. 2010).

For PFOS, the US EPA reviewed five studies relating to PFOS and various neurodevelopmental effects (Liew et al. 2014; Fei and Olsen, 2011; Høyer et al. 2015b; Fei et al. 2008a, Hoffman et al. 2010).

Three of the studies reviewed by US EPA (Fei and Olsen, 2011; Hoffman et al. 2010; Stein et al. 2013) were reviewed by the ATSDR.

The study by Liew et al. (2014) was on cerebral palsy, and is reviewed under the 'Prenatal, pregnancy and birth outcomes' section of this report.

Considerations and conclusions

PFOA

In the 'Executive Summary' of the 'Health effects support document for PFOA', the US EPA stated: "*The epidemiology studies did not find associations between PFOA and neurodevelopmental effects.*"

Under 'Nervous system effects', the US EPA stated: "*The data pertaining to neurotoxicity (including neurodevelopmental effects) of PFOA are limited, but do not indicate the presence of associations between PFOA and a variety of outcomes. Fei et al. (2008a) found no association between maternal serum PFOA concentrations and fine motor skills, gross motor skills, and cognitive abilities of children aged 6 and 18 months. Fei and Olsen (2011) found no association between behavioural or coordination problems in children aged 7 years and prenatal PFOA exposure. Epidemiology studies of children derived from the NHANES and C8 populations found a weak statistical association between serum PFOA with parental reports of ADHD (Hoffman et al. 2010; Stein et al. 2013).*"

PFOS

For PFOS, the US EPA made no statement or conclusions about neurodevelopment in the 'Executive Summary'.

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Summary of studies reviewed

PFOA

While several of the studies reviewed by the US EPA were also reviewed by the ATSDR, additional details reported about these studies by the US EPA are included below.

High-exposure community studies

The US EPA reviewed the study by Stein et al. (2013), and provided additional detail to that provided above under the ATSDR, including: "*The children had serum samples collected at enrollment in 2005–2006 with the current follow-up evaluation conducted in 2009–2010, when the children were 6–12 years old. Both the mother and teacher completed surveys to elicit information on each child's executive function, attention deficit hyperactivity disorder- (ADHD-) like behavior, and behavioral problems. Information on family demographics and other health conditions of the child were included as confounders. Linear regression was used to determine the association between PFOA levels and mother and teacher reports. The median PFOA level was 35.1 ng/mL with an IQR of 15.8–94.1 ng/mL. When comparing the highest to the lowest PFOA quartile, survey results from the mother for both executive function and ADHD showed a favorable association for males, but an adverse association for females. These findings were not replicated when males and females were analyzed together or with results from the teacher surveys. No association was found between PFOA levels and either mother or teacher scores for behavioral problems in females and males.*"

General population studies

The US EPA reviewed the study by Fei et al. (2008a), and reported details on this study, including: "*The mothers self-reported the infant's fine and gross motor skills and mental development at 6 and 18 months of age. There was no association between maternal plasma PFOA concentration and Apgar score or between maternal plasma PFOA concentration and fine motor skills, gross motor skills, or cognitive skills at 6 and 18 months of age. The children born to females having higher plasma PFOA concentrations reached developmental milestones at the same times as children born to females having lower plasma PFOA concentrations. The authors concluded that there was no association between maternal early pregnancy levels of PFOA and motor or mental developmental milestones in offspring. However, in children at 18 months, mothers with higher PFOS levels were slightly more likely to report that their babies started sitting without support at a later age.*"

The US EPA also reviewed Fei and Olsen (2011), and provided additional details of the study, to that provided under the ATSDR above, including: "*Behavioral problems were assessed using the Strengths and Difficulties Questionnaire (SDQ), and coordination problems were assessed using the Developmental Coordination Disorder Questionnaire (DCDQ) completed by the mothers. A total of 787 mothers completed the SDQ and 537 completed the DCDQ for children aged 7.01–8.47 years (mean age 7.15 years).*" The US EPA report the results as "*The mean maternal PFOA concentration was 5.7 ng/mL, and PFOA levels were divided into quartiles: <LLOQ-3.95, 3.96–5.32, 5.35–7.11, and 7.14–21.9 ng/mL. A child having higher scores in total difficulties, emotional symptoms, and hyperactivity was negatively associated with the second or third PFOA quartiles (OR=0.56, 95% CI 0.27–1.19; p<0.05 and OR=0.36, 95% CI 0.15–0.82; p<0.05, respectively) when compared with females in the lowest quartile. ORs adjusted for parity, maternal age, prepregnancy BMI, pregnancy smoking and alcohol consumption, socio-occupational status, child gender, breast-feeding, birth year, home density, gestational age at blood draw, and parental behavior problem as children did not show a positive association between prenatal PFOA exposure and behavior or coordination problems. Overall, no significant association between behavioral or coordination problems in children 7 years of age and prenatal PFOA (and PFOS) exposure was found.*"

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Of the study by Høyer et al. (2015a), the US EPA reported that: "*Similar to the above study [meaning Fei and Olsen, 2011], the association between maternal PFOA (and PFOS) levels and offspring behavior and motor development was investigated in a subset of the Biopersistent Organochlorines in Diet and Human Fertility study (INUENDO) birth cohort (Høyer et al. 2015a). Pregnant females were enrolled between May 2002 and February 2004 with a total of 1,106 mother-child pairs at follow-up between January 2010 and May 2012, when the children were 7–9 years old. The study population consisted of 526 pairs from Greenland, 89 pairs from Poland, and 491 pairs from Ukraine. Maternal blood samples for measurement of plasma PFOA levels were taken any time during pregnancy. Behavior of children was assessed with SDQ score, and logistic regression models were used in the analyses of PFOA tertile levels and behavioral problems. Motor development was assessed with DCDQ score, and linear regression was used for analyses. All analyses were performed on the entire cohort as well as by country, except that not all analyses could be performed on the Polish subset because of the small number of cases. The median maternal plasma PFOA level was 1.4 ng/mL for the combined population and 1.8, 1, and 2.7 ng/mL for the pregnant females from Greenland, Ukraine, and Poland, respectively.*"

The US EPA reported the findings of this study as: "*No associations were found between PFOA (and PFOS) levels and motor development score. Total SDQ score was not associated with PFOA levels; however, the OR of having an abnormal total SDQ score was 2.7 (95% CI 1.2, 6.3) for all groups combined. PFOS levels were associated with higher total SDQ score only in Greenland. The highest PFOA tertile was associated with a 0.5-point higher hyperactivity score in both the combined analysis and in Greenland, but no associations were found in Poland and Ukraine. The OR for hyperactive behavior in the combined analysis was 3.1 (95% CI 1.3, 7.2) for the highest tertile compared to the lowest PFOA tertile. In Greenland, the ORs for hyperactivity were increased for the middle (OR=5.4, 95% CI 1.1, 25.6) and highest (OR=6.3, 95% CI 1.3, 30.1) tertiles (Høyer et al. 2015a).*"

The US EPA also reviewed the study by Hoffman et al. (2010), and reported the following details about the study: "*Hoffman et al. (2010) examined the associations between perfluorochemicals, including PFOA, and diagnosis of ADHD using the NHANES data from 1999–2000 and 2003–2004. The study population comprised 571 children aged 12–15 years, including those who had been diagnosed as having ADHD (n = 48) and/or were taking ADHD medications (n = 21). Age, gender, and race/ethnicity were included as covariates; and socioeconomic status, health insurance coverage and having a routine health care provider, living with someone who smokes, birth weight, admittance to a neonatal intensive care unit, maternal smoking, and preschool attendance were confounders. Regression models were used to analyze the data. The median serum PFOA level was 4.4 ng/mL and ranged from 0.4 to 21.7 ng/mL. Serum PFOA was positively associated with parental report of ADHD (OR=1.12, 95% CI 1.01–1.23). The OR for serum PFOA and parental report of ADHD and ADHD medication use was 1.19 (95% CI 0.95–1.49). Both PFOS and perfluorohexane sulfonate also were positively associated with parentally reported ADHD. Data interpretation was limited by the cross-sectional study design, random misclassification error resulting from using current PFOA levels as proxy measures of etiologically relevant exposures, and other confounders not included in the available data.*"

In the 'Summary and conclusions' of the 'Human epidemiology studies' section, the US EPA concluded: "*Studies found a positive association with ADHD in children in the highly exposed community (Stein et al. 2013) and the general population (Hoffman et al. 2010). No other behavior end points in children were associated with maternal PFOA levels in either population.*"

In the 'Hazard characterisation – synthesis and evaluation of major non-cancer effects', the US EPA reported under 'Nervous system effects': "*The data pertaining to neurotoxicity (including neurodevelopmental effects) of PFOA are limited, but do not indicate the presence of associations between PFOA and a variety of outcomes. Fei et al. (2008a) found no association between maternal*

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serum PFOA concentrations and fine motor skills, gross motor skills, and cognitive abilities of children aged 6 and 18 months. Fei and Olsen (2011) found no association between behavioral or coordination problems in children aged 7 years and prenatal PFOA exposure. Epidemiology studies of children derived from the NHANES and C8 populations found a weak statistical association between serum PFOA with parental reports of ADHD (Hoffman et al. 2010; Stein et al. 2013)."

PFOS

The US EPA reported the literature they reviewed under the section 'Reproductive hormones and reproductive/developmental studies'. For the study by Fei and Olsen (2011), the US EPA reported the findings for PFOS in their summary above, under PFOA.

For the study by Høyer et al. (2015a), the US EPA reported the findings for PFOS as: "*The median maternal plasma PFOS level was 10 ng/mL for the combined population and 20, 5, and 8 ng/mL for the pregnant females from Greenland, Ukraine, and Poland, respectively. No associations were found between PFOS (and PFOA) levels and motor development score. Total SDQ score was not associated with PFOS levels; however, PFOS concentrations were associated with higher total SDQ score only in Greenland. The highest PFOS tertile was associated with a 0.5 point higher hyperactivity scores in the combined analysis in Greenland (0.3) and Poland (1.3), but no association was found in Ukraine. The adjusted OR for hyperactive behavior in the combined analysis was 1.4 (95% CI: 0.4–4.9) for the highest tertile compared to the lowest PFOS tertile, with comparable results found for Greenland and Ukraine. Although statistical adjustment in the regression models included country of participant, inter-country differences complicate interpretation of the study results especially given variability in exposure data collection periods and vastly different participation rates (e.g., 37% in Poland and 86% in Greenland). In addition to the potential for selection and information biases, the unknown critical exposure window(s), including the impact of unmeasured post-natal exposures, for these outcomes increases the uncertainty of these study results.*"

For the study by Fei et al. (2008a), the US EPA reported the findings for PFOS as: "*Using linear regression, no significant association between PFOS and APGAR score was observed after adjustment for potential confounders (OR = 1.20; 95% CI: 0.57–2.25). Although these data were limited by maternal reporting of the outcome data, there was no association between PFOS levels and motor or mental development as reported in the questionnaire at 6 months. In children at 18 months, mothers with higher PFOS levels were slightly more likely to report that their babies started sitting without support at a later age and "did not use word-like sounds to tell what he/she wants.*"

Of the study by Hoffman et al. (2010), the US EPA reported the findings for PFOS as: "*The median serum PFOS levels were 23 ng/mL and ranged from 2 to 90 ng/mL. Serum PFOS was positively associated with parental report of ADHD (OR = 1.03, 95% CI: 1.01–1.05). The adjusted odds ratio per each 1000 ng/L increase in serum PFOA for parental report of ADHD and ADHD medication use was 1.05 (95% CI: 1.02– 1.08). Both PFOA and perfluorohexane sulfonate were also positively associated with parentally reported ADHD. Data interpretation were limited by the cross-sectional study design, other potential confounders (e.g., alcohol consumption) that were not included in the available data, and measurement error resulting from using current PFOS levels as proxy measures of etiologically relevant exposures.*"

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6.9.3. Systematic reviews

Saikat et al. (2013)

Studies reviewed

Saikat et al. cited one study in their review that evaluated the association between blood serum PFOS and ADHD (Hoffman et al. 2010). This study was also reviewed by the ATSDR and the US EPA (summaries above).

Considerations and conclusions

Saikat et al. (2013), in their review of the impact of PFOS on health in the general population, stated in the 'Abstract': "*Small but statistically significant associations have been reported with PFOS and attention deficit/hyperactivity disorder (ADHD) [and a number of other health effects].*"

Saikat et al. did not make any specific conclusions about PFOS and ADHD, only a general conclusion about all of the studies they reviewed.

In addition to the information provided under the ATSDR and US EPA above for this study, Siakat et al. also noted: "*Socioeconomic status and environmental contaminants (lead, environmental tobacco smoke (ETS)) were considered as potential confounders. The adjusted odd ratio (OR) for reported ADHD in association with a 1 ng/mL increase in blood serum PFOS was 1.03.*"

Summary of studies reviewed

Roth and Wilks (2014)

Roth and Wilks conducted a systematic review of the epidemiological literature on neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals, using a quality assessment scheme.

Studies reviewed

Roth and Wilks reviewed seven studies on PFCs that had investigated neurodevelopmental and neurobehavioral end points covered in this section, including:

- three studies that analysed other neurodevelopmental end points and neurobehavioural end points (Chen et al. 2013; Gump et al. 2011; Stein et al. 2013);
- four studies that were questionnaire based/indirect evidence (Fei et al. 2008b[63]; Fei and Olsen, 2011; Hoffman et al. 2010; Stein and Savitz, 2011).

Considerations and conclusions

Roth and Wilks (2014) stated in the 'Abstract': "*Over the last decade there have been increasing reports in the epidemiological literature of the potential association of exposure to poly- bromo diphenylethers (PBDEs) and perfluorinated chemicals (PFCs) with neurodevelopmental and/or neurobehavioural effects in infants and children, such as adverse birth outcomes, cognitive deficits, developmental delay and attention deficit hyperactivity disorders (ADHD). However, direct evidence from epidemiology studies has been limited and contradictory.*"

The authors concluded in the 'Conclusion' of the 'Abstract': "*Collectively, the epidemiological evidence does currently not support a strong causal association between PBDEs and PFCs and adverse neurodevelopmental and neurobehavioural outcomes in infants and children. However, despite their*

[63] Note that Roth and Wilks refer to this study as Fei et al. 2008a, but it is in this report's Bibliography as 2008b.

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some limitations the studies raise questions that require further investigation through hypothesis-driven studies using more harmonized study designs and methodologies, more detailed exposure assessments and repeated testing with larger study populations."

In the 'Conclusion' section of the paper, Roth and Wilks also stated: "*The only consistent results were obtained for PFOA, for which none of the studies evaluated have shown any developmental or behavioural effects on all the different functional domains assessed."*

Summaries of studies reviewed

Roth and Wilks reported their review of the literature under three end points: motor function, cognitive development and behavioural. While Roth and Wilks reviewed the same studies that were reviewed by the ATSDR and/or US EPA, the findings of Roth and Wilks have been included below as the end point groupings are different and comments about some studies have been made.

Motor function

Roth and Wilks reported the following about the studies they reviewed that investigated PFAS and motor function: "*Only one study evaluated assessed motor function (Chen et al.2013). The authors reported a significant negative association between exposure to PFOS and motor coordination, primarily the gross motor domain, but no association with PFOA. In two questionnaire-based studies that were not evaluated in the present work, PFOA and PFOS exposure were not significantly related to maternal report of motor development (Fei et al. 2008[b]; Fei and Olsen, 2011). However, the testing methodologies differed substantially, making the comparison difficult."*

Cognitive development

Roth and Wilks reported the following on the studies they reviewed regarding cognitive development: "*Two studies investigated cognitive development (Chen et al. 2013; Stein et al.2013). Both studies reported no association for PFOA. They are of high quality and benefit from a prospective cohort design and larger sample sizes, but assessed the children at different ages (2y and 6–12y, respectively) and with different tests (see supplementary material). Models were adjusted for neurotoxicants such as smoking, alcohol or lead but no other environmental contaminants. Chen et al. (2013) found a significant negative association between exposure to PFOS and cognitive development (whole test performance), whereas an additional cross-sectional study by Stein and Savitz (2011) showed a similar trend for PFOS with learning problems, based on parental report of previous physician-diagnosed ADHD. A dose–response gradient was found by Chen et al. (2013) when PFOS levels were categorized into quartiles. In contrast with these findings, Fei et al. (2008[b]) reported no significant association between PFOS and maternal reporting of cognitive development."*

Behavioural end points

Roth and Wilks stated of the studies they reviewed: "*A few studies have assessed general behavioural end points such as attention (Stein et al.2013), impulsivity (Gump et al.2011; Stein et al.2013) or social competence (Chen et al. 2013). Regardless of their quality rating, none of the PFOA studies evaluated have shown any behavioural effects on the various functional domains assessed, in contrast to observations with PFOS. Chen et al. (2013) found a statistically significant negative association between social competence and self help skills and PFOS exposure, but no association for PFOA. Stein et al. (2013) reported no significant association between PFOA and sustained attention or impulsivity, a finding in line with a report by Gump et al. (2011) who found no significant association between PFOA and a behavioural measure of impulsivity assessed with an inhibition response test; however, Gump et al. (2011) reported a significant positive association between higher serum levels of*

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PFOS, perfluorodecanoate (PFDA), PFNA, PFHxS, perfluorooctanesulfonamide (PFOSA) and impulsivity. Less consistency was observed, most notably for PFOA, from three complementary studies based on teacher and/or parental reports of general behavioural health (Fei and Olsen, 2011) or of previously diagnosed ADHD (Hoffman et al. 2010) or of parental report or self-report of previous doctor-diagnosed ADHD with and without medication (Stein and Savitz, 2011)."

Priestly (2016)

Priestly, in his literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS), reviewed the human epidemiological literature on neurodevelopmental effects in humans that included possible links with intelligence (IQ), Attention Deficit Hyperactivity Disorder (ADHD), autism, and other effects on postnatal and adolescent behaviour.

Studies reviewed

Priestly reviewed 17 studies on the association between various aspects of the potential neurodevelopmental effects of PFAS (Fei et al. 2008b; Fei & Olsen 2011; Strøm et al. 2014; Chen et al. 2013; Forns et al. 2015; Donauer et al. 2015; Goudarzi et al. 2016c; Høyer et al. 2015a; Wang et al. 2015; Braun et al. 2014; Oulhote et al. 2016b; Gump et al. 2011; Vuong et al. 2016; Cincinatti 2003-06; Hoffman et al. 2010; Liew et al. 2015; Polanska et al. 2012).

Of these, six studies have been reported under the ATSDR, US EPA: Saikat et al. (2013) and Roth and Wilks (2014) above; Fei et al. 2008b; Fei & Olsen 2011; Chen et al. 2013; Høyer et al. 2015a; Gump et al. 2011; Hoffman et al. 2010).

These papers were also reviewed by Kirk et al. and Rappazzo et al. (2017). Priestly did additionally mention the paper by Polanska et al. (2012) on possible environmental and lifestyle factors influencing the development of ADHD that Rappazzo et al. and Kirk et al. did not review.

Considerations and conclusions

Priestly (2016) stated in the 'Executive Summary': "*The epidemiological studies are suggesting, but not yet proving, a possible link between PFOS/PFOA and perinatal neurodevelopment.*"

Priestly's 'Comment' on the studies he reviewed under 'Altered neurodevelopment' was: "*As with other end points, there are some studies that show a possible association between PFAS exposure with some forms of neurobehavioural development. The lack of consistency makes it difficult to attribute these possible associations as causally related.*"

Summary of studies reviewed

The studies reviewed by Priestly that were not reviewed by the ATSDR, US EPA, Saikat et al. (2013) and Roth and Wilks (2014) are reported below.

- Strøm et al. (2014): "*found no neurodevelopmental effects in children from a Danish cohort that could be associated with PFAS, or other POPs (PCBs, p,p'-DDE, HCB).*"
- *Forns et al. (2015): "assessed neuropsychological development to age 2 years in infants born to a cohort of mothers in the Norwegian HUMIS study. The exposure assessment was determined by measuring breast milk levels of only PFOS (median 110 ng/L) and PFOA (median 40 ng/L). No associations were found with either PFOS or PFOA in two batteries of cognitive, psychomotor or behavioural development."*
- Donauer et al. (2015): "*studied neurobehavioural development at age 5 weeks, using maternal serum concentrations of PFOS, PFOA and PBDEs at around 16 weeks gestation as*

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an indicator of prenatal exposure. The tests applied were the Neonatal Intensive Care Unit Network Neurobehavioural Scale (NNS) measuring social/easygoing, high arousal/difficult or hypotonic characteristics. None of the tests showed any association with PFOA, PFOS or PBDEs, although a tenfold increase in prenatal PFOA increased the odds of being categorized as hypotonfeic (OR 3.79; 95% CI 1.1-12.8)."

- Goudarzi et al. (2016c): "*studied neurodevelopment at 6 and 18 months in Japanese infants from the Hokkaido Study. Bayley Scales of Infant Development showed a very small PFOA-related decrement (-5.05 95% CI -10.66, 0.55; p=0.045) in female infants at 6 months, but not at age 18 months, or males at either age, or either sex with PFOS levels.*"

Priestly reviewed Wang et al. (2015), and noted that the authors: "*reported on an apparent association between PFAS exposure and IQ development in prenatally exposed children. In an analysis of the Taiwan Maternal & Infant Cohort Study of children at age 5 (n=120), two of the seven PFAS measured (PFUnDA and PFNA) suggested a reduction in IQ, with the adjusted correlation coefiicient for performance IQ for PFUnDA -1.6 (95% CI -0.2, -3.0) at age 5, and the PFNA coefficient for visual IQ at age 8 reaching statistical significance (-2.1; -0.2, -3.9). Most of the PFAS were also showing nonsignificant deficits in full-scale, performance and visual IQs at age 8.*"

Priestly reported about the study by Braun et al. (2014): "*Once again illustrating the difficulty of determining a relationship between PFAS and neurobehavioural effects when exposure involve multiple POPs, Braun et al. (2014) noted that there appeared to be an association between increasing serum PFOA levels and fewer autistic behaviours in a US cohort of children (the Health Outcome & Measures of the Environment – or HOME – study). The same study showed some weak positive, negative and no associations with other POPs and endocrine disrupting chemicals for increased autistic behaviour, but the authors conceded that the sample size may have been too small to interpret the findings.*"

Priestly reported on Oulhote et al. (2016b), describing the methodology and findings: "*In the Faroe Islands children cohort, measurements of PFOA, PFNA, PFDA, PFOS and PFHxS in maternal serum and in children at ages 5 and 7 were compared to scores on a strength and difficulties questionnaire (SDQ). PFOS and PFHxS levels declined over time, while PFOA, PFNA and PFDA tended to increase. There were no associations between maternal PFAS and SDQ scores, but a twofold increase in PFOA, PFNA and PFDA were associated with small, but significant increases in SDQ scores at age 5, mainly relating to hyperactivity, peer relationships and conduct problems. At age 7, girls had consistently positive associations between PFAS and PFAS, but boys did not.*"

Priestly reported on Vuong et al. (2016), noting that the authors: "*studied the effects of PFAS and PBDEs on 'executive function' in children. Executive function encompasses higher order neurocognitive processes, including cognitive flexibility, goal planning, and information processing. Deficits in executive functioning can hinder an individual's ability to formulate goals, effectively perform, and focus their behaviour. Maternal serum PBDEs and PFAS were measured prospectively at 16 ± 3 weeks gestation in 256 mother-child pairs in the HOME Study (Cincinatti 2003-06) and correlated with parent-rated assessment of the Behaviour Rating Inventory of Executive Function (BRIEF) in their children at ages 5 and 8 years. Regression analysis of BRIEF scores for each ln unit increase in PFOS level showed poorer behaviour regulation, metacognition and global executive function. No association was found with PFOA levels. Higher scores, indicating impairment of executive function were also associated with blood levels of BDE-153, a fire retardant.*"

For the study by Liew et al. (2015), Priestly noted that: "*In contrast, a nested case-control study of children from the Danish National Birth Cohort failed to find any relationship between serum PFAS and ADHD. The estimated RR values per ln ng/mL increase in PFAS all had 95% CI estimates encompassing*

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1.0 for both ADHD [PFOA 0.87, 0.74-1.02; PFOS 0.98 0.82-1.16] and autism [PFOA 0.98, 0.73 – 1.31; PFOS 0.92 0.69-1.22]. There were both positive and negative associations between higher PFASS quartiles and ADHD in models that simultaneously adjusted for all PFAS, but the estimates were not considered to be precise (Liew et al. 2015)."

Of the study by Polanska et al. (2012), Priestly reported that: "*In a review of possible environmental and lifestyle factors influencing the development of ADHD, Polanska et al. (2012) noted that there were only two studies that specifically address ADHD associated with PFAS (Hofmann et al. 2010 and Fei et al. 2008b; they did not cite the Fei et al. 2011 paper), and that both had different outcomes. They commented that the differences could have been due to the ages of the cohorts studied, but they agreed the matter needs further study.*"

Rappazzo et al. (2017)

Studies reviewed

Rappazzo et al. (2017) reviewed 19 studies on neurodevelopment and attention in children (Fei et al. 2008b; Fei and Olsen, 2011; Donauer et al. 2015; Chen et al. 2013; Forns et al. 2015; Goudarzi et al. 2016c; Stein et al. 2013; Wang et al. 2015; Stein and Savitz 2013; Vuong et al. 2016; Strøm et al. 2014; Gump et al. 2011; Hoffman et al. 2010; Høyer et al. 2015a; Liew et al. 2015; Lien et al. 2016; Quaak et al. 2016; Ode et al. 2014; Stein et al. 2014a; Bellinger et al. 2013; Roth and Wilks, 2014):

- twelve studies that investigated childhood developmental milestones or neurodevelopment (Fei et al. 2008b; Fei and Olsen, 2011; Donauer et al. 2015; Chen et al. 2013; Forns et al. 2015; Goudarzi et al. 2016c; Stein et al. 2013; Wang et al. 2015; Stein and Savitz 2013; Vuong et al. 2016; Strøm et al. 2014; Gump et al. 2011);
- nine studies on ADHD or related indicators of impulsivity (Gump et al. 2011; Stein and Savitz, 2013; Hoyet et al. 2015; Liew et al. 2015; Lien et al. 2016; Quaak et al. 2016; Ode et al. 2014; Strøm et al. 2014; Stein et al. 2014a).

The papers by Bellinger et al. (2013) and Roth and Wilks, (2014) were reviews on prenatal exposures to environmental chemicals in association with children's neurodevelopment.

Considerations and conclusions

Rappazzo et al. (2017), in the 'Abstract', did not make any conclusions about the studies on neurodevelopment in children they reviewed.

In the 'Neurodevelopment and attention' section, Rappazzo et al. made two overall statements about the literature they reviewed: "*Studies that examined childhood developmental milestones or neurodevelopment report primarily null results, though some observed positive associations*" and "*Nine studies examined either ADHD or related indicators of impulsivity; in general, results for these studies are mixed.*"

At the end of the section on 'Neurodevelopment and attention', the authors commented on the studies overall, stating that: "*Effects for observed neurological outcomes across studies are inconsistent; while some studies observe positive associations for both ADHD and neurodevelopment, there are also several studies that observe negative and null associations. Recent reviews on prenatal exposures to environmental chemicals in association with children's neurodevelopment report similar findings [Bellinger et al. 2013; Roth et al. 2014]... Despite the evidence from animal literature, the mixed nature of findings in humans precludes firm conclusions for the effects of PFAS on neurological outcomes.*"

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Summary of studies reviewed

The studies reviewed by Rappazzo et al. that have not been reviewed and reported on previously in this section are included below. Rappazzo et al. reported the following for these studies:

- Lien et al. (2016): "*In a cohort created by combining data from the Taiwan birth panel study and the Taiwan early-life cohort, Lien, et al. found cord blood PFNA concentrations to be associated with inattention, impulsivity/hyperactivity, and oppositional defiant disorder as measured by the Swanson, Nolan, and Pelham IV scale but not neurobehavioral symptoms measured by the Child Behavior Checklist (CBC) or the Strengths and Difficulties Questionnaire. They also found only null associations between PFOA, PFOS, and PFNA and neurobehavioral symptoms of ADHD.*"
- Quaak et al. (2016): "*Quaak, et al. [2016], using the Dutch Linking Maternal Nutrition to Child Health cohort (PFAS measured in cord blood), found higher PFOA tertiles associated with potential decreases in externalizing behavior using the CBC, but no associations with the ADHD scale, and no associations with PFOS concentrations; however, they did observe potential negative associations between summed PFAS and both externalizing behavior and ADHD. Other studies have also observed null or negative associations.*"
- Ode et al. (2014): "*Ode, et al. reported null ORs between cord serum PFAS and ADHD in a case-control study of Swedish children.*"
- Stein et al. (2014a): "*A study of C8 children examined serum PFOA with mother and teacher reports of executive function, ADHD like behavior, and behavioral problems using standardized score metrics. This study found that associations depended on who was reporting and that associations differed by child's sex. If mothers were reporting, boys had lower scores (indicated fewer behavioral issues) and girls had higher scores with doubling of PFOA concentrations. If teachers were reporting, boys still had lower scores with increasing PFOA, but no associations were observed in girls.*"

Kirk et al. (2018)

Studies reviewed

Kirk et al. (2018) evaluated 23 papers investigating the effect of PFAS exposure on neurodevelopmental and neurophysiological outcomes in children and adults. The authors report: "*The majority of studies centred on prenatal exposure to PFOA, PFOS, PFHxS and PFNA but the main health outcome discussed was childhood neurodevelopment.*"

The papers reviewed included the following papers on neurodevelopmental outcomes[64]:

- five studies on the association between PFAS exposure levels and neurodevelopmental outcomes in infants < 5 years old (Chen et al. 2013; Donauer et al. 2015; Forns et al. 2015; Goudarzi et al. 2016c; Fei et al. 2008b);
- four papers on the association between PFAS exposure levels and neurodevelopmental outcomes in childhood (≥ 5 years old) (Gump et al. 2011; Høyer et al. 2015a; Stein et al. 2013; Vuong et al. 2016);
- seven studies that investigated the association between PFAS exposure and the development of ADHD during childhood (Hoffman et al. 2010; Lien et al. 2016; Liew et al. 2015; Ode et al. 2014; Stein and Savitz, 2011; Stein et al. 2013; Strøm et al. 2014);

[64] Note that papers may have been reviewed in more than one subsection.

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- three studies that investigated the relationship between prenatal exposure to PFAS and autism in children (Liew et al. 2015; Braun et al. 2014; Oulhote et al. 2016b);
- three studies that investigated the effect of PFAS exposure on behavioural problems in children (Fei and Olsen 2011, Høyer et al. 2015a; Oulhote et al. 2016b);
- one study on childhood exposure to PFAS and learning problems in children aged between 5-18 years old (Stein and Savitz, 2011).

Kirk et al. also reviewed papers on neurophysiological outcomes:

- two studies that investigated the association between PFAS exposure and depression (Berk et al. 2014; Strøm et al. 2014);
- two studies on memory impairment in adults (Gallo et al. 2013; Power et al. 2013);
- One study on sleep effects (Shiue, 2016).

Of the papers on neurodevelopmental outcomes, only one paper has not been covered under previous reviews (Stein and Savitz 2011). Three of the papers on neurophysiological outcomes have been covered under previous reviews (Strøm et al. 2014; Gallo et al. 2013; Power et al. 2013).

Considerations and conclusions

Kirk et al. did not make specific any statements or conclusions about neurodevelopmental or neurophysiological outcomes in the 'Executive Summary'.

In the 'Neurodevelopment and neurophysiological effects' section, Kirk et al. made several overall statements about the literature they reviewed. These statements are included in the relevant sections below under 'Summaries of studies reviewed'.

The tables below are reproduced from Kirk et al. They show the reported associations determined by Kirk et al. for the various neurodevelopmental and neurophysiological outcomes, by PFAS.

Neurodevelopment:

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

From Kirk et al. (2018). Pp. 104

Neurophysical:

Associations at a glance

Health outcome	PFAS exposure	Evaluation of evidence

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Health outcome	PFAS exposure	Evaluation of evidence
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

From Kirk et al.(2018) p. 109.

Summaries of studies reviewed

Neurodevelopmental outcomes in infants < 5 years old

Kirk et al. reviewed five studies on the association between PFAS exposure levels and neurodevelopmental outcomes in infants < 5 years old, all of which have been discussed in previous reviews. Kirk et al. stated of the literature that: "*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.*", and concluded for PFOA that: "*...there is inconsistent evidence regarding a negative association between PFOA exposure levels and neurodevelopmental outcomes in infants.*" For PFOS, Kirk et al. concluded that: "*The evaluated literature does not suggest a decrease in infant neurodevelopment related to elevated PFOS levels.*"

Childhood (≥ 5 years old) neurodevelopment

For childhood (≥ 5 years old) neurodevelopment, Kirk et al. reviewed four papers, all of which have been discussed in previous reviews. Kirk et al. concluded of these four papers: "*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.*" At the end of the section on childhood neurodevelopment, Kirk et al. stated that: "*While these four studies report differences in the effects of PFAS on neurodevelopment in children, Gump et al. [2011] and Vuong et al. [2016] 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.*"

Development of ADHD during childhood

Kirk et al. reviewed seven studies that investigated the association between PFAS exposure and the development of ADHD during childhood. Of these, only Stein and Savitz (2011) has not been discussed in previous reviews. Kirk et al. noted about this study: "*In a study of 10,546 children aged 5 to 18-years old enrolled in the C8 Health Project, Stein & Savitz, [2011] found a significant association*

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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 [2011] reported non-significant results related to PFOA, PFOS and PFNA exposures."

In summarising the studies on ADHD, Kirk et al. concluded: "*Overall, the studies present inconsistent evidence to support an increased risk of ADHD related to PFAS levels, with both significant and non-significant results stated for PFOA, PFOS, PFHxS and PFNA exposures."*

Prenatal exposure to PFAS and autism in children

For autism, Kirk et al. reviewed three studies that investigated the relationship between prenatal exposure to PFAS and autism in children. Two of them have been discussed under previous reviews (Liew et al. 2015; and Oulhote et al. 2016b). Of the study undertaken by Braun et al. 2014; Kirk et al. provided the following information: "*In a cohort study of 175 mother-child pairs from the Health Outcomes and Measures of the Environment (HOME) study, Braun et al. [2014] 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 summarising the studies on autism, Kirk et al. commented: "*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 in children

Of the three studies (Fei and Olsen 2011; Høyer et al.2015a; Oulhote et al. 2016b) reviewed by Kirk et al. that investigated the effect of PFAS exposure on behavioural problems in children, all have been discussed in previous reviews. In summarising the studies, Kirk et al. commented that: "*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. [2016b]. 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. [2016b] 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."*

Childhood exposure to PFAS and learning problems in children

Of the one study on childhood exposure to PFAS and learning problems in children aged between 5-18 years old (this has been covered under previous reviews), Kirk et al. reported the study of Stein and Savitz (2011) and found no association related to exposure to PFOA, PFOS and PFNA. Kirk et al. stated: "*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."*

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NEUROPHYSIOLOGICAL OUTCOMES:

Association between PFAS exposure and depression

Kirk et al. evaluated two studies that investigated the association between PFAS exposure and depression. Of these, Berk et al. (2014) has not been discussed under previous reviews. Kirk et al. noted that the study: "*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 summarising the results from the two studies, Kirk et al. concluded that: "*The results reported in the studies are conflicting; however, the studies differed significantly in their design and outcome measurement.*"

Memory impairment in adults

Of the two studies Kirk et al. reviewed on PFAS exposure and memory impairment in adults, Kirk et al. noted: "*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

The one study on sleep effects reviewed by Kirk et al. has not been discussed under previous sections (Shiue, 2016). Shiue et al. (2016) 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. Kirk et al. reported that: "*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 [2016] 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 [2016] found no significant associations related to PFOA, PFOS, PFHxS, PFNA, PFDA, PFUdA, PFHpA and PFOSA.*"

Kirk et al. concluded: "*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.*"

6.9.4. Summary of considerations and conclusions from key national and international reports and systematic reviews

Recent key national and international reports:

- The ATSDR advised that the studies on PFAS and risk of ADHD are conflicting, preclude a weight of evidence determination, with the available studies not establishing causality.
- The US EPA concluded that the epidemiology studies did not find associations between PFOA and neurodevelopmental effects.

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Systematic reviews:

- Saikat et al. made no specific conclusion about the one study on PFOS and ADHD they reviewed.
- Roth and Wilks concluded the epidemiological evidence does not support a strong causal association between PFCs and adverse neurodevelopmental and neurobehavioural outcomes in infants and children, but further investigation is required. Additionally, the studies consistently showed no association between PFOA and developmental or behavioural effects assessed in infants and children.
- Priestly concluded the lack of consistency in studies of PFAS exposure with some forms of neurobehavioural development make it difficult to attribute the possible associations as causally related.
- Rappazzo et al. concluded the evidence for PFAS and neurodevelopment and attention outcomes in children is inconsistent and precludes firm conclusions.
- Kirk et al. concluded the evidence:
  - is inconsistent for PFOA and neurodevelopmental outcomes in infants, and for PFAS and autism and ADHD in children;
  - does not support a decrease in infant neurodevelopment related to PFOS levels;
  - is conflicting for PFAS exposure and depression in adults.

6.9.5. Expert Health Panel synthesis to support advice to the Minister

- The area of neurodevelopment is difficult to study. There are no biomarkers (as for cholesterol). There is inconsistency in definitions and diagnostic criteria for conditions such as autism and ADHD.
- Some studies had insufficient participants, making it difficult to draw statistically valid conclusions; others relied on parental report of behaviour and diagnosis.
- There is no established causal mechanism for PFAS to have an effect on neurodevelopment.

6.9.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and neurodevelopmental and neurophysiological effects:

- An association with PFAS and neurodevelopmental and neurobehavioural outcomes in infants and children is not consistently observed.
- There are many other significant influences on infant and child development including maternal alcohol, drug and medication intake, maternal smoking, socioeconomic status, parental education level, and heavy metal exposure e.g. lead.
- Four respondents identified autism (3) and ADHD (1) as a health concern.

To further investigate the association between PFAS exposure and neurodevelopmental and neurophysiological effects in an Australian setting, the Panel suggests the following research priorities:

- Studies that look for causal evidence are the key research need. Further, cross-sectional studies are unlikely to provide useful information. Well-designed longitudinal studies which take account of confounders (alcohol, drug and medication intake, smoking, socioeconomic status, parental education level, heavy metals including lead) may provide

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stronger epidemiological evidence that might indicate whether PFAS affects neurological development.

- Any measurement of neurodevelopment should be undertaken by trained examiners using a validated assessment instrument. Such studies are expensive, and thus this means the best value for money would be to add PFAS blood sampling to other prospective birth cohort/neurodevelopment studies that are being undertaken or planned.

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6.10. Diabetes, glycaemic control and metabolic syndromes and PFAS exposure

The World Health Organization defines diabetes as: "*a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The most common is type 2 diabetes, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin. Type 1 diabetes, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin by itself.*" Several of the key international reports and systematic reviews considered the human evidence on exposure to PFAS and diabetes, glycaemic control and metabolic syndromes.

6.10.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of four published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and four systematic reviews since 2013 that analysed the human epidemiological evidence regarding exposure to PFAS and diabetes:

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **United States Environmental Protection Agency (US EPA 2016a).** Health effects support document for Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA 2016b).** Health effects support document for Perfluorooctane Sulphonate (PFOS);
- **Food Standards Australia New Zealand (FSANZ 2017).** Hazard Assessment report (PFOS, PFOA, PFHxS).

Systematic reviews

- **Saikat et al. (2013).** The impact of PFOS on health in the general population: a review;
- **Priestly (2016).** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS). Monash University;
- **Rappazzo et al. (2017).** Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature;
- **Kirk et al. (2018).** The PFAS Health Study: systematic literature review. Australian National University.

No other systematic reviews or key national and international reports covered diabetes, glycaemic control or metabolic syndrome.

6.10.2. Key national and international reports

Agency for Toxic Substances and Disease Registry (ATSDR, 2015).

The ATSDR, in its draft toxicological profile for perfluoroalkyls, considered the human evidence for diabetes, including type I diabetes, type II diabetes and glucose homeostasis and deaths from diabetes.

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Studies reviewed

The ATSDR reviewed ten epidemiological studies that have reported an association between PFAS exposure and diabetes in human populations (MacNeil et al. 2009; Melzer et al. 2010; Lin et al. 2009; Nelson et al. 2010; Fisher et al. 2013; Leonard et al. 2008; Leonard 2006; Lundin et al. 2009; Steenland and Woskie 2012; Steenland et al. 2013), including:

- one study on type-I diabetes (Steenland et al. 2013);
- five studies on type II diabetes and glucose homeostasis (MacNeil et al. 2009; Melzer et al. 2010; Lin et al. 2009; Nelson et al. 2010; Fisher et al. 2013);
- four studies that investigated deaths from diabetes in occupationally exposed workers (Leonard et al. 2008; Leonard 2006; Steenland and Woskie, 2012; Lundin et al. 2009).

Considerations and conclusions

The ATSDR did not make any statements or conclusions on the human evidence regarding diabetes in either the 'Public health statement for perfluoroalkyls' or 'Relevance to public health' sections of the report.

Summaries and findings of studies reviewed

Type I diabetes

Under 'Immunological and lymphoreticular effects', the ATSDR reviewed the study by Steenland et al. (2013), and reported: "*The possible association between elevated serum PFOA levels and the occurrence of autoimmune diseases were examined in a cohort of 28,541 adults living or working in a community with elevated PFOA levels in the water (C8 Health Project participants) and 3,713 past and current workers at a nearby DuPont facility.....No other significant associations between serum PFOA levels and autoimmune disease (e.g., ..type-1 diabetes) were found.*"

Type II diabetes and glucose homeostasis

Under 'Health effects – endocrine effects', the ATSDR reported the following summaries for five studies (MacNeil et al. 2009; Melzer et al. 2010; Lin et al. 2009; Nelson et al. 2010; Fisher et al. 2013).

Of the study by MacNeil et al. (2009), the ATSDR reported that: "*No significant associations between serum PFOA levels and type II diabetes (self-reported and validated with medical records) were found in residents living near the Washington Works facility and participating in the C8 Health Project. Additionally, there was no exposure-response relationship between serum PFOA levels and fasting serum glucose levels.*"

The ATSDR reported that three studies utilised NHANES data to evaluate the possible association between diabetes or glucose homeostasis and serum perfluoroalkyl levels (Melzer et al. 2010; Lin et al. 2009; Nelson et al. 2010).

Of the study by Melzer et al. (2010) the ATSDR reported the findings as: "*Melzer et al. (2010) did not find a significant association between self-reported diabetes and serum PFOA or PFOS levels in adult men and women.*"

The study by Lin et al. (2009) was reported by the ATSDR to have found: "*In adolescents (12–20 years of age), no significant associations between serum PFHxS, PFOA, or PFOS and blood glucose or insulin levels insulin resistance status (measured via homeostasis model assessment of insulin resistance), or β-cell function were found. A negative association between β-cell function and serum PFNA levels was found with adjustments for age, sex, race, smoking status, alcohol intake, household income, waist circumference, C-reactive protein levels and medication use; PFNA was not significantly associated with*

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glucose or insulin levels, or insulin resistance status. In contrast, significant positive associations were found in adults (>20 years of age) between serum PFOA and insulin levels and β-cell function and serum PFOS and insulin levels, insulin resistance status, and β-cell function. No significant associations were found between these markers and serum PFNA or PFHxS levels in the adults."

The third study the ATSDR reviewed that used NHANES data (Nelson et al. 2010) was reported to have: "*also evaluated insulin resistance and found inconsistent results. Statistically significant (P<0.05) negative exposure -related trend was observed for PFHxS levels and insulin resistance in adolescent females (12-19 years of age) and a positive trend was observed for PFNA in females aged 20-59 years."*

Of a study of adults in Canada by Fisher et al. (2013), the ATSDR reported the study: "*did not find significant associations between serum PFOA, PFOS or PFHxS levels and plasma insulin or glucose levels."*

The ATSDR made the following observation about the above five studies: "*Overall, the studies in a highly exposed population and in the general population do not suggest an association between perfluoroalkyl exposure and alterations in glucose homeostasis or increased risk of diabetes. Although some significant associations have been found, they are not consistent across studies of similar populations."*

Deaths from diabetes in occupationally exposed workers

The ATSDR also cited four studies that had investigated deaths from diabetes among occupationally exposed workers (Leonard et al. 2008; Leonard 2006; Steenland and Woskie, 2012; Lundin et al. 2009).

Of the study by Leonard et al. (2008) the ATSDR reported: "*In the cohort mortality study by Leonard et al. (2008; Leonard 2006) of workers at the DuPont Washington Works facility in West Virginia exposed to APFO [Ammonium Perfluorooctanoate], a significant increase in deaths from diabetes (SMR 197, 95% CI 123–298) was found, as compared to workers at other DuPont facilities in the region."*

The Steenland and Woskie (2012) study was, according to the ATSDR, an update of the Leonard et al. (2008) study and: "*also found a significant increase in diabetes deaths (SMR 1.90, 95% CI 1.35–2.61) when compared to other regional DuPont employees, but not when compared to the U.S. population. However, when the workers were categorized by estimated cumulative exposure levels, the exposure-response trend was not statistically significant."*

The ATSDR reported the study by Lundin et al. (2009): "*also found an increase in deaths from diabetes in workers exposed to APFO at the 3M Cottage Grove facility in Minnesota, as compared to Minnesota death rates. The increase was only found in workers with probable exposure to APFO, but not with definite exposure (n=168); no deaths from diabetes were observed in the workers (n=513) with definite exposure to APFO."*

The ATSDR made the following observation: "*As noted by Steenland and Woskie (2012), diabetes mortality may not be a good surrogate for the underlying diabetes incidence data."*

United States Environmental Protection Agency (2016a, b)

In 2016, USEPA reviewed epidemiological studies on diabetes in the 'Health effects support documents' on PFOA and PFOS.

Studies reviewed

In the 'Health effects support document for PFOA', the US EPA reviewed:

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- three occupational exposure studies (Leonard et al. 2008; Steenland and Woskie, 2012; Steenland et al. 2015);
- one high-exposure community study (MacNeil et al. 2009);
- three general population studies (Zhang et al. 2015; Lin et al. 2009; Nelson et al. 2010), including studies on gestational diabetes and metabolic syndrome.

In the 'Health effects support document for PFOS', the US EPA reviewed the two general population studies by Zhang et al. (2015) and Lin et al. (2009).

Considerations and conclusions

The US EPA stated in the 'Executive Summary' of the 'Health effects support document for PFOA': "*The epidemiology studies did not find associations between PFOA and diabetes.*"

In the 'Summary and conclusions' from the 'Human epidemiology studies' section, the US EPA stated for PFOA and diabetes: "*No associations were observed between serum PFOA levels and type II diabetes incidence rate in general or worker populations with mean serum PFOA up to 91.3–113 ng/mL (MacNeil et al. 2009; Steenland et al. 2015). PFOA was not associated with measures of metabolic syndrome in adolescents or adults (Lin et al. 2009). However, one study found an increased risk for developing gestational diabetes in females with mean serum PFOA (measured preconception) of 3.94 ng/mL (Zhang et al. 2015).*"

For PFOS, the US EPA did not make any statement about diabetes in the 'Executive Summary'.

Summaries and findings of studies

PFOA

In the section 'Diabetes and related end points', the US EPA in its 'Health effect support document for PFOA' cited three occupational exposure studies (Leonard et al. 2008; Steenland and Woskie, 2012; Steenland et al. 2015), one high-exposure community study (MacNeil et al. 2009), and three general population studies (Zhang et al. 2015; Lin et al. 2009; Nelson et al. 2010), including studies on gestational diabetes and metabolic syndrome.

Deaths from diabetes in occupationally exposed workers- PFOA

Under the 'Occupational exposure studies' section, the US EPA provided summaries of the studies by Leonard et al. (2008), Steenland and Woskie (2012) and Steenland et al. (2015).

Of the study by Steenland and Woskie (2012), the US EPA provided different details to the ATSDR, including: "*Overall, the mean cumulative exposure was 7.8 ppm-years and the estimated average annual serum level was 350 ng/mL. Compared to the referent rates from other DuPont workers, cause-specific mortality rates were elevated for diabetes (n = 38; SMR=1.90; 95% CI 1.35, 2.61). These data are limited by the small number of cases and the restriction to mortality as an outcome.*"

The US EPA reviewed the follow-up study of this occupational cohort by Steenland et al. (2015); this study was not reviewed by ATSDR. The US EPA reported of this study: "*The most recent report on the above cohort included 6,026 workers evaluated for disease incidence, not just mortality (Steenland et al. 2015). Lifetime serum cumulative dose was estimated by combining occupational and nonoccupational exposures. Median measured serum level was 113 ng/mL based on samples collected in 2005. No association was found between PFOA level and type II diabetes incidence rate.*"

Type II diabetes-PFOA

Of the high-exposure community study by MacNeil et al. (2009), the US EPA provided more detail about the study and findings (in addition to the details provided by the ATSDR, above), including:

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"The mean serum PFOA concentration for the entire study population was 86.8 ng/mL and 91.3 ng/mL for subjects with type II diabetes validated by medical review (n = 3,539). There was no association between serum PFOA concentration and fasting serum glucose level in subjects characterized as nondiabetic. The mean serum PFOA concentration was 92.9 ng/mL in subjects who self-reported type II diabetes (n = 4,278) and 122.7 ng/mL in subjects diagnosed in the last 10 years (n = 1,055). No association was observed between type II diabetes and serum PFOA concentration. The OR by decile was 1.00, 0.71, 0.60, 0.72, 0.65, 0.65, 0.87, 0.58, 0.62, and 0.72."

The US EPA made the following observation about this study: "*The results of the analysis indicated that PFOA exposure is not associated with type II diabetes among the population studied. Data interpretation was limited by the cross-sectional study design, which made it difficult to determine if PFOA exposure preceded disease.*"

Also under the high-exposure community studies, the US EPA made the comment: "*The C8 Science Panel (2012) combined these data from the C8 general population cohort with follow-up data and data from worker cohorts, and concluded that there is no probable link between PFOA and type II diabetes.*" Under General Population Studies the US EPA concluded "*Overall, these studies show a lack of association of PFOA with diabetes, metabolic syndrome, and related end points.*"

The US EPA reviewed three general population studies for PFOA (Zhang et al. 2015; Lin et al. 2009; Nelson et al. 2010).

Gestational diabetes – PFOA

Of the study by Zhang et al. (2015), in which preconception serum levels of PFOA, PFOS (and other PFASs) were evaluated in females attempting pregnancy in relation to risk of developing gestational diabetes, the US EPA reported: "*The 258 participants were members of the Longitudinal Investigation of Fertility and the Environment (LIFE) study with blood samples taken during 2005–2009. The ORs and 95% CIs of gestational diabetes associated with each SD increment of preconception serum PFOA concentration (log-transformed) (and six other PFASs) were estimated with the use of logistic regression after adjusting for confounders. Preconception mean serum PFOA levels were 3.3 ng/mL for the entire cohort, 3.94 ng/mL in females with gestational diabetes and 3.07 ng/mL in females without gestational diabetes. A significant positive association was found between PFOA and risk of gestational diabetes in the fully adjusted model (OR=1.86; 95% CI 1.14, 3.02). Associations for six other PFAS were slightly increased (e.g., PFOS OR=1.13), but did not attain statistical significance.*"

Metabolic syndrome[65] *– PFOA*

The US EPA reviewed the study by Lin et al. (2009) and provided information about this study, in addition to that provided by the ATSDR, above. The US EPA noted the study investigated the association between serum PFOA (plus three other PFASs) and glucose homeostasis and metabolic syndrome in adolescents (aged 12-20 years) and adults (aged > 20 years) by analysing the 1999-2000 and 2003-2004 NHANES data. The US EPA reported the findings for PFOA as: "*Serum PFOA concentration was not associated with metabolic syndrome, metabolic syndrome waist circumference, glucose concentration, homeostasis model of insulin resistance, or insulin levels in adults or adolescents. Both PFOS and PFNA were positively associated with some of the end points associated with metabolic syndrome.*"

The US EPA also reviewed the study by Nelson et al. (2010), which is mentioned above under ATSDR, and reported additional information: "*Nelson et al. (2010) examined the relationship between*

[65] Metabolic syndrome is a combination of medical disorders and risk factors that increase the risk of developing cardiovascular disease and diabetes. (Source: US-EPA Health effects support document 2016a)

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polyfluoroalkyl chemical serum concentration, including PFOA, and insulin resistance as previously described for data from NHANES. Fasting insulin and fasting glucose were used to determine the homeostatic model assessment for insulin resistance. No association was found between serum PFOA concentration, or any other PFAS, and insulin resistance."

At the end of the section 'Diabetes and related end points' in the 'Health effects support document for PFOA' (US EPA 2016a), the US EPA made the following statement: *"Overall, these studies show a lack of association of PFOA with diabetes, metabolic syndrome, and related end points."*

PFOS

Gestational diabetes – PFOS

The US EPA reviewed the study by Zhang et al. (2015) (study details reported above under PFOA) and reported the findings, including: *"Preconception mean serum PFOS levels were 13.1 ng/mL in females with gestational diabetes and 12 ng/mL in females without gestational diabetes (p-value for mean difference = 0.10). A positive association was found between PFOS and risk of gestational diabetes in the fully adjusted model (OR = 1.13; 95% CI: 0.75–1.72). PFOA was the only PFAS that was significantly associated with developing gestational diabetes in this analysis."*

Metabolic syndrome[66] – PFOS

The US EPA reported the findings of the study by Lin et al. (2009) (study details above under PFOA) as: *"Log-transformed PFOS concentration was 3.11 ng/mL and 3.19 ng/mL for adolescents and adults, respectively. In adults, serum PFOS concentration was associated with increased β-cell function (β coefficient 0.15, p < 0.01). Serum PFOS concentration was not associated with metabolic syndrome, glucose concentration, homeostasis model of insulin resistance, or insulin levels in adults or adolescents."*

Food Standards Australia New Zealand (FSANZ)

In 2017, FSANZ made a number of statements about the evidence on PFAS and diabetes in the 'Hazard assessment report for PFOA, PFOS and PFHxS'.

Considerations and conclusions

For PFOA, under 'Endocrine effects', FSANZ stated: *"Evidence for increased risk of diabetes mellitus as a result of exposure to PFOA is equivocal (based on data from Lin et al. 2009 and MacNeil et al. 2009, presented in Bull et al. 2014)."* FSANZ also reported in the 'Other effects' section on PFOA, *"No consistent associations have been reported between serum PFOA levels and... indicators of metabolic syndrome..."*

Regarding PFHxS and diabetes, FSANZ noted that from the ATSDR and other more recent data: *"Most studies have found no association between serum PFHxS and evidence of diabetes, although one study found a negative association between PFHxS levels and insulin resistance in adolescent females."* With respect to gestational diabetes, FSANZ reported on the US EPA report: *"A small set of studies reported associations with gestational diabetes, pre-eclampsia and pregnancy induced hypertension. These outcomes were also associated with increased serum PFOA levels (reviewed by US EPA 2016)."*

[66] Metabolic syndrome is a combination of medical disorders and risk factors that increase the risk of developing cardiovascular disease and diabetes. (Source: US-EPA Health effects support document 2016a)

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6.10.3. Systematic reviews

Saikat et al. (2013)

In 2013 Saikat et al. reviewed the literature on the impact of PFOS on health in the general population.

Studies reviewed

Saikat et al. reviewed two studies (Nelson et al. 2009; Lin et al. 2009). Both studies were reviewed by the ATSDR and US EPA, with details provided in those sections, above.

Considerations and conclusions

The authors stated in the 'Coherence with evidence' section: "*There were two studies in this review that looked at glucose metabolism but only one [Lin et al. 2009] demonstrated some significant associations, observed only in adults.*"

Priestly (2016)

In 2016 Priestly considered, in the section 'Miscellaneous end points', four studies published between 2014 and 2016 that investigated PFAS exposure and diabetes, glycaemic control and insulin resistance.

Studies reviewed

The studies Priestly reviewed were Timmermann et al. (2014), Kim et al. (2015), Shapiro et al. (2016), and Su et al. (2016). None of these studies were reviewed by the ATSDR or US EPA.

Considerations and conclusions

Priestly did not make any specific comment or conclusions about these four studies, only an overall comment about all of the studies he reviewed in the 'Miscellaneous end point' section: "*With only one or two studies addressing each end point, and inconsistent findings, it is too early to draw definitive conclusions on whether PFAS have a role in any of the diseases discussed in this section. Many of the studies point out the difficulty of discerning between PFAS causation, or reverse causation, where the condition under study results in a tendency to accumulate higher plasma levels.*"

Summaries and findings of studies reviewed

Type II diabetes and glucose homeostasis

Of the paper by Timmermann et al. (2014), Priestly reported that the study of glycaemic control in children aged 8-10 years from the Danish component of the European Youth Heart Study found that: "*there was no association between PFAS and adiposity and glycaemic control factors in children with normal weight. However, in over weight children, an increase in serum PFOS of 10 ng/mL was associated with a 16.2% (95% CI 5.2-28.2%) higher insulin concentration, 17.6% (CI 1.2-16.5%)*[67] *increase in insulin resistance, and 8.6% (CI 1.2-16.5%) higher triglycerides. For PFOA, the effects were more marked. A 10 ng/mL rise was associated with 71.6% (CI 2.4-187.5[%]) higher insulin, 67.5% (CI 5.5-166%) higher β-cell function, 73.9% (CI 0.2-202%) higher insulin resistance and 76.2% (CI 22.8-153%) higher triglycerides.*" Priestly noted "*the authors did not rule out reverse causation and suggested that the findings needed confirmation in longitudinal studies.*"

[67] Note that this is correct verbatim from Priestly (2016), however the Panel believes it to be an error. The Abstract by Timmermann et al. 2014 published on 'PubMed' shows the 95% CI to be 5.8%-30.8%.

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The study by Kim et al. (2015) investigated insulin resistance in an elderly Korean population. Priestly reported the study: "*also found an association between serum PFAS (PFOS and PFDoA; but NOT three out of ten other perfluoroalkyl sulphonates and acids).*" Priestly also reported: "*These effects disappeared after treatment with Vitamin C, and the authors speculated that the effects may have been associated with PFAS-induced oxidative stress.*"

In Shapiro et al.'s 2016 study on glycemic control in pregnant Canadian women (MIREC Study), Priestly noted that: "*an increased OR [Odds Ratio] for impaired glucose tolerance associated with gestational diabetes mellitus (GDM) was found in the second quartile of PFHxS 2nd trimester serum levels, but with no clear dose-response and with no other PFAS.*"

Priestly noted that "*similarly inconsistent results*" were found by Su et al. (2016) in their study of glucose tolerance and diabetes in an adult Taiwanese population study. Priestly noted the findings as: "*PFOS appeared to have a weak effect, with increases in various glycaemic control measures increasing by 3-8% with a doubling of PFOS serum levels: The effects of PFOA, PFNA and PFUA were, if anything, in the opposite direction.*"

Rappazzo et al. (2017)

Rappazzo et al., in their systematic review of the epidemiologic literature on exposure to perfluorinated alkyl substances and health outcomes in children, reviewed four studies on glucose regulation in children, under the 'Cardiometabolic' section.

Studies reviewed

Rappazzo et al. reviewed four studies (Lin et al. 2009; Lin et al. 2011; Timmermann et al. 2014; Halldorsson et al. 2012).

Considerations and conclusions

Rappazzo et al. stated of the studies they reviewed: "*Studies of glucose regulation in children have generally reported mixed effects, with limited agreement between studies.*"

Summaries and findings of studies reviewed

The study by Lin et al. (2009) was also reviewed by the ATSDR and US EPA. The study by Timmermann et al. (2014) was reviewed by Priestly (2016).

Glucose homeostasis

Of the study by Halldorsson et al. (2012), Rappazzo reported that: "*In the 20years follow-up of the Danish Pregnancy Cohort, there were positive associations with percent changes of insulin and leptin association and log-unit increases in prenatal serum PFOS.*"

The study by Lin et al. (2011) was reported by Rappazzo et al. as: "*In a small Taiwanese cohort, metabolic indicators (insulin, glucose, etc.) were not associated with increasing serum concentrations of PFOA, PFOS, PFNA, or perfluoroundecanoic acid (PFUA).*"

Rappazzo et al. then referred back to both studies when talking about studies on adiponectin[68], stating of Lin et al. (2011): "*In a cohort of hypertensive young people from Taiwan (aged 12–30), higher PFNA serum concentration was associated with elevated serum adiponectin concentration, while PFOA, PFOS, and PFUA were not. Although in this study only the p-value for trend and mean response values were reported, rather than comparison effect estimates and the population included adults.*"

[68] Adiponectin. A hormone that plays a role in glucose regulation and fatty acid oxidation, and is important for metabolic homeostasis. (Source Rappazzo et al. 2017, p7)

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Of the study by Halldorsson et al. (2012), Rappazzo et al. noted: "*In the Danish Pregnancy Cohort, prenatal PFOA exposure (serum) was negatively associated with adiponectin concentrations.*"

Rappazzo et al. also noted: "*Both the study of the Danish Pregnancy Cohort and a cross-sectional study in Denmark observed no associations between PFOS and adiponectin [Halldosrsson et al. 2012; Timmermann et al. 2014].*"

Kirk et al. (2018)

Kirk et al. (2018) evaluated 11 papers focused on the effect of PFAS exposure on diabetes outcomes in children and adults. The papers' main exposure of interest was PFOA and a range of health outcomes were investigated in the studies, including type I and type II diabetes, gestational diabetes and diabetic mortality. All reviewed papers were determined by Kirk et al. to have a moderate or high risk of bias. Under 'Other metabolic outcomes', Kirk et al. also reviewed five papers on metabolic syndrome; glycaemic control and metabolic function.

Studies reviewed

The 11 papers on 'Diabetes' evaluated by Kirk et al. were Karnes et al. 2014; Leonard et al. 2008; Lin et al. 2011; Lind et al. 2014; Lundin et al. 2009; MacNeil et al. 2009; Predieri et al. 2015; Shapiro et al. 2016; Steenland and Woskie, 2012; Su et al. 2016; Zhang et al. 2015. These included:

- two studies on Type I diabetes (Predieri et al. 2015; Steenland and Woskie, 2012);
- three studies on type II diabetes (Karnes et al. 2014; Lin et al. 2011; MacNeil et al. 2009);
- two studies on gestational diabetes (Shapiro et al. 2016; Zhang et al. 2015);
- five studies on unspecified diabetes including mortality caused by diabetes (Leonard et al. 2008; Lind et al. 2014; Lundin et al. 2009; Steenland and Woskie, 2012; Su et al. 2016).

The five papers on 'Other metabolic outcomes' included:

- two studies on metabolic syndrome (Lin et al. 2009; Fisher et al. 2013);
- two studies on glycaemic control (Timmermann et al. 2014; Lin et al. 2011);
- one study on metabolic function (unspecified) (Fleisch et al. 2016).

Considerations and conclusions

The tables below are the 'Associations at a glance' tables compiled by Kirk et al. following their review of the human epidemiological literature on PFAS exposure and diabetes and other metabolic outcomes.

Associations at a glance (Diabetes)

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

Source: Kirk et al. (2018), page 116.

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Associations at a glance (Other metabolic outcomes)

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

Source: Kirk et al. (2018), page 85.

Summaries and findings of reviewed studies

Type I diabetes

Kirk et al. reviewed two studies on Type I diabetes (Predieri et al. 2015; Steenland and Woskie, 2012). The study by Steenland and Woskie has been summarised previously in this section regarding mortality. Kirk et al. provided additional information: "*Steenland and Woskie [2012] reported no significant relationship between PFOA exposure and the development of type I diabetes in a cohort of exposed workers. This study did not evaluate the effects of exposure to PFOS.*" None of the key international reports or other systematic reviews reviewed the study by Predieri et al. (2015).

Of this study, Kirk et al. reported that: "*Predieri et al. [2015] 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. [2015] stated that there was no difference between serum PFOA measurements in cases and controls.*"

Kirk et al. then commented: "*Predieri et al. [2015] 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

Kirk et al. reviewed three studies on type II diabetes (Karnes et al. 2014; Lin et al. 2011; MacNeil et al. 2009). Summaries of the studies by Lin et al. (2011) and MacNeil et al. (2009) have been provided elsewhere in this section.

Of the study by Karnes et al. (2014), Kirk et al. reported only: "*Karnes et al. [2014] Lin et al. [2011] and MacNeil et al. [2009] all investigated PFAS exposure and type II diabetes and reported no association for PFOA.*"

The authors note the same caveat in relation to results from studies on Type II diabetes: "*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.*"

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Gestational diabetes

Kirk et al. reviewed two studies on gestational diabetes. The study by Zhang et al. (2015) has been covered previously in this section.

Of the study by Shapiro et al. (2016), Kirk et al. reported: "*Shapiro et al. [2016] found no relationship between PFOA, PFNA, PFDA, PFHxS, Et-PFOSA-AcOH and Me-PFOSA-AcOH and gestational diabetes, and Zhang et al. [2015] 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.*"

Of both studies, Kirk et al. made the following observation: "*The use of self-reported measures to determine gestational diabetes by Zhang et al. [2015] and the high percentage of missing data on gestational diabetes in Shapiro et al. [2016] were potential areas of concern relating to study design.*"

Unspecified diabetes, death from diabetes in occupationally exposed workers

Kirk et al. reviewed two studies on unspecified diabetes (Lind et al. 2014; Su et al. 2016). Kirk et al. noted that: "*Lind et al. [2014] and Su et al. [2016] 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.*" Kirk et al. reported the findings as "*Lind et al. [2014] 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. [2016] 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)).*"

They then concluded that: "*Overall, these papers each conclude no association for PFOA exposure and diabetes, and present conflicting results for the association for PFNA and PFOS.*"

Looking closer at the association between PFAS exposure and mortality caused by diabetes, based on occupation cohorts of workers exposed to PFOA in the United States, Kirk et al. noted of the three studies they reviewed (Leonard et al. 2008; Lundin et al. 2009; Steenland and Woskie, 2012) that: "*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.*"

Metabolic syndrome

Kirk et al. also reviewed two papers on metabolic syndrome (Lin et al. 2009; Fisher et al. 2013), and concluded: "*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

Two papers were also reviewed on glycaemic control (Timmerman et al. (2014), Lin et al. (2009)). Both papers have been reviewed previously in this section. Kirk et al. noted: "*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.*"

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Metabolic function

The one paper reviewed (Fleisch et al. 2016) investigated the associations between serum PFAS levels and several metabolic outcomes (unspecified) in a prospective birth cohort in the United States. Kirk et al. stated: "*Fleisch et al. [2016] reported no evidence for adverse effects of prenatal and early-life PFAS exposure on metabolic functions in children.*"

6.10.4. Summary of key national and international reports and systematic reviews

Key national and international reports:

- ATSDR concluded that, overall, studies in both a highly exposed population and in the general population do not suggest an association between perfluoroalkyl exposure and alterations in glucose homeostasis or increased risk of diabetes. Among occupationally exposed workers, studies showed some suggestive, but inconsistent, evidence for an association of death from diabetes but mortality is not a good measure of diabetes incidence.
- The US EPA reported that the C8 Science Panel had found no probable link between PFOA exposure and type I or type II diabetes. Also, general population studies had shown a lack of association between PFOA and diabetes, metabolic syndrome and related end points. US EPA reported one study showed a significant positive association between PFOA and risk of gestational diabetes.
- FSANZ reported the findings of their own review, which were that there were no consistent associations with either diabetes of markers of metabolic syndrome.

Systematic reviews:

- Saikat et al. concluded from the two studies on the general population that only one demonstrated some significant associations and only in adults.
- Priestly concluded it is too early to draw any definitive conclusions based on the small number of studies, and inconsistent findings.
- Rappazzo et al. concluded that studies of glucose regulation in children have reported mixed effects with limited agreement between studies.
- Kirk et al. noted the issue with the temporality of exposure-disease relationship in studies on both type I and type II diabetes. They concluded that, overall, studies on PFAS exposure and unspecified diabetes showed no association with PFOA and conflicting results for PFOS, and no studies showed an association between PFOA exposure and mortality due to diabetes. All outcomes were considered to have inadequate evidence, either because of limited studies, problems with study design and/or inconsistent findings.

6.10.5. Expert Health Panel synthesis to support advice to the Minister

- Epidemiological studies do not generally document consistent associations between PFAS and diabetes, glucose metabolism or metabolic syndrome. One of the two studies of gestational diabetes found an association.
- An association of PFOA concentration with increased diabetes mortality, but not diabetes incidence, was found in one study of workers; however there was no relationship with estimated exposure to PFAS, or increased risk over the general population.
- There are inconsistent associations in some selected populations, mostly based on weak study designs. Any associations in cross-sectional studies may be due to reverse causation or confounding with other conditions, such as kidney function.

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- Any association of PFAS with diabetes does not have an established causal mechanism. PFAS interact with PPAR receptors which leads to multiple metabolic changes, but PPAR agonist drugs generally improve glucose control.
- Diabetes was not a direct concern of those who reported being exposed to PFAS who responded in the public consultation (although cholesterol, kidney and cardiovascular diseases that might be affected by diabetes were a concern).

6.10.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and diabetes:

- Consistent associations of PFAS with diabetes or metabolic syndrome have not generally been observed. The most concerning signals are for diabetes mortality (but not diabetes incidence) and gestational diabetes, but these might be explained by confounding by kidney function.
- The known biological effects of PFAS on metabolism do not suggest this is a likely effect of PFAS.

To further investigate the association between PFAS exposure and diabetes in an Australian setting, the Panel suggests the following research priorities:

- Studies on diabetes risk would best be combined with other studies of overall health effects in exposed workers or communities or pregnant women. Conversely, any studies of cholesterol, kidney, weight gain, and cardiovascular disease should include a consideration of interactions with diabetes and hyperglycemia.
- Studies that look for causal evidence might also be useful. Relevant studies would (for example) investigate direct evidence for activation of causal biochemical mechanism(s) in humans, or investigate whether reducing PFAS concentrations in individuals alters glucose metabolism.

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6.11. Obesity, overweight, BMI and PFAS exposure

The World Health Organization (WHO) defines overweight and obesity as abnormal or excessive fat accumulation that may impair health, and body mass index (BMI) as a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. The WHO states: "*Overweight and* obesity *are major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer*"[69]. Several international authority reports and systematic reviews have reviewed the human evidence on exposure to PFAS and obesity, overweight and BMI.

6.11.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of three published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published in 2015 and 2016 and four systematic reviews since 2013 that analysed the human epidemiological evidence regarding exposure to PFAS and obesity, overweight, and BMI:

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR 2015).** Draft Toxicological Profile for Perfluoroalkyls;
- **United States Environmental Protection Agency (US EPA 2016a).** Health effects support document for Perfluorooctanoic Acid (PFOA);
- **United States Environmental Protection Agency (US EPA 2016b).** Health effects support document for Perfluorooctanoic Acid (PFOS).

Systematic reviews and reviews

- **Saikat et al. (2013).** The impact of PFOS on health in the general population; a review;
- **Priestly (2016).** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS). Monash University;
- **Rappazzo et al. (2017).** Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature;
- **Kirk et al. (2018).** The PFAS Health Study: systematic literature review. Australian National University.

While the Panel acknowledges that FSANZ cited one study under the section 'Effects on offspring of PFOA-exposed parents', FSANZ did not review the epidemiological evidence on PFAS and obesity, overweight and BMI in its 'Hazard assessment report (PFOS, PFOA, PFHxS)'. For this reason, the FSANZ 'Hazard assessment report' is not considered further in this section. No other key international authority report evaluated the human evidence on PFAS exposure and obesity, overweight and BMI.

6.11.2. Key national and international reports

US Agency for Toxic Substances and Disease Registry (ATSDR, 2015)

In 2015, the ATSDR in its draft toxicological profile for perfluoroalkyls reviewed the human evidence on obesity, overweight and BMI in adults and children.

[69] http://www.who.int/topics/obesity/en/

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Studies reviewed

In the section on 'Developmental effects', the ATSDR reviewed five studies that investigated PFAS exposure and obesity in adults and children (Andersen et al. 2010; Andersen et al. 2013; Halldorsson et al. 2012; Maisonet et al. 2012; Barry et al. 2014).

Considerations and conclusions

The ATSDR did not make any statements or conclusions about obesity, overweight and BMI effects in the 'Public health statement for perfluoroalkyls' or 'Relevance to public health' sections of the draft toxicological profile.

Summaries of studies reviewed

Childhood overweight and obesity

Of the study by Andersen et al. (2010), the ATSDR reported the following: "*Follow-up studies of the infants of mothers participating in the Danish National Birth Cohort study monitored growth at 5 months, 12 months, and 7 years of age. No significant associations between maternal PFOA levels and body weight or infant BMI were found at 5 or 12 months of age (Andersen et al. 2010). However, when grouped by sex, inverse associations between maternal PFOA levels and body weight and BMI were found in male infants at 5 and 12 months of age (Andersen et al. 2010). Maternal PFOS levels were inversely associated with infant body weight and BMI at 12 months, but not at 5 months; grouping by sex resulted in significant association in 12-month-old male infants.*"

Of the study by Andersen et al. (2013), the ATSDR reported: "*At age 7 years, there were no significant associations between maternal PFOS or PFOA levels and child BMI or waist circumference and the risk of being overweight was not significantly associated with maternal serum PFOS or PFOA levels.*"

Of the study by Maisonet et al. (2012), the ATSDR reported the following: "*Maisonet et al. (2012) found a positive association between body weight at 20 months and maternal PFOS levels in a study of girls in Great Britain.*"

The ATSDR did not make any overall statements or conclusions statements in the 'Health effects – oral exposure – developmental effects' section about childhood overweight and obesity.

Adult overweight and obesity

Of the study by Halldorsson et al. (2012), the ATSDR reported: "*Halldorsson et al. (2012) examined 665 offspring of women participating in a birth cohort study in Denmark and found significant positive associations between BMI and waist circumference in females and maternal serum PFOA levels (median level of 3.7 ng/mL), but no association in male offspring. Biomarkers of adiposity (insulin, leptin, and leptin-adioponectin ratio) were also positively associated with maternal serum PFOA levels in the female offspring.*"

Of the study by Barry et al. (2014), the ATSDR reported: "*A follow-up study of C8 participants found no association between early life PFOA exposure (estimated average PFOA serum concentration over the first 3 years of life) and overweight or obesity risk in men and women.*"

The ATSDR did not make any overall statements or conclusions in the 'Health effects – oral exposure – developmental effects' section about adult overweight and obesity.

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United States Environmental Protection Agency (US EPA, 2016a and 2016b)

In 2016, the US EPA (2016a and 2016b) in their health effects support documents for PFOA and PFOS reviewed evidence relating to the link between PFOA / PFOS and obesity, overweight and BMI.

Studies reviewed

The US EPA (2016a), in their 'Health effects support document for PFOA', reviewed four studies on BMI and being overweight under 'Postnatal development'. Three of these studies were reviewed by ATSDR (Andersen et al. 2010; Andersen et al. 2013; Halldorsson et al. 2012), and the fourth study was Høyer et al. (2015b).

The US EPA (2016b), in their 'Health effects support document for PFOS', reviewed one study by Andersen et al. 2013. This study was also reviewed by ATSDR.

Considerations and conclusions

The US EPA did not make any statements or conclusions about obesity, overweight or BMI in the 'Executive Summaries' of the 'Health effects support documents' for PFOA or PFOS.

Summaries of studies reviewed

Childhood overweight and obesity

The studies and findings of Andersen et al. (2010) and Andersen et al. (2013) are provided above under the ATSDR section. Detail about the study by Høyer et al. (2015b), not reviewed by the ATSDR, is provided below, as is more detail on the study by Andersen et al. (2013).

Of the study by Høyer et al. (2015b), the US EPA (2016a) for PFOA reported: "*Pregnant females were enrolled between May 2002 and February 2004 with a total of 1,022 mother-child pairs at follow-up between January 2010 and May 2012, when the children were 7–9 years old. The study population consisted of 531 pairs from Greenland and 491 pairs from Ukraine. Maternal blood samples for measurement of plasma PFOA levels were taken at a mean gestational age of 24 weeks. Each child's weight and height were measured and BMI calculated. All analyses were performed on the entire cohort as well as by country.*" The findings were reported as "*The median maternal plasma PFOA level was 1.8 ng/mL in pregnant females from Greenland and 1.0 ng/mL in pregnant females from Ukraine. No associations were found between PFOA (and PFOS) levels and risk of being overweight in the combined analysis or in Ukraine. In Greenland, the risk of being overweight was slightly increased only for females (RR=1.81, 95% CI 1.04, 3.17). PFOA association with risk of having waist-to-height ratio >0.5 was slightly increased for the combined analysis (RR=1.30, 95% CI 0.97, 1.74), but statistical significance was not attained. PFOS levels were significantly associated with waist-to-height ratio >0.5 in the combined analysis.*"

For PFOS, the US EPA (2016b) reported more detail about the study by Andersen et al. (2013): "*Andersen et al. (2013) evaluated the association between maternal plasma PFOS levels and the children's body mass index, waist circumference, and risk of being overweight at 7 years of age. From the subset of 1,400 randomly selected females from the DNBC who provided blood samples during their first trimester, only those children with weight and height information (n = 811) or waist measurements (n = 804) at age 7 years were included in the analysis. Maternal plasma PFOS levels were evaluated as both continuous and categorical exposures. Maternal PFOS concentrations were inversely associated with all of the children's anthropometric end points, but statistical significance was not attained and a dose-response relationship was not observed. Neither maternal PFOS nor PFOA levels were associated with anthropometric measures in either boys or girls at age 7 in this prospective birth cohort.*"

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Adult overweight and obesity

The US EPA (2016a) reviewed the study by Halldorsson et al. (2012) in greater detail than the ATSDR. It examined prenatal exposure to PFASs, including PFOA, and the risk of being overweight at 20 years of age in a prospective study in the 'General population studies' section. Of the study by Halldorsson et al. (2012), the US EPA (2016a) reported the findings as: "*Maternal PFOA levels were measured in serum samples collected during week 30 of gestation for assessment of in utero PFOA exposure and offspring anthropometry at 20 years... Three PFASs, including PFOS, perfluorooctane sulphonamide, and perfluorononanoate, increased across quartiles of PFOA concentration, but eight other PFASs did not. In covariate-adjusted analyses, female offspring whose mothers were in the highest quartile had 1.6 kg/m2 higher BMI (95% CI: 0.6, 2.6) and 4.3 cm larger waist circumference (95% CI: 1.4, 7.3) than offspring whose mothers were in the lowest quartile. Female offspring of mothers in the highest versus lowest PFOA quartile were also more likely to be overweight [RR 3.1 (95% CI: 1.4, 6.9)] and to have a waist circumference >88 cm at 20 years of age [3.0 (95% CI: 1.3, 6.8)]. Among female participants who provided blood samples at clinical examination (n = 252), maternal PFOA concentration was positively associated with insulin, leptin, and the leptin-adiponectin ratio; and inversely associated with adiponectin levels. PFOA was not associated with being overweight or obesity in male offspring. The other PFASs were not significantly associated with any end point after adjustment for PFOA.*"

6.11.3. Systematic reviews

Saikat et al. (2013)

Saikat et al. reviewed one study on the impact of PFAS on BMI in the 'BMI/waist circumference' section.

Studies reviewed

Saikat et al. reviewed Nelson et al. (2009). This study was not reviewed and reported on by any of the other key reports or systemic reviews.

Considerations and conclusions

Saikat et al. in the Abstract stated that: "*Small but statistically significant associations have been reported with PFOS and ...body mass index (BMI).*" The authors made no specific conclusion about PFOS and BMI, only a conclusion for the one study they reviewed.

Saikat et al. considered Nelson et al.'s paper in the 'Coherence with evidence' section, stating: "*Nelson et al. demonstrated an association between body size and PFOS in males only and the direction of the association was different in men under and over 60. This reduces the confidence in this being a "true" toxicological effect and it may be due to unmeasured or unknown confounder.*"

Summaries of studies reviewed

Saikat et al. cited the findings of Nelson et al. (2009): "*Nelson et al. further considered the association between PFOS and BMI and waist circumference and found that the relationships varied by sex and age group. Males under 60 years had a negative association between PFOS and BMI (those aged 12–19 years in the highest PFOS exposure quartile had a BMI 2.76 kg m^{-2} (95% CI: -4.08 to -1.43) lower than the lowest PFOS exposure quartile and those aged 20–59 years in the highest PFOS exposure quartile had a BMI 1.8 kg m^{-2} (95%CI: -4.02 to -0.43) lower than the lowest exposure quartile). Whilst males between 60 and 80 years had the opposite association (highest PFOS exposure quartile had a BMI 1.55 kg m^{-2} higher than the lowest exposure quartile), there was no evidence of an association in women.*

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Although no results were presented in the paper, Nelson et al. report that the association between PFOS and waist circumference was similar to that for BMI. Although this study used appropriate sampling weights in the analysis, the exclusion criteria led to 61% of the potential sample being excluded."

Priestly (2016)

Priestly (2016) considered the exposure of PFAS in the section 'Miscellaneous end points' on adiposity in children and overweight in adulthood.

Studies reviewed

Priestly evaluated four papers (Braun et al.2016, Høyer et al. 2015b; Halldorsson et al. 2012; and Karlsen et al. 2016), which included:

- three studies on adiposity in children (Braun et al. 2016, Høyer et al. 2015b; Karlsen et al. 2016);
- one study on overweight in adulthood (Halldorsson et al. 2012).

The studies by Høyer et al. (2015b), and Halldorsson et al. (2012) were reviewed by the ATSDR and US EPA with summaries of those studies provided above.

Considerations and conclusions

Priestly did not make any specific overall conclusion about the studies he reviewed on adiposity and BMI in his 'Comment' in the 'Miscellaneous end point' section. Priestly did make comment about individual studies, and these are reported below in the 'Summaries of studies' section.

Summaries of studies reviewed

While summaries of the studies by Høyer et al. (2015b), and Halldorsson et al. (2012) were reviewed by the ATSDR and US EPA, Priestly did make comment about these studies, and these comments are included below.

Childhood overweight and obesity

The two studies that Priestly reviewed that the ATSDR and the US EPA did not review were by Braun et al. (2016) and Karlsen et al. (2016).

Of the paper by Braun et al. 2016), Priestly reported that: "*Braun et al. (2016) reported on adiposity in children born to mothers who lived (2003-06) downstream from a fluoropolymer manufacturing plant in Cincinnati, Ohio. Data was drawn from the HOME prospective cohort study of early-life exposure to environmental chemicals. Children's weight (n=204) was recorded at ages 2, 3, 4, 5 and 8 years, with BMI and body fat measured at age 8. Maternal PFOA levels (GM[70] 5.4 ng/mL) was generally within the normal range, as were PFOS (13), PFHxS (1.4) and PFNA (0.9). Only for PFOA was there an apparent increase in adiposity at age 8, with small increases in waist circumference (4.3cm 95%CI 1.7 – 6.9; 2.2cm -0.5 -4.9) in the 2nd and 3rd terciles, and similar small gains in BMI from age 2 to 8 years.*"

Of the study by Karlsen et al. (2016), Priestly reported that: "*In a study aimed at investigating the effects of prenatal exposure to endocrine disrupting persistent environmental pollutants (POPs) in children from the Faroe Islands, Karlsen et al. (2016) reported a trend for increased BMI (>85th percentile WHO z-scores) at ages 18 months and 5 years with maternal serum PFOA, PFOS and HCB,*

[70] Geometric mean

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but not for PFHxS, PFNA or PFDA, or the other POPs examined (p-p'-DDE, PCBs). Paradoxically, the relationship between child serum POPS and BMIz scores and 'overweight RR at age 5 was inverse."

Of the study by Høyer et al. (2015b), Priestly made the following comment: "*These findings do not support any significant effect of prenatal exposure to either PFOA or PFOS affecting adiposity in children."*

Adult overweight and obesity

Of the study by Halldorsson et al. (2012), Priestly commented: "*Halldorsen et al. (2012) reported that the odds ratio for being overweight at age 20 among female offspring (but not male) was 3.1 (95% CI 1.2 – 6.8) for highest:lowest quartiles of PFOA serum concentrations (median 5.8 vs 2.3 ng/mL). These findings may have been associated with increasing insulin and leptin levels and decreasing adiponectin levels in these girls. A possible mechanistic basis for such findings could include PPAR-Y-receptor mediated stimulation of adipocyte differentiation (Watkins et al, 2015)."*

Rappazzo et al. (2017)

Rappazo et al. (2017) reviewed the literature on exposure to PFAS and health outcomes in children.

Studies reviewed

Rapazzo et al. (2017) reviewed nine studies under the 'Cardiometabolic' section that examined anthropometric outcomes such as weight (Andersen et al. 2010; Andersen et al. 2013; Braun et al. 2016; Maisonet et al. 2012; Halldorsson et al. 2012; Høyer et al. 2015b; Wang et al. 2016; Timmermann et al. 2014; Kristensen, et al. 2013).

Considerations and conclusions

Rappazzo et al. stated at the end of the Cardiometabolic section: "*The evidence for the effects on weight or BMI in children across PFAS is mixed with PFOA most frequently associated with overweight status in females but some PFOA studies show null results. It may be the small positive associations such as those reported by [Maisonet et al. 2012], have cumulative effects over time, which lead to being overweight in adulthood."*

Summaries of studies reviewed

The studies by Andersen et al. (2010), Andersen et al. (2013), and Maisonet et al. (2012), Braun et al. (2016), Halldorsson et al. (2012), and Høyer et al. (2015b) were reviewed by the ATSDR, US EPA or Priestly, and summaries of these studies are provided above.

Childhood overweight and obesity

In the 'Cardiometabolic' section, Rappazzo et al. reviewed three studies that none of the other key reports or systematic reviews reviewed (Wang et al. 2016; Timmermann et al. 2014; Kristensen et al. 2013).

Of the study by Wang et al. (2016), Rappazzo et al. reported that: "*Height z-score, but not weight, was negatively associated with 3rd trimester maternal serum concentrations of several PFAS (excepting PFOA) for children aged 2 to 11, particularly in girls, in the Taiwan Maternal and Infant Cohort study."*

The study by Timmermann et al. (2014) was reported by Rappazzo et al. as: "*In a cross-sectional study, Timmermann, et al. [2014] found no associations between serum PFOA or PFOS at age 8 years and anthropometric measures, including BMI."*

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Of the study by Kristensen et al. (2013), Rappazzo et al. reported: "*A Danish pregnancy cohort from 1988 to 1989 reported BMI significantly increased across tertile of maternal serum PFOA measured at gestational week 30 in female offspring at age 20, though no effect estimates were reported and interquartile ranges were within normal values.*"

However, Rappazzo et al. provided more detail about the study by Maisonet et al. (2012), than the ATSDR. Maisonet et al. (2012) is the only study that Rappazzo et al. provided an indication of the magnitude of the effect. Rappazzo et al. stated of this study: "*In Bristol England, the Avon Longitudinal Study of Parents and Children (ALSPAC) examination of prenatal PFAS (maternal serum) and weight in girls at 20 months of age found an increase in weight of 580 g (301, 858 g) when adjusting for birthweight and height at 20 months for the highest tertile of PFOS compared to the lowest; they observed no associations between PFOA or PFHxS and weight at 20 months in girls.*"

Kirk et al. (2018)

In their systematic review, Kirk et al. evaluated the effect of PFAS exposure on overweight and obesity in children and adults in the 'Overweight and obesity' section. The main health outcome evaluated in the studies was overweight in childhood, adulthood and during pregnancy, reported as measured using BMI and waist circumference.

Studies reviewed

Kirk et al. evaluated nine papers (Andersen et al. 2013; Ashley-Martin et al. 2016; Barry et al. 2014; Braun et al. 2016; de Cock et al. 2014a; Halldorsson et al. 2012; Høyer et al. 2015; Jaacks et al. 2016; Rylander et al. 2009). Kirk et al. reported that: "*The studies mainly investigated exposure to PFOA and PFOS, however, the effect of additional PFAS exposure was also considered.*"

These studies included:

- Four studies on childhood overweight and obesity (Andersen et al. 2013; Braun et al. 2016; de Cock et al. 2014a; and Høyer et al. 2015);
- Two studies on adult overweight and obesity (Barry et al. 2014; and Halldorsson et al. 2012);
- Three studies on gestational weight gain (Ashley-Martin et al. 2016; Jaacks et al. 2016; and Rylander et al. 2009).

Considerations and conclusions

Kirk et al. did not make any statements or conclusions about PFAS and obesity and overweight in the 'Executive Summary' or 'Discussion' sections. However, at the end of the section on 'Childhood overweight and obesity', Kirk et al. made the following conclusion about the four studies they reviewed: "*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. [2013] and de Cock et al. [2014a], the findings should be considered with caution.*"

Kirk et al. stated that "*All papers were determined to have a moderate to high risk of bias.*"

The table below shows the associations determined by Kirk et al. for all health outcome end points evaluated, by PFAS.

Associations at a glance (Overweight and obesity)

Health outcome	PFAS exposure	Evaluation of evidence

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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

Source: From 'Associations at a glance' page 120, Kirk et al. 2018.

Summaries of studies reviewed

Childhood overweight and obesity

Of the nine studies cited by Kirk et al., four studies investigated the association between PFAS exposure and childhood overweight and obesity in the 'Childhood overweight and obesity' section (Andersen et al. 2013; Braun et al. 2016; de Cock et al. 2014a; and Høyer et al. 2015). The studies by Andersen et al. (2013), Braun et al. (2016) and Høyer et al. (2015) were reviewed by other key reports and reviews in this section, with details of these studies provided in the respective paragraphs above. Kirk et al. provided additional detail about the study by Braun et al. (2016), which is included below.

Kirk et al. noted that the four studies they reviewed: "*were based on analysis of mother-child pairs enrolled in prospective birth cohorts. The research mainly focused on the effect of prenatal exposure to PFOA and PFOS on BMI and waist measurements in young children.*"

Of the studies by de Cock et al. (2014a) and Braun et al. (2014), Kirk et al. reported: "*Andersen et al. [2013] and de Cock et al. [2014a] 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. [2014], supported this conclusion of exposure to PFOS, PFNA and PFHxS in children aged 18 years or younger.*"

Kirk et al. made the following observations about the four studies they reviewed: "*Whilst most of the studies concluded no association between PFAS exposure and indicators of childhood overweight and obesity, Braun et al. [2014] and Høyer et al. [2015] reported conflicting results. In the HOME study, Braun et al. [2014] 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. [2015] 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. [2015] identified similarly elevated results for PFOA, which were not statistically significant.*"

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Adult overweight and obesity

Kirk et al. reviewed two studies on adult overweight and obesity and PFAS exposure. The two studies, Barry et al. (2014) and Halldorsson et al. (2012), were reviewed by ATSDR, US EPA (2016a), Priestly, and Rappazzo with details on these studies provided under the respective sections above.

In the 'Adulthood overweight and obesity' section, Kirk et al. made the following observations about these two studies: "*The two studies presented conflicting results and the measurement of overweight and obesity was not the same... 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

Kirk et al. reviewed three studies that evaluated PFAS exposure and weight gain during pregnancy (Ashley-Martin et al.2016; Jaacks et al. 2016; and Rylander et al. 2009) in the 'Gestational weight gain' section.

Kirk et al. reported the following about these three studies: "*Ashley-Martin et al. [2016], Jaacks et al. [2016] and Rylander et al. [2009] studied the effects of PFAS exposure on weight gain during pregnancy. Ashley-Martin et al. [2016] and Jaacks et al. [2016] 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. [2016] 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. [2016] reported a significantly positive association with gestational weight gain in women with a BMI < 25 kg/m2 (adjusted regression coefficient (95% CI); 280.29 (13.71, 546.86)) but not in women with BMI ≥ 25 kg/m2. 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. [2016] justified their measurement of gestational weight gain through only including female participants that had a normal BMI before their pregnancy.*"

Kirk et al. made the observation: "*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.*"

6.11.4. Summary of key national and international reports and systematic reviews

Recent key national and international reports:

- The ATSDR cited five studies which reported inconsistent findings; the ATSDR did not make any statements or conclusions about overweight, obesity or BMI.
- The US EPA cited four studies, also with conflicting findings, and made no statements or conclusions about PFOS/PFOA and overweight, obesity or BMI.

Systematic reviews:

- Saikat et al. reviewed the one paper which found an association in males, but this may be due to an unmeasured or unknown confounder.

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- Priestly commented that of two studies in children the findings do not support any significant effect of prenatal exposure to either PFOS or PFOA affecting adiposity in children. He did not make any specific conclusions about overweight, obesity or BMI.
- Rappazzo et al. noted the evidence for the effects on weight or BMI in children across PFAS is mixed, with some studies showing a positive effect for PFOA in females, while others show null results.
- Kirk et al. concluded that there was inadequate evidence for all outcomes. The strongest, although still weak, evidence was that PFOA and PFOS exposures may increase childhood overweight and obesity, but that the findings should be considered with caution. Studies on adults showed conflicting findings. Three studies showed no association between PFAS exposures and overweight and obesity measurement during pregnancy.

6.11.5. Expert Health Panel synthesis to support advice to the Minister

- There were some inconsistent associations between PFAS and obesity in various age groups, but any associations found related to very small increases and these are unlikely to represent important differences at a clinical or population level.
- There was little consistent evidence for associations with PFOS or other fluorinated substances.
- Any association of PFAS with obesity does not have an established causal mechanism. However, PFAS do interact with PPAR receptors and these are involved in energy regulation; PPARγ agonists used in diabetes (rosiglitazone, and pioglitazone) cause weight gain.
- The current evidence is largely from cross-sectional studies, which is generally a weak study design, and stronger evidence would come from future cohort studies with standardised measures and those that could demonstrate a causal mechanism (to exclude confounding and reverse causation).
- Obesity and weight gain were not a concern of those exposed to PFAS who responded in the public consultation (although cardiovascular diseases that might be affected by weight gain were a concern).

6.11.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and obesity:

- An association of PFAS with excessive weight gain has been observed in some studies, but the relationship is conflicting across studies and poorly characterised. Evidence to date does not establish whether or not PFAS exposure is causally related to increased weight gain in any age group, but if there is a causal link, then any weight gain is likely to be small.
- Study limitations, such as weak study designs, limited adjustment for confounders, inconsistent measures, the possibility of reverse causation, and the lack of any measured causative mechanism, hinder firm conclusions to be drawn.
- Due to the limitations noted above, the existing scientific evidence does not warrant any change in obesity prevention programs or to peoples' medical management for obesity or related disorders. Established risk factors for obesity, such as poor diet, excessive alcohol, some prescription medications, and lack of exercise, are likely to be of a much greater magnitude than those potentially caused by PFAS.

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To further investigate the association between PFAS exposure and obesity in an Australian setting, the Panel suggests the following research priorities:

- Studies that look for causal evidence are the key research need. Further cross-sectional studies are unlikely to provide this information, but well-designed longitudinal studies in occupational groups or highly exposed community groups may provide stronger epidemiological evidence. Relevant studies would (for example) investigate direct evidence for activation of causal biochemical mechanism(s) in humans, or determine whether reducing PFAS concentrations in individuals alters weight, or adipose tissue distribution.

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6.12. Cardiovascular effects and PFAS exposure

The World Health Organization defines cardiovascular diseases (CVDs) as disorders of the heart and blood vessels, including coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions. The WHO notes individuals at risk of CVD may demonstrate raised blood pressure, glucose, and lipids as well as overweight and obesity[71]. Two international authorities and three systematic reviews have reviewed the effect of PFAS on cardiovascular outcomes in humans.

6.12.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of two published key (inter)national authority/intergovernmental/governmental reports ('key national and international reports') published between 2015 and 2017 and three systematic reviews since 2016 that analysed the human epidemiological evidence regarding exposure to PFAS and cardiovascular effects:

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR 2015**). Draft Toxicological Profile for Perfluoroalkyls;
- **United States Environmental Protection Agency (US EPA 2016a).** Health effects support document for Perfluorooctanoic Acid (PFOA).

Systematic reviews

- **Priestly (2016).** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS). Monash University;
- **Rappazzo et al. (2017).** Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature;
- **Kirk et al. (2018).** The PFAS Health Study: systematic literature review. Australian National University.

While the Panel acknowledges that FSANZ did comment on cardiovascular diseases, stating: "*There is a lack of consistent evidence that PFOA is associated with increased risk of cardiovascular disease (reviewed by EFSA 2008)*" and cited the findings of the C8 Science Panel, FSANZ did not review epidemiological studies on cardiovascular effects in the Hazard Assessment report for PFOA, PFOS and PFHxS. For this reason, the FSANZ report is not considered further in this section. The US EPA reviewed studies on PFOS serum lipids and cardiovascular diseases, the findings of which are included in the section on 'Cholesterol'.

No other systematic reviews or key national and international reports covered cardiovascular effects.

Please note that studies reviewed on PFAS and pregnancy-induced hypertension by key national and international reports and systematic reviews are covered in the 'Neonatal, infant and maternal outcomes from exposure during pregnancy' section of this report.

[71] http://www.who.int/cardiovascular_diseases/en/

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6.12.2. Key national and international reports

US Agency for Toxic Substances and Disease Registry (ATSDR, 2015)

The ATSDR, in its 'Draft toxicological profile for perfluoroalkyls', considered the human evidence on cardiovascular effects of PFAS in the 'Cardiovascular effects' section.

Studies reviewed

The cardiotoxicity of PFAS was examined by the ATSDR in nine studies:

Under inhalation exposure route: four cohort mortality studies of workers (Leonard 2006; Lundin et al. 2009; Sakr et al. 2009; Steenland and Woskie 2012); and one cross-sectional study of nonlethal cardiovascular effects in workers (Sakr et al. 2007b);

Under oral exposure route: two studies of residents living near a PFOA facility (Anderson-Mahoney et al. 2008; Steenland et al. 2010); and two studies of the general population (Min et al. 2012; Shankar et al. 2012).

Considerations and conclusions

The ATSDR did not make any statements or conclusions about cardiovascular effects in the 'Public health statement for perfluoroalkyls'.

In the 'Relevance to public health' section of the toxicological profile, the ATSDR commented on hypertension in relation to increased levels of uric acid: "*Based on the weight of evidence, there is support for identifying several health effects in humans that appear to be related to perfluoroalkyl exposure...; increases in uric acid, a possible biomarker for hypertension.*"

In the section 'Populations that are unusually susceptible', the ATSDR made the following comment about cardiovascular risk: "*The available epidemiology data identify several potential targets of toxicity of perfluoroalkyls, and individuals with pre-existing conditions may be unusually susceptible. For example, it appears that exposure to PFOA or PFOS can result in increases in serum lipid levels, particularly cholesterol levels. Thus, an increase in serum cholesterol may result in a greater health impact in individuals with high levels of cholesterol or with other existing cardiovascular risk factors. Similarly, increases in uric levels have been observed in individuals with higher perfluoroalkyl levels; increased uric acid may be associated with an increased risk of high blood pressure. Thus, individuals with hypertension may be at greater risk.*"

Summaries of studies reviewed

Inhalation exposure route – cardiovascular effects for workers

The ATSDR reviewed the cardiotoxicity of PFOA in four cohort mortality studies of workers (Leonard 2006; Lundin et al. 2009; Sakr et al. 2009; Steenland and Woskie 2012) and a study of nonlethal cardiovascular effects in workers (Sakr et al. 2007b), under the section 'Inhalation exposure – systemic effects – cardiovascular effects – human exposure studies' section.

Of the study by Leonard (2006), the ATSDR reported that: "*Leonard (2006) conducted a cohort mortality study of DuPont employees at the Washington Works, West Virginia, polymer manufacturing facility. The cohort (n=6,027; 80% males) was defined as all individuals who had ever worked at the plant at any time between January 1, 1948 (plant start-up) and December 31, 2002. Results from the cross-sectional study indicated that workers in all areas across the entire plant site showed some measurable level of serum PFOA ranging from 5 to 9,550 ng/mL. The standardized mortality ratios (SMRs) for cerebrovascular disease, all heart disease, and ischemic heart disease were not significantly*

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increased, as compared to the United States and West Virginia population rates or to a population of DuPont workers residing in West Virginia and seven neighboring states. Cox proportional hazard modeling using an average exposure intensity categories and cumulative PFOA exposure categories (calculated for each member of the cohort based on categorization of jobs) for white male workers showed an increase in the ischemic heart disease mortality based on equal distribution of cases across cumulative exposure categories in one lagged analysis (the 10-year lag period). Proportional hazards calculated with 5-, 15-, or 20-year lags showed no effect, and results for a second set of models using a different set of exposure cutpoints were attenuated toward the null. Moreover, none of the hazard estimates themselves were statistically significant."

For the study by Sakr et al. (2009) the ATSDR reported: "*Sakr et al. (2009) extended the Leonard (2006) study by using individually measured serum PFOA levels to categorize job titles into three categories: high, medium, and low; a fourth group with minimal PFOA exposure was used as a referent. Exposure intensity was assigned using the mean serum PFOA levels of all jobs in an exposure category. Of the 4,747 male and female workers (98% male), 239 died from ischemic heart disease, 534 died of other causes, and 3,974 were alive at the end of follow-up. No statistically significant increases in the relative risk of ischemic heart disease were found; however, there was a significant trend for increasing risk from the 10-year lagged exposure categories.*"

The cohort mortality study by Steenland and Woskie (2012) was reported by the ATSDR as: "*A third study of workers at this facility (Steenland and Woskie 2012) extended the follow-up period through 2009 and estimated serum PFOA levels for 5,801 workers in the cohort based on job histories and serum PFOA levels collected between 1974 and 2004 from a subset of workers. No significant increases in SMRs for ischemic heart disease were found when U.S. population or DuPont regional employees were used as referent populations. Dividing the workers into quartiles or deciles based on cumulative PFOA exposure did not result in significant increases in SMRs for ischemic heart disease, as compared to DuPont regional employees, regardless of the lag period.*"

Lundin et al. (2009) used a different occupational population for the sample: the 3M manufacturing facility in Cottage Grove, Minnesota. The ATSDR reported: "*Lundin et al. (2009) conducted a cohort mortality study of 3,993 workers (80% male) at the 3M manufacturing facility in Cottage Grove, Minnesota. The workers were divided into three categories: definite occupational exposure to APFO, probable occupational exposure, and no or minimal occupational exposure. Exposure intensity and cumulative exposure were estimated based on job categories. No increases in the SMRs for cerebrovascular disease, all heart disease, or ischemic heart disease, as compared to mortality rates for the state of Minnesota, were found. Hazard ratio (HRs), estimated with time-dependent Cox regression models, for cerebrovascular disease were significantly increased in workers with high exposure intensity (HR 4.6, 95% confidence interval [CI] 1.3–17.0) and with exposure durations of ≥5 years (HR 2.1, 95% CI 1.0–4.6). The HRs were not significantly increased for ischemic heart disease.*"

When reporting on the study of nonlethal cardiovascular effects by Sakr et al. (2007b), ATSDR stated: "*No alterations in the electrocardiograms (EKG) were observed in a cross-sectional study of 1,025 workers potentially exposed to PFOA (Sakr et al. 2007b); the mean serum PFOA levels ranged from 5 to 9,550 ng/mL.*"

Oral-exposure route – cardiovascular effects of the general population

The ATSDR also reported that the potential of perfluoroalkyls to induce cardiovascular effects in the 'Oral exposure – systemic effects – cardiovascular effects – human exposure studies' section. The ATSDR reviewed two studies of residents living near a PFOA facility (Anderson-Mahoney et al. 2008;

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Steenland et al. 2010), and two studies of the general population (Min et al. 2012; Shankar et al. 2012).

The ATSDR reported that Anderson-Mahoney et al. (2008) was: "*A study of 566 white adults in West Virginia and Ohio exposed to PFOA in contaminated drinking water from a nearby manufacturing facility calculated standardized prevalence ratios (SPRs) by comparing self-reported cardiovascular effects to expected rates from NHANES 2001–2002 (Anderson-Mahoney et al. 2008). Significant increases in cardiovascular problems (including myocardial infarction, stroke, and angina) were observed; the SPR was 4.29 (95% CI 3.47–5.29). The prevalence of high blood pressure was not significantly altered; however, when the subjects were categorized by age and sex, significant increases in prevalence rates were observed in males 18–34, 35–49, 50–64, and ≥65 years old and in females 18–34, 50–64, and ≥65 years old.*"

The ATSDR noted: "*The study did not measure serum PFOA levels in the subjects and approximately 15% of the subjects worked at the PFOA facility, which likely resulted in inhalation exposure to PFOA.*"

ATSDR did not provide any summary text of the study by Steenland et al. (2010) in this section.

Of the study by Shankar et al. (2012), the ATSDR reported: "*Using NHANES data for 1,216 adults (≥40 years of age), Shankar et al. (2012) found significant increases in the risk of self-reported cardiovascular disease in adults with serum PFOA levels in the third (4.0–5.6 ng/mL in women and 4.4–6.1 ng/mL in men) and fourth (>5.6 ng/mL in women and >6.1 ng/mL in men) quartiles (ORs 1.77 [95% CI 1.04–3.02] and 2.01 [95% CI 1.12–3.60], respectively) and an increased risk of peripheral arterial disease in adults with PFOA levels in the fourth quartile (OR 1.78, 95% CI 1.03–3.08); cardiovascular disease was defined as physician-diagnosed coronary heart disease, heart attack, or stroke and peripheral arterial disease was defined as the ratio of <0.9 for ankle systolic blood pressure to arm systolic blood pressure. The results were similar when the subjects were categorized by sex, smoking status, and BMI, although the OR was not always statistically significant. When cardiovascular disease was divided into types of disease, significant increases in the risk of coronary artery disease and stroke were significantly higher in adults with PFOA levels in the fourth quartile.*"

The ATSDR reported of Min et al.'s (2012) study:"*Another study using the NHANES data for 2,263 adults (>20 years of age; Min et al. 2012) found a significant positive association between serum PFOA levels and systolic blood pressure (adjusted for various factors including obesity, physical activity, smoking status, total cholesterol, and kidney function) when analyzed by linear regression and risk analysis (OR 2.62 [95% CI 2.09–3.14] when subjects with serum PFOA levels in the 80th percentile were compared to those in the 20th percentile). Categorizing subjects by serum PFOA quartiles and adjusting for serum PFOS levels also resulted in significantly elevated ORs in comparisons of the third and fourth quartiles to the first quartile. A positive association between serum PFOA and homocysteine levels, considered a marker for cardiovascular disease, was also found.*"

The ATSDR also reviewed several studies on pregnancy-induced hypertension in this section. These studies are reviewed in this report under the 'Neonatal, infant and maternal outcomes' section.

United States Environmental Protection Agency (US EPA, 2016a)

The US EPA reported on the impact of PFOA on cardiovascular disease in the Health effects support documents for PFOA. The US EPA's (2016a) reported information in two sections: 'Serum lipids and cardiovascular disease' and 'Cardiovascular diseases'. For PFOS, the US EPA reported information in

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the section 'Serum lipids and cardiovascular disease'. For this report, only the studies reviewed under the section 'Cardiovascular diseases' were considered.

The studies reviewed by the US EPA for PFOA and PFOS under the section 'Serum lipids and cardiovascular disease' are covered in the section 'Metabolic biomarkers – concentrations of cholesterol and triglycerides in the blood' of this report.

Studies reviewed

Under 'Cardiovascular diseases', the US EPA cited five studies of occupational exposure that examined cardiovascular-related cause of death among PFOA-exposed workers, including:

three studies at the West Virginia Washington Works plant (Leonard et al. 2008; Sakr et al. 2009; Steenland and Woskie 2012);

two studies at the 3M Cottage Grove plant in Minnesota (Lundin et al. 2009; Gilliland and Mandel 1993).

The US EPA also reviewed one study (Geiger et al. 2014) on the effect of PFAS on postnatal development and cardiovascular disease in the 'Hazard identification – human studies – Noncancer – postnatal development' section.

Considerations and conclusions

The US EPA did not make any statements or conclusions about PFOA/ PFOS and cardiovascular disease in the 'Executive Summaries' of the 'Health effects support documents' for PFOA and PFOS. In the 'Health effects support document for PFOA', the US EPA did make a statement about pregnancy-induced hypertension, which is covered under the 'Neonatal, infant and maternal outcomes' section of this report.

Summaries of studies reviewed

Occupational exposure

The US EPA reported about the studies on occupational exposure (Leonard et al. 2008; Sakr et al. 2009; Steenland and Woskie 2012; Lundin et al. 2009; and Gilliland and Mandel 1993): "*This type of mortality is of interest because of the relation between lipid profiles (e.g., LDL) and the risk of cardiovascular disease. The most recent West Virginia study included 5,791 individuals who had worked at the plant for at least 1 year between 1948 and 2002, with mortality follow-up through 2008. No associations were found between cumulative PFOA levels and ischemic heart disease (IHD) mortality (standardized mortality ratio [SMR] 1.07, 1.02, 0.87, and 0.93 across four quartiles of cumulative exposure, compared to U.S. referent group). Based on these data from the worker cohorts, the C8 Science Panel (2012) concluded that there is no probable link between PFOA and stroke and coronary artery disease.*

The analysis of the Minnesota plant (n = 3,993 workers who began work between 1983 and 1997, with follow-up through 2002) also found no association between cumulative PFOA exposure and IHD risk, but an increased risk of cerebrovascular disease mortality was seen in the highest exposure category (HR 2.1, 95% CI 1.0, 4.6). These studies are limited by the reliance on mortality (rather than incidence) data, which can result in a substantial degree of under ascertainment and misclassification."

Hypertension in children

Under the section 'Postnatal development', the US EPA reviewed the study by Geiger et al. (2014). It reported the following: "*Geiger et al. (2014b) used data from the NHANES to determine whether there was a relationship between serum PFOA levels and hypertension in children. A total of 1,655*

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participants (aged 12–18 years) from the 1999–2000 and 2003–2008 cycles of the survey who had PFOA measurements available were examined. Blood pressure was measured to determine the presence of hypertension, and linear regression modeling was used to study the association between increasing quartiles of serum PFOA and mean changes in systolic and diastolic blood pressures. Mean PFOA level was 4.4 ± 0.1 ng/mL. No association was found between serum PFOA (or PFOS) levels and hypertension in either unadjusted or multivariable-adjusted analyses. Compared with the lowest quartile, the multivariable-adjusted OR (95% CI) of hypertension in the highest quartile of exposure was 0.69 (0.41–1.17) (P-trend >0.30)."

6.12.3. Systematic reviews

Priestly (2016)

Priestly (2016) considered one study on cardiovascular disease in his literature review.

Studies reviewed

In the section 'Miscellaneous end points', Priestly reviewed one study by Lin et al. (2013). This study examined PFAS in relation to carotid artery intima width taken from a Taiwanese cohort in the section 'Miscellaneous end points'.

Considerations and conclusions

Priestly did not make any specific conclusion about the one study he reviewed. His 'Comment' at the end of the' Miscellaneous end points' section was general and referred to all of the studies he reviewed.

Summaries of studies reviewed

Priestly reported about Lin et al. (2013): "*Also possibly linked with stimulation of inflammatory or atherosclerotic responses, Lin et al. (2013b) reported a small, but significant increase in the width of the carotid artery intima in adolescents and young adults from a Taiwanese cohort. The thicknesses across the four quartiles of PFOS were: 0.434, 0.446, 0.458 and 0,451 mm (p<0.001 for trend). The effects were strongest in females, non smokers, BMI<24 and those with apolipoprotein E alleles (APOE genotype of E2 carrier and E3/E3 genotypes). No associations were found between PFOS, PFOA, PFNA and serum lipids, except for a slight trend for serum triglycerides to decrease with increasing PFOA concentrations.*"

Rappazzo et al. (2017)

Rappazzo et al. (2017) reviewed studies on cardiovascular disease in children under the 'Cardiometabolic' section of their systemic review of the epidemiological literature on exposure to perfluorinated alkyl substances and health outcomes in children.

Studies reviewed

Rappazzo et al. (2017) reviewed two studies (Geiger et al. 2014b; Lin et al. 2013).

The findings of the study by Geiger et al. (2014b) were also reviewed by the US EPA (2016a). The study by Lin et al. (2013) was also reviewed by Priestly, above.

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Considerations and conclusions

Rappazzo et al. (2017) did not make any specific conclusions about PFAS and cardiovascular effects in children. At the end of the section on 'Cardiometabolic effects', the authors stated: "*A single study of carotid intima-media thickness found an association with PFOS concentration.*"

Summaries of studies reviewed

A summary of the study by Geiger et al. (2014b) is reported in the US EPA section.

Of the study by Lin et al. (2013), there is a summary of this study above under Priestly (2016). Rappazzo et al. provided additional information in his summary, and this is included below: "*Another cross-sectional study examined carotid artery intima-media thickness in a Taiwanese population of which 38% had elevated blood pressure during childhood, finding increased carotid artery intima-media thickness in adolescents aged 12–19 with increasing quartiles of plasma PFOS.*"

Kirk et al. (2018)

In their systematic review, Kirk et al. evaluated the effect of PFAS exposure on cardiovascular outcomes in children and adults. Kirk et al. reported that: "*Most of the 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.*"

Studies reviewed

Kirk et al. evaluated nine papers in total under cardiovascular effects (Geiger et al. 2014b; Lin et al. 2013a; Lundin et al. 2009; Mattsson et al. 2015; Min et al. 2012; Sakr et al. 2009; Shankar et al. 2012; Steenland and Woskie 2012; Winquist and Steenland 2014a). These include:

- six studies on cardiovascular effects (Winquist and Steenland 2014a; Steenland and Woskie 2012; Lundin et al. 2009; Mattsson et al. 2015; Sakr et al. 2009; Shankar et al. 2012);
- three studies on hypertension (Geiger et al. 2014b; Min et al. 2012; Winquist and Steenland 2014a); and
- one study on carotid intima-media thickness (Lin et al. 2013a).

The findings of papers by Geiger et al. (2014b), Lin et al. (2013a), Lundin et al. (2009), Min et al. (2012), Sakr et al. (2009) Shankar et al. (2012), and Steenland and Woskie (2012) have been provided above under ATSDR, US EPA, and Priestly.

The papers by Mattson et al. (2015) and Winquist and Steenland (2014a) were not reviewed by other studies. The findings are included below.

Considerations and conclusions

Kirk et al. did not make any statements or conclusions specifically about cardiovascular effects in the 'Executive Summary' or 'Discussion' sections of the systematic review. However, they make a number of references to cardiovascular disease, particularly in relation to cholesterol. Please see the 'Metabolic markers – concentrations of cholesterol and triglycerides in the blood' section of this report.

The authors noted that: "*All papers were determined to have a moderate or high risk of bias.*"

The following table, reproduced from Kirk et al. (pg. 118) shows the reported associations for cardiovascular outcomes by PFAS, following Kirk et al.'s review of the literature

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Associations at a glance (Cardiovascular outcomes)

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

Source: Associations at a glance, taken from Kirk et al. 2018, pp. 118

Kirk et al. made the following comment about the six studies they reviewed on PFAS and cardiovascular disease: "*The studies presented conflicting results. However, it is difficult to make direct comparisons, as the health outcomes were not the same for all studies.*" The authors also noted: "*...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.*"

Of the three studies Kirk et al. reviewed on hypertension, they made the following overall comment: "*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.*"

Of the one study Kirk et al. reviewed on carotid intima-media thickness, they provided the following comment: "*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.*"

Summaries of studies reviewed

Cardiovascular disease

Kirk et al. evaluated six papers (Lundin et al. 2009, Mattsson et al. 2015, Sakr et al. 2009, Shankar et al. 2012, Steenland and Woskie 2012, Winquist and Steenland 2014a) that investigated the association between PFAS exposure and cardiovascular disease.

Of the study by Mattsson et al. (2015) that was not reviewed by the ATSDR, US EPA, Priestly or Rappazzo et al. Kirk et al. reported: "*In contrast, Mattsson et al. [2015], Sakr et al. [2009] and Steenland & Woskie [2012] 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. [2015]. The case-control study concluded no association between PFOS, PFNA, PFDA, PFHpA, PFHxS, PFUdA and PFDoA and coronary heart disease diagnosis in adults.*"

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Hypertension

The authors evaluated three papers that investigated the relationship between PFAS exposure and diagnosis of hypertension (Geiger et al. 2014b, Min et al. 2012, and Winquist and Steenland 2014a). The studies by Geiger et al. (2014b) and Min et al. (2012) have been reported previously in this section.

For Winquist and Steenland (2014a), Kirk et al. reported: "*Winquist & Steenland [2014a] used the C8 Health Project cohort to study the association between PFOA exposure and hypertension in adults and concluded there was no association.*"

Carotid intima-media thickness

Kirk et al. evaluated one paper (Lin et al. 2013a) that investigated the association between PFAS exposure and carotid intima-media thickness as an indicator of carotid atherosclerotic vascular disease. This study was also reviewed by Priestly and Rappazzo et al. above.

6.12.4. Summary of key national and international reports and systematic reviews

Recent key national and international reports:

- The ATSDR did not make any specific conclusions about PFAS and cardiovascular disease but noted increased uric acid through exposure to PFAS may be a possible biomarker for hypertension.
- The US EPA did not make any conclusion about PFOA and cardiovascular disease from the studies they reviewed, but noted the findings of the C8 Science Panel.

Systematic reviews:

- Priestley reviewed one study on carotid artery intima width and made no specific conclusion.
- Rappazzo et al. did not make any specific conclusions about the two studies on childhood cardiovascular effects they reviewed.
- Kirk et al. reported all studies evaluated on cardiovascular disease are conflicting. The association between PFOA and stroke and peripheral arterial disease is unclear. For coronary artery disease, studies showed no association between PFOA exposure and disease diagnosis and mortality in adults, possibly indicating PFOA exposure is not related to coronary heart disease. No clear conclusions could be made for PFAS and hypertension.

6.12.5. Expert Health Panel synthesis to support advice to the Minister

- Epidemiological studies do not generally document associations between PFAS and cardiovascular diseases. There are inconsistent associations, mostly based on weak study designs, with various cardiovascular risk factors (i.e. lipids, weight, hypertension).
- The association of PFAS with cardiovascular disease does not have an established causal mechanism. However, PFAS do interact with PPAR receptors and one potent PPARγ agonist used in diabetes (rosiglitazone) has been linked to heart failure and ischaemic heart disease. This could be a potential biological mechanism for increasing the risk of cardiovascular disease. Alternatively, the lack of a consistent association may be due to a small effect being swamped by the wide variation in intake of naturally occurring PPARγ modulators in foods.

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- Several studies investigated the link between PFAS and hypertension, based on self-report of hypertension or taking medication. When actual blood pressure was measured in children, there was no association with hypertension and exposure to PFOS or PFOA.
- The current evidence for cardiovascular disease risks is limited, and based on studies of mortality and cross-sectional self-reported health in PFAS exposed workers and in residents exposed to PFAS in drinking water. Changed risk factors for heart disease may take decades to manifest as disease, and stronger evidence would come from very long-term cohort studies, and those that could demonstrate causal mechanisms (to exclude confounding and reverse causation).
- Cardiovascular disease, often linked to cholesterol, was a common concern of those exposed to PFAS who responded in the public consultation.

6.12.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and cardiovascular disease:

- Associations of PFAS with cardiovascular disease have not generally been observed but the relationship is poorly characterised. The known biological effects of PFAS on metabolism suggest this should be the primary concern from excessive exposure in adults.
- As noted in other sections of this report, there are consistent associations with biomarkers linked to cardiovascular disease (e.g. uric acid, cholesterol, kidney function).
- Evidence to date does not establish whether PFAS at exposure levels seen in Australia might increase risks of cardiovascular disease, due to weak study designs, limited adjustment for confounders, the possibility of reverse causation, and the lack of any measured causative mechanism.
- Due to the small number of studies, and their limitations noted above, the existing scientific evidence does not warrant any change to peoples' medical management. Established risk factors for cardiovascular disease such as smoking, poor diet, excessive alcohol, diabetes, some prescription medications, and lack of exercise are likely to be of a much greater magnitude than those potentially caused by PFAS.

To further investigate the association between PFAS exposure and cardiovascular disease in an Australian setting, the Panel suggests the following research priorities:

- Further cross-sectional studies are unlikely to provide useful information, but well-designed long-term cohort studies may provide stronger epidemiological evidence.
- Studies that look for causal evidence are a key research need. Relevant studies would (for example) investigate direct evidence for PFAS concentrations that activate potential causal biochemical mechanism(s) in humans (e.g. PPAR activation), or determine whether as PFAS concentrations in individuals reduce, biomarkers associated with cardiovascular risk also decrease eg cholesterol, weight, insulin resistance and blood pressure.

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6.13. Respiratory effects and PFAS exposure

Studies that have investigated exposure to PFAS and respiratory effects in humans, while very limited, have been reviewed recently by an international authority and one systematic review.

6.13.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of one key international authority report published in 2015 and one systematic review that analysed the human epidemiological evidence regarding exposure to PFAS and respiratory effects:

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR, 2015).** Draft Toxicological Profile for Perfluoroalkyls.

Systematic reviews

- **Kirk et al. (2018).** The PFAS Health Study: systematic literature review. Australian National University.

The Panel acknowledges that FSANZ commented on respiratory effects, citing the US EPA's comment about the C8 Science Panel's findings in the 'Hazard assessment report for PFOS, PFOA and PFHxS'. However, FSANZ did not review epidemiology studies on PFAS exposure and respiratory effects, and the 'Hazard assessment report' is not considered further in this section. The US EPA did not review the epidemiology studies on respiratory effects; instead they reported on the findings of the C8 Science Panel. For this reason, the US EPA reports are not considered further in this section. No other key international reports or systematic reviews reviewed the human evidence on respiratory effects and PFAS exposure.

Note: Asthma and wheezing are included in the 'Immunological effects' section.

6.13.2. Key national and international reports

US Agency for Toxic Substances and Disease Registry (ATSDR, 2015)

In 2015, the ATSDR published the 'Draft toxicological profile for perfluoroalkyls'. In this profile, the ATSDR considered one human exposure study on respiratory effects.

Studies reviewed

The study reviewed by the ATSDR was by Sakr et al. (2007b).

Considerations and conclusions

The ATSDR did not make any statements or conclusions about respiratory effects in the 'Public health statement for perfluoroalkyls' or' Relevance to public health' sections of the report. The one study reviewed was included under 'Systemic effects – respiratory effects'.

Summaries of studies reviewed

Of the study by Sakr et al. (2017), the ATSDR reported that: "*Pulmonary function tests conducted on workers potentially exposed to PFOA in a fluoropolymers production plant were within normal limits (Sakr et al. 2007b). This cross-sectional study assessed a total of 1,025 workers whose serum PFOA levels ranged from 5 to 9,550 ng/mL.*"

The studies on asthma reviewed by the ATSDR are included in the section 'Immunologic effects'.

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6.13.3. Systematic reviews

Kirk et al. (2018)

Kirk et al. (2018) evaluated three papers that investigated the effect of PFAS exposure on the respiratory system in adults. These were different studies to the one study reviewed by ATSDR.

Studies reviewed

The three studies reviewed by Kirk et al. were by Leonard et al. (2008), Nolan et al. (2010), and Steenland and Woskie, (2012). Of these studies:

- Two investigated the association between occupational exposure to PFOA and respiratory disease mortality (Leonard et al. 20018; Steenland and Woskie, 2012).
- One determined the relationship between PFAS exposure and lung disease in pregnant women living in a region of the USA with drinking water contaminated with PFOA (Nolan et al. 2010).

Considerations and conclusions

Kirk et al. undertook a risk of bias assessment for the studies they reviewed and determined that all of the papers on respiratory effects had a moderate risk of bias, and that: "*All respiratory health outcomes were ineligible for meta-analysis.*"

The table below shows the associations determined by Kirk et al. for all end points evaluated:

Associations at a glance (Respiratory health outcomes)

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

Source: Associations at a glance, taken from Kirk et al. 2018, pp. 140.

Summaries of studies reviewed

Chronic obstructive pulmonary disease (COPD)

Kirk et al. noted: "*Chronic obstructive pulmonary disease (COPD) is an umbrella term used for three respiratory conditions; emphysema, chronic bronchitis and chronic asthma.*"

Of the two studies on COPD (Leonard et al. 2008 and Steenland and Woskie, 2012), Kirk et al. reported that: "*Leonard et al. [2008] and Steenland and Woskie [2012] 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. [2008] began with exposure in 1948, 4 years prior to the period of exposure in the analysis by Steenland and Woskie, [2012]. Neither analysis reported a statistically significant association between PFOA exposure and COPD related death in the cohort. Leonard et al. [2008] also reported on bronchitis mortality and emphysema mortality separately and found no significant associations between them and estimated PFOA exposure.*"

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Lung disease

Of the one study that investigated the association between modelled PFOA exposure level and lung disease in pregnant women (Nolan et al. 2010), Kirk et al. stated that: "*Nolan et al. [2010] 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.*"

6.13.4. Summary of key national and international reports and systematic reviews

Recent key national and international reports:

- The ATSDR reported no associations were found for PFOA exposure and pulmonary function tests in a cross-sectional study of occupationally exposed workers.

Systematic reviews:

- Kirk et al. evaluated three papers; no significant association was found between PFOA exposure and COPD, bronchitis or emphysema mortality in manufacturing workers, or lung disease in pregnant women. The authors consider that there was inadequate evidence for all respiratory outcomes.
- Very little research has been completed and no significant results have been found.

6.13.5. Expert Health Panel synthesis to support advice to the Minister

- There is no known direct effect of PFAS on the lungs, but effects through other pathways, such as altered immune function, may be possible.
- There is very limited research and none of it supports any associations.
- The public consultation indicated respiratory effects were not a common concern of those who participated in the public consultation.

6.13.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and respiratory effects:

- An association with respiratory effects has not been demonstrated in human studies, and there is no known biological mechanism. As the main exposure pathway is through ingestion, research into respiratory disease is not considered a high priority for research.

To further investigate the association between PFAS exposure and respiratory effects in an Australian setting, the Panel suggests the following research priorities:

- Specific research on respiratory effects is not a high priority.
- Any research on respiratory effects should be done as part of a global health assessment e.g. analysing whether elimination of PFAS alters biomarkers of immune function including those relevant to the respiratory system.

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6.14. Skeletal effects and PFAS exposure

Studies that have investigated exposure to PFAS and effects on the human skeleton, while limited in scope and numbers, have been reviewed recently by an international authority and two systematic reviews.

6.14.1. What evidence did the Panel consider?

The Panel considered the findings and conclusions of one published key international authority report published in 2015 and two systematic reviews since 2016 that analysed the human epidemiological evidence regarding exposure to PFAS and skeletal effects.

Key national and international reports

- **Agency for Toxic Substances and Disease Registry (ATSDR, 2015).** Draft Toxicological Profile for Perfluoroalkyls.

Systematic reviews and reviews

- **Priestly (2016).** Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS). Monash University;
- **Kirk et al. (2018).** The PFAS Health Study: systematic literature review. Australian National University.

While the Panel notes that FSANZ commented that: "*various epidemiological studies have found an association between PFOA and increased risk of osteoarthritis*", FSANZ did not review epidemiological studies on skeletal effects, and the 'Hazard assessment report for PFOS, PFOA and PFHxS' is therefore not considered further in this section.

Chang et al. (2016) also reviewed three studies on osteoarthritis (Innes et al. 2011; Steenland et al. 2015; Uhl et al. 2013), and the findings can be found in the autoimmune section under Section 6.8.3. No other key international reports or systematic reviews reviewed the human evidence on PFAS exposure and skeletal effects.

6.14.2. Key national and international reports

Agency for Toxic Substances and Disease Registry (ATSDR, 2015)

In 2015, the ATSDR, in its draft toxicological profile for perfluoroalkyls, considered the human evidence on musculoskeletal effects in the 'Health effects – systemic effects – musculoskeletal effects – oral exposure' section.

Studies reviewed

The ATSDR reviewed two studies on osteoarthritis (Uhl et al. 2013, Innes et al. 2011) in the 'Health effects – systemic effects – musculoskeletal effects – oral exposure' section.

Considerations and conclusions

The ATSDR did not make any statements or conclusions about musculoskeletal effects in the 'Public health statement for perfluoroalkyls' or 'Relevance to public health' sections of the draft toxicological profile.

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Summaries of studies reviewed

Osteoarthritis

The ATSDR reported in the 'Health effects – systemic effects – musculoskeletal effects – oral exposure' section that: "*Two studies have examined the possible association between serum PFOA and PFOS levels and the risk of osteoarthritis; the possible mechanisms associated with these findings have not been elucidated.*"

Of the study by Uhl et al. (2013), the ATSDR reported the following: "*In a study of NHANES participants (2003–2008) aged 20–84 years (n=1,888 males and 1,921 females), the odds of self-reporting osteoarthritis were significantly higher in women with serum PFOA levels in the highest quartile (>5.88 ng/mL), as compared to women with serum PFOA levels in the first quartile (≤2.95 ng/mL) (adjusted OR 1.35, 95% CI 1.02–1.79) (Uhl et al. 2013). An elevated OR was also observed for women with serum PFOS in the fourth quartile (>20.97 ng/mL), but it was not statistically significant (adjusted OR 1.34, 95% CI 0.97–1.63). When males and females were combined, subjects with the highest PFOS levels had a significantly higher risk of osteoarthritis (adjusted OR 1.77, 95% CI 1.05–5.96). No significant associations between serum PFOA or PFOA and odds of osteoarthritis were found in males only.*"

Of the study by Innes et al. (2011), the ATSDR reported: "*Innes et al. (2011) examined 49,432 male and female adult (3,731 subjects reporting physician-diagnosed osteoarthritis) participants in the C8 Health Project. After adjustment for potential confounders, the odds of a subject reporting osteoarthritis were significantly higher in subjects with serum PFOA levels in the fourth quartile (≥72.0 ng/mL) compared to subjects in the first quartile (0.25–13.5 ng/mL) (OR 1.42, 95% CI 1.26–1.59). When segregated by age and BMI, there were stronger associations between serum PFOA levels and osteoarthritis in subjects under 55 years of age and in nonobese (BMI<30) subjects. In contrast to the serum PFOA findings, there was a lower risk of osteoarthritis in subjects with serum PFOS levels in the fourth quartile ≥29.4 ng/ml) compared to the first quartile (0.25-13.6ng/ml) OR 0.76, 95% CI 0.68-0.85).*"

6.14.3. Systematic reviews

Priestly (2016)

Studies reviewed

In the section 'Miscellaneous end points', Priestly reviewed two studies on osteoarthritis by Innes et al. (2011) and Uhl et al. (2013). These studies were both reviewed by the ATSDR, with summaries provided above. Priestly also reviewed a study by Khalil et al. (2016) on osteoporosis and bone mineral density.

Considerations and conclusions

Priestly did not make any specific overall conclusions about the three studies he reviewed under 'Miscellaneous end points'.

Summaries of studies reviewed

Osteoarthritis

Of the paper by Innes et al. (2011), Priestly commented that: "*The authors conceded that the cross-sectional nature of the study limits any conclusion about causality, but they suggested the divergent findings with PFOA and PFOS were worthy of further study.*"

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Regarding the findings of Uhl et al. (2013), Priestly commented that: "*One difference between this and the Innes et al. (2011) study was that PFOS levels in the NHANES database are higher than PFOA, where this is reversed in the C8 Health Study.*"

Osteoporosis and bone mineral density

Priestly reviewed the paper by Khalil et al. (2016) and reported the following summary: "*In a study attempting to link PFAS exposures to bone mineral density and osteoporosis, Khalil et al. (2016) interrogated the NHANES database (2009-10) on the relationship between serum PFAS and osteoporosis, as measured by bone mineral density for the total femur (TFBMD; n=1914), femoral neck subregion (FNBMD; n= 1914) and lumbar spine (LSBMD; n=1605). They found PFOS serum levels were inversely related to FNBMD in both sexes; but only TFBMD and FNBMD in women... In postmenopausal women, the negative associations were: PFOS with TFBMD and FNBMD, and PFNA with all three measures. Neither the gender specificity nor the selectivity of the effects for different PFAS and different bone sites could be explained.*"

Kirk et al. (2018)

In their systematic review, Kirk et al. evaluated the human epidemiological evidence that investigated the development of skeletal conditions in adults. The authors reviewed the evidence on osteoarthritis, osteoporosis, bone mineral density and bone fractures.

Studies reviewed

Kirk et al. evaluated five papers (Uhl et al. 2013, Innes et al. 2011, Steenland et al. 2015, Khalil et al. 2016, Lin et al. 2014), including:

- three studies on osteoarthritis (Steenland et al. 2015; Innes et al. 2011; Uhl et al. 2013);
- one study on osteoporosis (Khalil et al. 2016);
- two studies on bone mineral density (Khalil et al. 2016; Lin et al. 2014);
- one study on bone fractures (Lin et al. 2014).

The studies by Uhl et al. (2013), Innes et al. (2011), and Steenland et al. (2015) were also reviewed by the ATSDR (2015), Chang et al. (2016), and Priestly (2016). The study by Khalil et al. (2016) was also reviewed by Priestly (2016).

Considerations and conclusions

Kirk et al. undertook a risk of bias assessment for the studies they reviewed and determined that all of the papers on skeletal effects had a high risk of bias, and that: "*All skeletal health outcomes were ineligible for meta-analysis.*"

The table below shows the associations determined by Kirk et al. for all end points evaluated.

Associations at a glance (Skeletal health outcomes)

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

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Health outcome	PFAS exposure	Evaluation of 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

Source: From page 132, Kirk et al. 2018.

Summaries of studies reviewed

Osteoarthritis

Of the study by Steenland et al. (2015), Kirk et al. reported that: "*Steenland et al. [2015] found no significant association between occupational exposure to PFOA and osteoarthritis in a cohort of DuPont workers.*"

Kirk et al. made the following comments about the three studies they reviewed: "*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. [2011] and Steenland et al. [2015] studying highly exposed communities and Uhl et al. [2013] using NHANES data that is broadly of the United States population.*"

Osteoporosis

Kirk et al. reviewed one study on osteoporosis by Khahil et al. (2016). This study was also reviewed by Priestly (2016) and a summary of this study is provided above. Kirk et al. made the following comment on this study: "*As the results stated by Khalil et al. [2016] 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

Kirk et al. reviewed two studies on bone mineral density (Khalil et al. 2016; Lin et al. 2014). Priestly (2016) also reviewed the study by Khahil et al. (2016), and a summary of findings is provided above under the studies reviewed by Priestly.

Of the two studies, Kirk et al. reported the following about the study populations and measurements: "*Khalil et al. [2016] and Lin et al. [2014] 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.*"

Kirk et al. made the following observation about these two studies before providing summaries of the studies: "*Overall, the results were conflicting of the association between PFAS and bone mineral density, with inconsistencies within each study and between the two studies.*"

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Of the study by Lin et al. (2014), Kirk et al. reported that: "*Lin et al. [2014] 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. [2016] did not identify an association. Khalil et al. [2016] 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. [2016] and Lin et al. [2014] found no association between PFOA exposure and bone mineral density of the lumbar spine.*"

Kirk et al. made the following comments about these two studies: "*The findings reported by Khalil et al. [2016] and Lin et al. [2014] 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. [2016], 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. [2014] 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. [2016].*"

Bone fractures

Kirk et al. reviewed one study on bone fractures (Lin et al. 2014) and reported: "*Lin et al. [2014] 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.*"

6.14.4. Summary of key national and international reports and systematic reviews

Recent key national and international reports:

- ATSDR noted that two studies have examined serum PFOS and PFOA levels and increased risk of osteoarthritis but the mechanisms associated with the findings have not been elucidated.

Systematic reviews:

- Priestly concluded it was too early to draw definitive conclusions due to the small number of skeletal studies, and pointed to the findings on osteoarthritis worthy of further study.
- Kirk et al. evaluated all five skeletal studies to be at high risk of bias due to study design and noted that evidence was inadequate for associations between PFAS exposure and all skeletal outcomes evaluated i.e. osteoarthritis, osteoporosis, bone mineral density and bone fracture risk.

6.14.5. Expert Health Panel synthesis to support advice to the Minister

- There are a small number of cross-sectional studies on skeletal effects and PFAS exposure in a few adult study populations.
- Current data suggest that the limited evidence of significant associations relate to small changes in end-points such as osteoarthritis, osteoporosis/bone mineral density.
- The small amount of evidence which is available relates to associations with PFOA, PFOS, PFHxS or PFNA exposure.

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- Skeletal and rheumatological effects were not a concern of those exposed to PFAS who responded in the public consultation.

6.14.6. Expert Health Panel advice to the Minister

In considering the evidence, the Panel has the following advice to the Minister regarding exposure to PFAS and skeletal effects:

The evidence does not support PFAS being a major cause of skeletal or rheumatological diseases in highly-exposed communities and nor was it a concern noted in the public consultation.

To further investigate the association between PFAS exposure and skeletal effects in an Australian setting, the Panel suggests the following research priorities:

- Specific research on skeletal effects is not considered to be a high priority. Effects on bone growth would be best integrated within other studies of PFAS and childhood development, e.g. include measures of weight/growth/length from birth through childhood and into young adult age. This would be complemented by analyses of hormone levels relevant to bone formation (e.g. growth, thyroid and sex hormones).
- Rheumatological diseases would be best integrated with studies of overall health and/or immune function.

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6.15. Reverse causality and confounding

This term reverse causation is used when there is an association between an exposure and a health outcome, but it is possible that the outcome came before the exposure, so it is difficult to distinguish which is the cause and which is the effect. In the case of PFAS this could occur when there is an observed association between a PFAS concentration and certain types of health effects (e.g. kidney function, hormone levels) whereby the health effect could have resulted in greater accumulation of the PFAS. When it is written that there might be 'reverse causality' this means these conditions might increase PFAS concentrations. Determining the direction of causation is particularly a problem in cross-sectional studies (where both factors are measured at the same time).

The elimination of PFAS provides several possible mechanisms for reverse causality. The two main routes of PFAS elimination are via the kidneys and blood loss. So factors that reduce kidney filtration (e.g. kidney, heart or liver diseases) or blood loss (e.g. sex or thyroid hormonal disturbances in women altering menstruation) would be expected to lead to slower elimination and higher concentrations of PFAS. Further, kidney elimination is very slow due to very active reuptake of PFAS by the kidney tubules. The expression and activity of these transporters is under hormonal control, which might be affected by sex or thyroid hormone levels or chronic inflammation.

Confounding is a related concept, where a third factor related to both the exposure and the health outcome might explain the association and needs to be considered and, if possible, adjusted for in the analysis. For example, an association of a disease with PFAS concentrations needs to account for age and sex, as these two factors are likely linked to PFAS accumulation/elimination (due to general age-related decline in kidney function and increased elimination in pre-menopausal women) and also most diseases are more common in the elderly and in either males or females. Important confounding variables may be unknown or not recorded (e.g. smoking status or socioeconomic status) but still potentially explain the associations.

The possibility of reverse causation and/or confounding influencing the results does not necessarily refute there also being causation in either direction (e.g. kidney disease might cause higher PFAS while PFAS caused kidney disease). It does provide a strong argument for doing long-term prospective studies, where important potential confounding factors are recorded and the order in which things occur can be determined to assess the possibility of reverse causation.

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6.16. Limitations and issues about the human evidence base highlighted in the key international reports and systematic reviews

The key (inter)national authority reports and systematic reviews reviewed by the Panel raised many issues with the epidemiological studies that have investigated exposure to PFAS and health outcomes among adults and children. Such issues included the large number of cross-sectional studies, estimated serum levels of PFAS, confounding, bias and paucity of evidence. Many of the key reports and reviews also provide advice about study design needs and interpretation of the studies, particularly regarding causality.

Many of the issues that were raised about specific studies or groups of studies have been captured in the various health sections in this reort. This section is included to provide the overall limitations and issues identified in the various key reports and systematic reviews.

6.16.1. Key national and international reports

Agency for Toxic Substances and Disease Registry (ATSDR, 2015). Draft toxicological profile for perfluoroalkyls

The ATSDR made statements about exposure to PFAS and provided context about associations found in studies and what this means about causality, including:

"*If you are exposed to perfluoroalkyls, many factors determine whether you'll be harmed. These include how much you are exposed to (dose), how long you are exposed to it (duration), and how you are exposed (route of exposure). You must also consider the other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.*"

"*A number of epidemiology studies have evaluated potential health effects associated with exposure to perfluoroalkyl compounds. The three primary sources of this information are occupational exposure studies, studies of a communities living near a PFOA manufacturing facility with high levels of PFOA in the drinking water, and studies of populations exposed to background levels of perfluoroalkyl compounds (referred to as general population studies). One limitation of most of the available epidemiology studies is the lack of reliable environmental monitoring data. However, most studies measured serum perfluoroalkyl levels that were used as biomarkers of exposure.*"

"*The majority of the epidemiology studies using serum perfluoroalkyl levels as a biomarker of exposure examined possible associations between serum perfluoroalkyl levels and a specific health outcome. In statistics, an association is any relationship between two measured quantities that renders them statistically interdependent. Although a study may find a statistical association between serum perfluoroalkyl levels and a particular health outcome, it does not necessarily indicate causality or biological significance. ATSDR examined the consistency of the finding across studies, dose-response, and plausibility of an effect in assessing whether the data provide evidence of a relationship between perfluoroalkyl compounds and a specific health outcome.*"

"*A large number of studies have examined the possible health effects of PFOA and PFOS in humans. The effect of inhalation exposure to PFOA and PFOS has been examined in workers exposed to high concentrations of these compounds. Studies have also examined a large community exposed to high levels of PFOA in the drinking water and compared this community to the general population; ingestion was the primary route of exposure for these two groups. Most human studies have looked for a relationship between levels of perfluoroalkyls in the blood and a health effect. It is difficult to interpret the results of these studies because they are not consistent; some studies have found associations, but*

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others looking at the same health effect have not found these associations. Even though some studies have found significant associations between serum perfluoroalkyl levels and adverse health effects, it does not mean that perfluoroalkyls caused these effects. The effects may have been due to other factors that were not considered by the researchers."

United States Environmental Protection Agency (US EPA, 2016a, b). Health effects support document for perfluorooctanoic acid (PFOA, PFOS)

The USEPA did not have a specific section on limitations, rather the Agency commented on the limitations of the studies they reviewed. These comments are captured in the 'Health effects' sections.

National Toxicology Program (NTP) (2016). NTP monograph on immunotoxicity associated with exposure to perfluorooctanoic acid (PFOA) or perfluorooctance sulfonate (PFOS)

The National Toxicology Programme included a section on 'Limitations of the evidence base' and reported the following on the studies they reviewed that investigated exposure to PFOA/ PFOS and immunological effects.

Potential for multiple PFAS exposures

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

Antibody response

"While the association between both PFOA and PFOS and the antibody response is relatively well studied, additional epidemiological studies that address the dose-response relationship and can control for effects of other PFAAs would increase confidence in the bodies of evidence. Additional studies that examine the antibody response to the same vaccine across multiple populations would also increase

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confidence. For other measures of the immune system, there are no human data, including NK cell activity and DTH response. Few specific infectious disease end points have been examined in epidemiological studies and the study with the most power/largest sample size used a potentially less sensitive measure (i.e., hospitalization for any infectious disease, which would only capture the most severe outcomes and could miss potential associations with individual diseases) (Fei et al. 2010)."

Asthma and hypersensitivity

"The limitations of the epidemiological data on asthma and hypersensitivity are typical of studies with cross-sectional study design. Although the long half-life of PFOA in humans (2 to 8 years) (Olsen et al. 2007a, Kudo 2015) increases the likelihood that current serum measurements represent past exposure that would be biologically relevant for development of asthma, there is likely to be some exposure misclassification. Prospective studies that evaluate asthma, IgE and other hypersensitivity-related outcomes in children relative to early childhood exposures could increase confidence in this body of evidence. The results of two epidemiological studies show PFOA-associated increases in the incidence of ulcerative colitis in residents of the Ohio Valley, a region associated with elevated PFOA levels in drinking water, and workers exposed to PFOA that were a subset of the original analysis (Steenland et al. 2013, Steenland et al. 2015). There is low confidence in this body of evidence because the studies are from a single population and therefore there are no independent results from a separate population. Given the low confidence in the human body of evidence and the absence of animal studies, the data are inadequate to classify whether or not PFOA exposure is associated with the incidence of ulcerative colitis. These are the only studies of the potential association between PFOA or PFOS and autoimmunity. Studies of PFOA in animal models of ulcerative colitis [e.g., (Low et al. 2013)] or epidemiological studies of ulcerative colitis in other populations would increase confidence in this body of evidence."

New Jersey Drinking Water Quality Institute (DWQI, Public Review draft 2016). Health-based maximum contaminant level support document: perfluorooctanoic acid (PFOA)

In the 'Human studies' section, the DWQI made the following comments about co-exposure to PFAS, bias, and the limitations of observational studies to definitively prove causation. The DWQI also provided context and raised issues about the epidemiological evidence for the three exposure groups reviewed.

Confounding

"In human environmental health effect studies in general, confounding by co-exposure to contaminants other than the one being evaluated may be particularly important since it may bias results. In some instances, PFOA has been shown to be strongly correlated with other co-occurring PFCs which may not have been controlled for, and the same may be true for other environmental contaminants. This confounding bias could impact studies in any type of population, but may play a more important role in occupational populations which may be more likely than the general population to be exposed to co-occurring contaminants at meaningful levels. In general, co-exposure to other chemicals could also be more likely in communities where there are high levels of environmental contamination. However, this is not likely the case in the C8 Health Project, a large community study of populations with drinking water exposure to PFOA (discussed in more detailed below), since PFOA is the only contaminant that was reported to be present at elevated levels in drinking water or other environmental media. As is the case for epidemiologic studies of environmental contaminants in general, the nature of these observational epidemiology studies, in contrast to experimental studies, limits our ability to definitively conclude that PFOA causes health effects. However, the findings from observational epidemiology

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studies are useful in assessing consistency, strength of association, exposure response, temporality, specificity, and biologic plausibility – criteria which are useful in assessing causation."

Occupational worker studies

"Occupational studies are often considered useful for evaluating effects of environmental contaminants because exposure levels are generally higher than in general population or in communities exposed through site-specific environmental contamination. Mean or median serum PFOA levels in occupational studies reviewed in this report were generally over 1,000 ng/ml (ppb), several orders of magnitude higher than the median concentrations in the general population or in communities with drinking water exposure.

Associations of PFOA with some clinical parameters, including cholesterol, liver enzymes, and uric acid, exhibit a steep dose-response curve in the lower exposure range found in the general population, with a much flatter slope (approaching a plateau) at higher exposure such as those found occupationally (discussed in more detail below). For dose-response curves of this type, the associations found in populations with lower exposures may not be observed in workers because even the least exposed workers used as the comparison/reference group in occupational studies may have exposure levels that are high enough to fall on the much flatter upper portion of the dose-response curve.

Occupational studies may also have a selection bias from a "healthy worker effect" whereby workers usually have lower overall mortality and morbidity than individuals of the same age as a whole, since severely ill and disabled persons are typically not included in the workforce, especially in industrial settings (Shah, 2009). Longer duration of employment may also increase the effects of this bias, since sick people will be more likely to leave or change to safer work. Therefore, data based on duration of employment may not accurately reflect higher prevalence or larger magnitude of effects that are associated with longer exposures to the contaminant being evaluated.

Another issue with occupational studies of PFOA is the small number of exposed female employees which limits the ability of the occupational epidemiology to adequately address specific effects among women. An additional issue is the possibility of effect modification due to exposure to other chemicals. Exposure to other PFCs, including PFOS at the 3M Decatur plant, may have played a role in the observed associations. Differences in exposures to other chemicals among manufacturing facilities may result in differences in degree of association with various effects."

General population studies

"For the end points that were comprehensively reviewed, the majority of studies evaluated the general population and/or study populations with general population-level exposures to PFOA. Twenty nine (29) studies with general population, low-level exposures were identified. The serum PFOA concentrations (based on a measure of central tendency, which was presented as median, mean, or geometric mean) in these studies range from 0.9 to 7.1 ng/ml. A strength of the general population studies is their use of serum PFOA levels as the basis for exposure assessment. Because of the long human half-life of PFOA, serum levels do not rapidly fluctuate with short term variations in exposure, and serum levels taken at a single time therefore reflect long-term exposures. Serum levels thus provide an accurate measure of internal exposure for each study participant, an advantage over studies based on external exposure metrics such as drinking water concentrations. Among these studies, the large majority are cross-sectional (23 studies, plus one which includes a cross-sectional component). A general limitation of cross-sectional studies is that they evaluate information on both exposure and outcome at the same point in time, limiting their ability to establish temporality."

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Studies in highly exposed communities

"For the end points selected for comprehensive evaluation, 15 studies evaluated highly-exposed individuals residing in communities with known PFOA drinking water contamination or in close proximity to a factory utilizing or producing PFOA. A large majority of these studies (14) occurred among communities in the Mid-Ohio Valley near the DuPont Washington Works plant in Parkersburg, WV. This industrial facility used large amounts of PFOA in the manufacturing of a fluoropolymer, polytetrafluoroethylene (PTFE), and discharged PFOA to the environment resulting in widespread drinking water contamination. Many of the studies in this population are the result of the settlement of a class-action lawsuit by residents exposed to PFOA-contaminated drinking water which mandated that DuPont fund a health study called the C8 Health Project. Additional epidemiologic studies of associations with PFOA and health end points in this population have also been published by other researchers. The C8 Health Project is a community health study of approximately 70,000 Ohio and West Virginia residents of all ages (infants to very elderly) with at least one year of exposure to drinking water contaminated with PFOA at >50 ng/L to over 3000 ng/L (Frisbee et al, 2009; C8 Science Panel, 2014).

The C8 Health Project was conducted by the C8 Science Panel, which consisted of three epidemiologists chosen jointly by the parties involved in the legal settlement. This study is notable because of its large size, the wide range of exposure levels, and the large number of parameters evaluated. Data collected included serum levels of PFOA and other PFCs, clinical laboratory values, and health histories. The median serum PFOA concentration in this population was 28 ng/ml (ppb), and serum concentrations in the lowest two deciles were within the U.S. general population range at the time (<10ng/ml).

The C8 Science Panel was charged with determining if "probable links" exist between diseases and PFOA exposure in the C8 study population, based on the results of their studies and other information from the scientific literature. Probable links were defined as ".... given the scientific evidence available, it is more likely than not that a connection exists between C8 exposure and a particular human disease among class members...." Probable links were established with PFOA exposure and six health end points (clinically defined high cholesterol, kidney and testicular cancer, ulcerative colitis, thyroid disease, and pregnancy-induced hypertension). For a number of other end points, no probable link with PFOA exposure was reported. Associations were also found with additional health end points for which no probable link evaluation was conducted because they were not considered to be clinically defined diseases. These end points include increased serum levels of liver enzymes, uric acid, C-reactive protein, and others. C8 Science Panel reports and citations for peer-reviewed publications presenting the results of these studies are found at the C8 Science Panel website (C8 Science Panel, undated, b)."

Dutch National Institute for Public Health and the Environment (RIVM, 2017). PFOA exposure and health: A review of scientific literature

The RIVM noted in particular that end points were evaluated differently in different reviews leading to differing conclusions. RIVM noted "*Nine biological and physiological parameters and diseases were evaluated as being associated with higher PFOA concentrations in the blood by at least one (inter)national organization: increased concentrations of levels of liver enzymes in blood, concentrations of (total and LDL-) cholesterol in blood, thyroid effects, kidney and testicular cancer, pregnancy-induced hypertension and preeclampsia, reduced birth weight, increased uric acid concentrations in the blood, decreased vaccination response, and ulcerative colitis. The level of evidence for an association with PFOA concentrations in the blood differs between the various end points. Also, the evidence for a particular end point was evaluated differently in the different reviews. For example, The ATSDR (2015) described the evidence for an association between PFOA and uric acid concentrations in the blood as 'consistent evidence' (ATSDR, 2015), while the US EPA (2016a) mentions that 'an association was*

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observed, but potentially confounded'. In the present review, the evaluations of the epidemiological evidence for these end points from the previous reviews have been summarized."

Food Standards Australia New Zealand (FSANZ, 2017). Hazard assessment report (PFOS, PFOA, PFHxS)

FSANZ undertook three literature reviews of the human evidence for the 'Hazard assessment report for PFOS, PFOA, and PFHxS': the effects of PFAS on immunomodulation, birth weight and cholesterol concentrations in the blood. FSANZ raised a number of concerns abut the data and studies in all three reviews.

Limitations of data in immunological studies

"A number of limitations with the available data were noted. These included comparisons of 'low' and 'high' exposure groups where the differences are over a very low and narrow serum concentration range (0.002 – 0.05 mg/L), and potential co-exposures to other environmental chemicals that are known to have immunomodulating effects. It was noted that many of the associations are weak and the effects are small and of questionable clinical significance."

Residual confounding in birth weight studies

In the 'Commentary' of 'Appendix 1: 'Observational studies of PFAS and birthweight', FSANZ raised the issue of residual confounding, stating:

"In addition to the usual considerations about residual confounding, such as whether smoking has been adequately measured and adjusted for, there are several specific gaps in the analyses examined. One notable feature in the papers is that many authors describe the effect of PFOA and PFOS on birthweight after adjusting for various factors which might confound the relationships, such as gestational age, parity, maternal smoking of body habitus. These analyses are performed separately for PFOS and PFOA. Authors sometimes describe the correlation between PFOS and PFOA in their data sets (Table A1.9). However, there does not seem to have been any consideration of whether the analysis examining PFOA should be adjusted for PFOS concentrations and vice versa. For example, in the study of Chen et al. (2012) PFOS has a much larger coefficient than PFOA and so it is possible that the PFOA result might be confounded by PFOS.

An exception is the analysis of the C8 cohort by Darrow et al. (2013) who found that simultaneously including both PFOS and PFOA in the same model halved the small effect on birthweight observed for PFOA did not change the effect for PFOS importantly (Table A1.10). In other words, the effect seen for PFOA was partly due to PFOA acting as a surrogate for PFOS. The correlation between the two PFAS in this study was lower than any other shown in Table A1.9 and raises questions about whether there may be confounding of the PFOA result shown in the Johnson meta-analysis. This study is unusual among the available studies in that the median concentration of PFOS and PFOA was almost the same in their subjects and it has a larger sample size than any study included in the meta-analysis of Johnson et al. (2014).

Furthermore, some authors have measured other PFAS and sometimes other chemicals such as PCBs in the same blood sample and these may or may not have associations with birthweight. Only rarely do authors comment on whether any of these other contaminants confound the relationships of PFOS and PFOA with birthweight. For example, Lauritzen et al. (2016) state that only the odds ratio for the association between PFOA and being born small-for-gestational age remained statistically significant when PFOA, PFOS and five organo-cholorine chemicals were included in the same model."

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"This analysis excludes a number of studies which did not report their results in a suitable format for inclusion. It also assumes that the relationship is linear whereas many of the authors of the underlying paper used a logarithmic transformation when analysing their data. It is possible that the body of evidence contains selective reporting or publication bias in the body of literature leading to an overrepresentation of studies reporting significant adverse effects on birthweight. Furthermore most studies examined associations for PFOA and PFOS separately and did not conduct a mutually-adjusted analysis despite often noting a substantial correlation between PFOA and PFOS. Other explanations of the association are also possible, such as the presence of a physiological change leading to increases in blood PFAS and decreases in birthweight."

Cholesterol

FSANZ raised several issues with respect to the epidemiological studies they reviewed in the 'Commentary' section of 'Appendix 2: Observational studies of PFAS and cholesterol concentrations', including potential publication bias, confounding. FSANZ also commented on the possible mode of action, including the role of the kidney in cholesterol levels, and some concurrent biological process that is not yet understood.

Below are the issues and observations highlighted by FSANZ from their literature review on PFAS and cholesterol concentrations:

Publication bias

"This report has focused on describing the results from the studies of PFAS chemicals and cholesterol concentrations but has not considered either p-values or standard errors for several reasons. Firstly, p-values are not a measure of effect; they describe the probability of obtaining the observed result, or a more extreme result, if the null hypothesis is true. It is customary to set some value, such as <0.05 to reject the null hypoth[e]sis and this is called 'statistically significant'. If there is truly no effect or association (the null hypothesis seems to be correct), then this will not be statistically significant, by definition. It is not possible to determine whether the inconsistent presentation of information across the studies occurs because the samples were not tested for certain cholesterol fractions or whether the authors have failed to report non-significant associations. It is also notable that some papers do not report all results, possibly because they were not statistically significant. For example, Lin et al. (2013) provide numerical data for their non-significant LDL-C analyses, but not for their total-C or HDL-C analyses. Gilliland et al. (1996) found non-significant associations between all three cholesterol fractions and total serum fluorine (as a surrogate for PFAS), but then presented a significant reduction in HDL-C from a regression analysis in which a categorised HDL-C variable appears to have been analysed as a continuous variable. Therefore the question of whether there is publication bias affecting this body of literature must be raised."

Bias and issues with analysis

"Studies have been included in this review regardless of whether or not they have reported their results in a common format because failure to do this may have introduced a bias into the body of evidence. As far as it is possible to tell, the results of studies which could not be graphed do not contradict the results of studies which could be graphed in a qualitative sense although it is not possible to make a quantitative comparison.

In addition, some of the methods used to analyse data in some papers seem questionable and so their p-values and standard errors are also questionable. For example, in one paper it seems that the PFAS data have been grouped into quantiles and then the quantiles have been entered into the regression equation as a continuous variable instead of being treated as a set of dummy variables. No quantitative

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results can be derived from some studies because one or both of the PFAS and cholesterol variables have been logarithmically transformed but the authors do not state the base used in the transformation or provide any back-transformed results. In this case, it is only possible to state the direction of the association found in the study."

Confounding

"The extent of control for confounding across the studies is variable depends on the other characteristics that were included. A number of studies note the correlation between the concentrations of PFOA and PFOS but they do not adjust the results of each PFAS for the other. For example, Nelson et al. (2010) report a correlation of 0.65 between PFOS and PFOA in the NHANES dataset. Lin et al. (2013) is an exception as they measured four PFAS (PFOS, PFOA, perfluorononanoic acid and perfluorodecanoic acid) in adolescents and young adults and conducted a composite analysis of effects on carotid intima medial thickness, which was their focus, but not of other outcomes such as cholesterol concentrations. Similarly, populations with high exposure to PFAS due to occupation or environmental contamination might have exposure to other contaminants and these have not been considered in the studies. Most studies do not adjust for diet. Skuladottir et al. (2015) is an exception; they used semiquantitative food frequency questionnaires and examined various associations. In their population of pregnant women, the highest quartile of saturated fat intake had total-C concentration that was 0.61 mmol/L higher than women in the lowest quartile. Intake of PFOS was positively associated with saturated fat intake but intake of PFOA was not. However, adding dietary factors to an adjusted model already containing age, parity, education, smoking and prepregnancy BMI did not alter the effect associated with either PFOS or PFOA. Hence, the non-inclusion of dietary measurement might be more or less important depending on what other factors have also been measured and controlled for."

Mode of action considerations

"Although some studies have examined a range of other biochemical parameters, such as thyroid function, kidney function does not seem to have been examined together with cholesterol concentrations. This may be relevant because PFAS concentrations increase as glomerular filtration rate decreases. This is a possible factor that might also lead to changes in cholesterol metabolism.

No randomised controlled trials were found. One striking feature is that the observational studies in humans have the opposite finding to studies in animals. A conference abstract reporting on a Phase 1 study investigating an ammonium salt of PFOA as a cancer treatment was found but did not meet the inclusion criterion of being conducted in healthy people. This study used a dose-escalation strategy until a dose-limiting toxicity was found. Among 41 middle-aged adults with advanced cancer who received a single dose of between 50 and 1200 mg of the ammonium PFOA salt for a median of 6.5 weeks, the authors reported "reductions in LDL-C consistent with a PD effect" (MacPherson et al. 2011). While there is no randomised control group, this is a longitudinal study with a clearly defined exposure. Patients had a range of cancers and so it is unlikely that they all had disturbed lipid metabolism. It adds to the debate concerning whether the increases in LDL-C observed in the human epidemiological studies are an adverse effect caused by PFAS exposure or reflect some concurrent biological process that is not yet understood."

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6.16.2. Systematic reviews

Saikat et al. (2013). The impact of PFOS on health in the general population: a review

Saikat et al. included a limitations section in their review of the literature of 15 studies on PFOS on health in the general population. The authors raised a number of limitations and issues, the most notable being study design.

Study design

"The main limitation of the reviewed studies was the study design. Two of the 15 papers were descriptive and the remaining epidemiological studies were of varying quality and design (ESI Table 1†). To determine whether there is a causal relationship between an exposure and an outcome a cohort design should be used, but only three of the identified papers had this design and these studies also had limitations. The first, Eriksen et al. had a long follow-up period (12 years), and a robust method of case ascertainment; however the exposure measurement was only conducted once despite the follow-up period being longer than the half life of PFOS. The lack of a significant association between PFOS and cancer is reassuring, and expected due to the lack of mutagenic properties; however this may also have been due to the sample size, and further studies may be needed to explore whether there are any associations.

The second cohort study, Fei et al. demonstrating a significant association between blood serum PFOS and self- reported female infertility was limited by selection bias. They chose a population of women with a successful pregnancy to study the risk of infertility. Therefore it is possible that the detected association is underestimated and may actually be higher. This study was further limited by the self-reported outcome measurement which has the potential to introduce recall bias. In another cohort study Fei et al. indicated that higher maternal concentrations of PFOS may be associated with a shorter duration of breastfeeding. However this association was restricted to multiparous women and no consistent association was observed among primiparous women. The association observed may be non-causal as studies indicate that the women who previously breastfed are more likely to do so again and a reduction in PFOS may occur through excretion (as shown in Karrman et al.; Tao et al.).

Ten of the epidemiological studies had a cross-sectional design where the exposure and outcome were measured simultaneously and therefore they were not able to demonstrate any causal association between PFOS and health outcome. However their findings can be used to develop further research hypotheses.

Most of the studies in this review were based on samples from large population based cohorts, including the well validated NHANES and the DNBC. The sampling methods used in these studies improve the generalisability of the findings and reduce the potential for selection bias. However the sample size available for study from the NHANES study was reduced to a third due to the small proportion of participants randomly selected to have PFC measurements."

Exposure to other PFAS

"There is a possibility that participants may have been exposed to unmeasured PFCs. Adequate control of other relevant exposures is likely to be a major limitation. All of the studies described how the PFOS exposure was measured: thirteen used blood serum PFOS and two used blood plasma PFOS. One study adjusted their analysis for the presence of albumin and found that it did not generally alter the results. None of the studies provides any information about environmental sources of PFOS (e.g. drinking water, diet) to characterise the association between environmental levels, exposure pathways, human levels and health outcomes."

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Evaluation of surrogate outcomes rather than end points

"Eight studies in this review looked at health end points (cancer, infertility, thyroid disease, hyper-cholesterolaemia, breastfeeding, ADHD); the remaining studies looked at surrogate outcomes (e.g. cholesterol levels). Therefore more work is needed to determine the significance of surrogate outcomes and how they relate to health status. Furthermore, in one study approximately 95% of the participants had thyroid hormone levels within the normal range which makes it difficult to determine the significance of the reported association between PFOS and thyroid hormones."

Recall bias

"The use of self-reported outcome variables in two of the studies is unlikely to introduce recall bias as participants are unlikely to be aware of their blood serum PFOS levels. However, Meltzer et al.[24] combined all thyroid diseases into a single category which limits the scientific interpretation of the findings."

Lam et al. (2014). The navigation guide – evidence-based medicine meets environmental health: integration of animal and human evidence for PFOA effects on fetal growth

Lam et al. included a 'Limitations' section in their paper on the use of the Navigation Guide to integrate animal and human evidence on PFOA and human fetal growth.

"One benefit of our adoption of the IARC approach is that it was transparent and simple to integrate the evidence from human and non human bodies of available evidence once we rated each stream's strength of evidence separately. However, this meant that quantitative evaluations of the effect estimates for each body of evidence were kept separate and not integrated earlier on in the process. There has been much discussion recently in several research fields to utilize quantitative methods that can integrate diverse sources of data, such as human and non human toxicity evidence, into a single quantitative model that can account for the different sources of data and expected contribution of each data set to the evidence for human toxicity (DuMouchel and Harris 1983; Jones et al. 2009; Peters et al. 2005). Future investigation into methods for quantitatively integrating these diverse sources of data (e.g., in a hierarchical Bayesian model) is warranted and would be an important contribution to advancing strength of evidence conclusions in environmental health. The nomenclature of the overall strength of the human evidence (i.e., the terms "known," "probably," and "toxic") generally had differing connotations among review authors despite agreement on the underlying definitions that supported the final conclusion. Some of the review authors found "known to be toxic" to be an accurate descriptor of the body of evidence, whereas others felt the descriptor "probably toxic" was more appropriate. Our discussions of the variability of our own subjective reactions to "known" and "probably" emphasized the need for further delineation of pre specified objective criteria for the strength of the evidence definitions."

"Specifically, there is currently no consensus in environmental health on how to name and communicate the strength of the evidence, and indeed there are many examples of similar terms that are commonly used to characterize varying strengths of evidence; for example, terms used to describe "moderate" evidence include "balance of evidence," "balance of probabilities," "reasonable grounds of concern," and "strong possibility" (Gee 2008). Research related to climate change has shown that the public consistently misinterprets probabilistic statements such as "unlikely" or "very unlikely," used in Intergovernmental Panel on Climate Change reports, and there are large individual differences in the interpretation of the statements that are associated with the public's views and beliefs on climate change (Budescu et al. 2012). Research on better ways to communicate uncertainty is critical, and

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discussion of improved communication needs to include the users of the information, such as policy makers and the public."

"Finally, exposures to environmental contaminants that lead to chronic disease or adverse reproductive and developmental health outcomes are complex and poorly understood. Such harm can be irreversible and can span across generations, making a strong case for timely decision making and actions to prevent harm. However, having limited data or multiple studies of varying quality and findings can often hinder the ability to take such action. Criteria for evaluating diverse sources of scientific evidence to support action on the science is lacking and is therefore a critical unmet research need (Krauth et al. 2013)."

Roth and Wilks (2014). Neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals: a systematic review of the epidemiological literature using a quality assessment scheme

In the 'Discussion' section of their systematic review, Roth and Wilks comment on issues regarding the literature they reviewed for neurodevelopmental and neurobehavioural effects. The authors investigated studies on PBCs and PFCs. THe PFCs are the chemicals of interest in this review by the Expert Health Panel. Roth and Wilks discuss the issues in the evidence base on PBCs and PFCs together in the Discussion. Their comments are as below:

"Our systematic review of the literature largely confirmed the difficulty of appraising the body of evidence for a given neurodevelopmental or neurobehavioural outcome. Collectively, when looking at general effects that may be attributed to either or both the brominated or the perfluorinated class ("class effects"), studies suggest a certain number of potential neurodevelopmental and neurobehavioural adverse effects in various functional domains such as fine motor skills, cognitive performance and general behavioural health, including attention deficits, impulsivity or hyperactivity. However, upon closer examination of the evidence for each individual chemical on a case-by-case basis, many inconsistencies emerge with some associations being observed only for a specific health out- come in relation to a specific chemical. This considerably increases the difficulty of fully appraising of the overall body of evidence due to: (i) the general lack of comparability across studies, most notably in term of exposure characterization, age of the children, and functional domains assessed; (ii) the limited number of available studies, in particular for PFCs; (iii) the general lack of consistency of effects for a given chemical between studies; (iv) the lack of individual data on the specific toxicological profile of each PBDE congener which are often assessed as the sum of their total concentrations. We also identified several frequently observed shortcomings that may diminish the strength of evidence for certain specific effects and more generally contribute to questioning the validity of certain studies: (i) the lack of consideration of certain confounding factors; (ii) uncertainties regarding exposure characterization (tim ing of exposure or life stage of assessment); (iii) the inadequacy of sample size (underpowered studies); (iv) the lack of a clear dose response) the representativeness/generalizability of the results."

Confounding

"In general, we found that assessment of causality was difficult. In many instances, the reported associations could be confounded by other factors that could influence neurodevelopment and that were not controlled for in the statistical models. This may lead to inappropriate inference. The selection of relevant confounding variables from a larger set of potential confounds should be determined a priori based on empirical evidence from previous research, thus avoiding over-fitting the statistical model (Babyak, 2004). In practice though, there is a need to find the right balance between a limited, manageable set of covariates and an adequate control of the potential confounders, especially when the study population size is modest. Confounders and effect modifiers may be important both at the

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individual level and in the environment, such as poor education and low socio-economic status of the family, various maternal and pregnancy characteristics, smoking or alcohol consumption during pregnancy, as well as co-exposures to other environmental contaminants (see supplementary material S5). Most notably, with the exception of a few studies that controlled for PCBs and OCs (DDE, HCB) (Gascon et al. 2012), PCBs and OPs (Eskenazi et al. 2013) or heavy metals (lead, mercury) (Gump et al. 2011), exposure to other neurotoxicants could have interfered with the outcome of these studies. Interestingly, none of the PBDE studies adjusted for PFCs, and vice-versa. When linking exposure to effects, limitations may result from the study design and an inadequate exposure characterization; e.g. in Gascon et al. (2011) postnatal exposure to PBDEs was measured at the same time as the neurodevelopmental tests were administered, which makes the interpretation of any association difficult, whereas in Hertz- Picciotto et al. (2011), current children PBDEs blood levels were measured after children were assessed for autism or developmental delay and were used as a proxy for exposures that preceded the neuropathologic changes leading to those health outcomes. Cohorts and cross sectional studies usually include a comparison group, whereas case series typically do not. Ten studies (56%) divided their partipants into quartiles or percentiles a posteriori once the exposure measurement was done, the lowest quartile being typically used as the referent group. However, without an appropriate control group in the general population, it becomes more difficult to evaluate the association between an exposure and an outcome."

Study size and lack of power calculations

"Collectively, a major identified limitation is that none of the studies evaluated appeared to have performed a power calculation to assess if the study size was appropriate to detect an effect or no effect. Only a single nationwide, population-based birth cohort (Fei et al. 2008b) had a large enough study sample (n = 1399) to possibly warrant the assumption of an adequately powered study. Many studies had a population below 100 participants. An appropriate sample size is crucial for the statistical power of a study, because underpowered studies can give an overestimation of the effect. This is particularly true for those evaluated small sample size studies that suffer from loss to follow up or missing data (Herbstman et al. 2010; Lee et al. 2013; Shy et al. 2011). The need for adequately powered studies was already recognized by Roze et al. (2009). If failing to do a power calculation is understandable for early, small exploratory studies for which it is a priori difficult to hypothesize an expected effect because the association between the variable and the outcome has simply not been investigated before, subsequent studies designed to confirm suspected associations should perform a sample size calculation as a prerequisite (Amler et al. 2006) and include it in the reporting. Large birth cohort studies with a long-term follow up are therefore needed to better evaluate the role of environmental contaminants exposure in the development of adverse neurological and neurobehavioural disorders to detect a sufficient number of those cases above the low background incidence rate typically observed in the general population (Savitz, 2007)."

Lack of dose response relationship common

"A large majority of studies did not report a clear dose–response relationship between levels of PBDEs or PFCs and the measured health outcomes. Potential effects of mixtures due to the combined effect of possibly numerous environmental contaminants should also be taken into account. If prenatal exposure to neurotoxicants during critical developmental periods leads to irreversible effects, the possibility of reversibility of certain effects following postnatal exposures in later life stages should also be taken into account."

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Generalisability

"Some studies raise the question of the representativeness of the study population and generalizability of their findings, due to: (i) differences in term of ethnicity, e.g. study participants were pre- dominantly non-Hispanic white (C8 Health Project), Afro-American (THREE study) or Mexican-American (CHAMACOS study); (ii) poor education, low socio-economic status population background (e.g. CHAMACOS and THREE studies); (iii) unusual exposure scenarios following accidents or outbreaks (C8 Health Project and 9/11 WTC cohorts)."

Chang et al. (2014). A critical review of perfluorooctanoate and perfluorooctanesulphonate exposure and cancer risk in humans

Chang et al. did not have a specific limitations section. Instead, they discussed the strengths and limitations of each study they reviewed.

Johnson et al. (2014). The navigation guide – evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth

In the 'Discussion' section, Johnson et al. raised the following limitations about the data and the need to follow standardised reporting criteria:

"A limitation to this review, and to all reviews in general, is that reviews are based on the available data, which may be insufficient in depth or breadth or may be otherwise limited. Future reviews could be strengthened if more investigators followed standardized reporting criteria such as the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (von Elm et al. 2008), enabling improved quality assessment. In addition, we found that contacting study authors was essential to obtaining the data necessary to include some of the studies in the meta-analysis. Not all study authors were able to provide data that could be included in the meta-analysis. Future efforts in meta-analysis could also be supported by data repositories. Our risk of bias tool also had limitations. Although there is existing guidance for assessing risk of bias of human observational studies (Viswanathan et al. 2012; Wells 2014), there is no universally accepted tool (Sanderson et al. 2007). The risk of bias domains "exposure assessment" and "confounding" were less developed than other domains that were transferred more directly from established evidence-based risk of bias tools. Additionally, in future reviews, we will consider the assessment of outcome as a separate risk of bias domain. For this case study, potential bias resulting from outcome misclassification fell under "other" risk of bias and was not a problematic risk because the outcomes were standard birth measurements that did not vary across study groups. However, it is possible that in future cases of other outcomes more attention will need to be given to potential bias in the assessment of those outcomes."

Chang et al. (2016). A critical review of perfluorooctanoate and perfluorooctanesulphonate exposure and immunological health conditions in humans

Chang et al. raised issues and provided comment about exposure assessment, confounding, selection bias and statistical considerations in their critical review of the studies they reviewed on PFOS and PFOA and immunological effects in humans. The comments by Chang et al. on each of these criteria are reported below.

Exposure assessment

"Exposure assessment is as vital to the validity and interpretation of a study as is outcome assessment. Of the 24 epidemiologic studies included in this review, 19 measured PFOA and/or PFOS in the serum or

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plasma of individual subjects using liquid chromatography tandem mass spectrometry, the standard method for quantitative analysis of these chemicals (Ashley-Martin et al. 2015; Dong et al. 2013; Emmett et al. 2006b; Fei et al. 2010; Grandjean et al. 2012; Granum et al. 2013; Humblet et al. 2014; Innes et al. 2011; Kielsen et al. 2015; Lin et al. 2011; Looker et al. 2014; Okada et al. 2012, 2014; Olsen et al. 2003; Osuna et al. 2014; Pennings et al. 2015; Smit et al. 2015; Uhl et al. 2013; Wang et al. 2011). The other five studies estimated PFOA exposure based on place of residence (Anderson-Mahoney et al. 2008), employment at a polymer manufacturing plant (Leonard et al. 2008) or within a PFOA production department at another chemical production plant (Costa et al. 2009), or an environmental fate and transport model for PFOA linked with a pharmacokinetic model and individual-level residential history and water consumption data, validated against recent serum PFOA measurements in a subset of the study subjects (Steenland et al. 2013, 2015). Compared with the five studies that used exposure estimates or proxies, the studies that measured PFOA and/or PFOS levels had the advantage of direct exposure assessment, thereby theoretically reducing exposure misclassification.

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

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

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levels and is not taken during an etiologically important time window, the pertinent exposure will be misclassified."

Confounding

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

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

Selection bias

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

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

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al. 2012; Granum et al. 2013; Okada et al. 2012, 2014; Pennings et al. 2015; Smit et al. 2015; Steenland et al. 2013, 2015; Wang et al. 2011) raise varying degrees of concern about potential selection bias.

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

Statistical considerations

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

Conversely, low statistical power should be taken into account as an explanation for statistically non-significant findings in studies with a small number of subjects.

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

Bach et al. (2015). Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: a systematic review

In the 'Discussion', Bach et al. included a specific section on 'Selection bias, confounding and effect modification'. Their comments under each criterion are reported below.

Selection bias

"In the studies by Stein et al. (2009) and Darrow et al. (2013), the participants were aware of their exposure levels. However, in the remaining studies, individual knowledge about exposure category seems unlikely. Therefore, we do not consider selection bias to be very likely, even though it cannot be ruled out that selection depended on other factors associated with both PFAS levels and birth weight."

Confounding

"We considered parity, body mass index (BMI), and socioeconomic status to be the most important potential confounders, as these are associated with both exposure and outcome in the literature. Most

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included studies considered several potential confounders (Table 5), but as in all observational studies, residual confounding cannot be excluded. The magnitude of observed associations was small and therefore more likely to be explained by confounding or bias than strong associations, even if the extent of this was modest. Overall, crude estimates failed to change substantially when adjustments were made in multivariate models. In most studies, associations became somewhat stronger with adjustments. However, a few studies did not include some of the potential confounders we considered to be important. Apelberg et al. (2007), Arbuckle et al. (2013), Hamm et al. (2010), Inoue et al. (2004), Lee et al. (2013), Maisonet et al. (2012), and Monroy et al. (2008) failed to consider socio-economic status in their analyses of PFOA or PFOS and birth weight. It was previously demonstrated that women in higher socio-economic groups tend to have higher PFAS levels (Brantsæter et al. 2013). As women with high socio-economic status often give birth to children with higher birth weights (Luo et al. 2004, Moser et al. 2003), a lack of adjustment for socio-economic status may potentially explain the higher birth weight associated with higher PFOS (although statistically insignificant) in the study by Hamm et al. (2010). A lack of adjustment could have obscured a potential decrease in birth weight with PFOA exposure (Apelberg et al. 2007, Arbuckle et al. 2013, Hamm et al. 2010, Lee et al. 2013, Maisonet et al. 2012, Monroy et al. 2008) or PFOS exposure (Apelberg et al. 2007, Inoue et al. 2004, Lee et al. 2013, Maisonet et al. 2012, Monroy et al. 2008). Adjusting for socio-economic status is likely to be insufficient to control for behavioral factors such as smoking and alcohol consumption, but associations for PFASs with such behaviors have not been established.

Only Apelberg et al. (2007) adjusted for maternal weight gain during pregnancy, which is probably a relevant proxy for the size of plasma volume expansion during pregnancy. Poor plasma expansion as well as low pregnancy weight gain is associated with impaired fetal growth (Salas et al. 1993, Viswanathan et al. 2008), but may also cause higher PFAS concentrations due to a smaller distribution volume (Apelberg et al. 2007, Olsen et al. 2009). Thus, if blood samples are taken in late pregnancy or from cord blood, in utero exposure might be overestimated in smaller fetuses and vice versa, thereby creating a noncausal association between PFAS exposure and birth weight. Adjusting for maternal weight gain during pregnancy may not solve the problem, due to collinearity. On the other hand, if two pregnant women initially (e.g. at conception) have the same PFAS concentrations, a woman with lower pregnancy weight gain will probably preserve a higher concentration later in pregnancy, resulting in higher fetal PFAS exposure. With early pregnancy PFAS measurements, overall pregnancy exposure might thus be systematically underestimated in women with less gestational weight gain that may carry smaller fetuses and cause bias against no association. However, there were no systematic differences in the study results depending on the timing of exposure assessment."

Effect modification

"Other physiologic and metabolic changes during pregnancy may also impact the association between PFAS levels and fetal growth parameters. No studies adjusted for maternal glomerular filtration rate (GFR). Since renal excretion of PFASs is proportional to the GFR and higher maternal GFR during pregnancy is associated with higher birth weight (Morken et al. 2014), GFR may be an important confounder for the association between PFAS exposure and birth weight. Morken et al. (2014) investigated the association between PFOA exposure and birth weight in a group of participants from the Norwegian Mother and Child Cohort Study [diff erent from the group studied by Whitworth et al. (2012)], and found that adjusting for maternal GFR attenuated the estimate by 66%. High pre-pregnancy BMI has been shown to be associated with higher birth weight (Papachatzi et al. 2013, Wahabi et al. 2013), and higher BMI might be associated with higher levels of PFASs (Brantsæter et al. 2013). Thus, a lack of adjustment for pre-pregnancy BMI can potentially bias the association towards no association. However, Wu et al. (2012) and Stein et al. (2009) found associations between PFOA and

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birth weight, and PFOS and LBW, respectively, even though they did not adjust for pre-pregnancy BMI. Maternal diet during pregnancy is another potential confounder. Fish contains considerable amounts of PFASs (Brantsæter et al. 2013, Haug et al. 2010, Rylander et al. 2010), but ingestion during pregnancy has been associated with increased birth weight as well (Brantsæter et al. 2012). Only Fei et al. (2007) attempted to control for diet, but they did not include it in their main analysis.

All studies except those by Arbuckle et al. (2013), Darrow et al. (2013), Inoue et al. (2004), Lee et al. (2013), and Monroy et al. (2008) adjusted for parity. Since average PFASs levels are lower in parous women (Brantsæter et al. 2013), and higher parity is associated with increased birth weight (Wilcox et al. 1996), lack of adjustment for parity could create a noncausal association between higher PFAS exposure and lower birth weight, but none of the studies that did not adjust for parity demonstrated any statistically significant associations.

All studies except those by Arbuckle et al. (2013), Darrow et al. (2013), Inoue et al. (2004), Monroy et al. (2008), and Stein et al. (2009), adjusted for gestational age. However, Darrow et al. (2013) and Monroy et al. (2008) restricted the study population to term births. Fei et al. (2007) also did this in supplementary analyses, and this did not change the result. It would be interesting to discover whether PFASs cause reduced fetal growth or whether a low birth weight may be due to a shorter gestational duration. However, it is debated whether adjustment for gestational age is appropriate when studying impacts on pregnancy outcomes (Wilcox et al. 2011). A way to distinguish effects on birth weight from effects on gestational age is to consider gestational age as an outcome. Many of the studies included in this review investigated associations between PFOS or PFOA and gestational age (Apelberg et al. 2007, Chen et al. 2012, Hamm et al. 2010, Maisonet et al. 2012, Wu et al. 2012) or preterm birth (Arbuckle et al. 2013, Chen et al. 2012, Darrow et al. 2013, Fei et al. 2007, Stein et al. 2009, Whitworth et al. 2012). Chen et al. (2012) found lower gestational age and higher odds for preterm birth with PFOS exposure, but not with PFOA exposure, and Wu et al. (2012) found lower gestational age with higher PFOA. Arbuckle et al. (2013) found an association between term gestational age and higher PFOS. However, in the other studies, there was no significant association between PFOA or PFOS and gestational length or preterm birth, and no tendency for estimates to point in a certain direction. In three out of four studies corresponding to 992 out of 1421 pregnancies, there was no association between PFOS and gestational age, and in five out of six studies corresponding to 9293 out of 9722 pregnancies there was no association between PFOS and preterm birth. For PFOA, four out of fi ve studies corresponding to 1421 out of 1588 pregnancies did not demonstrate any association with gestational age, and in five studies of 6205 pregnancies there was no association with preterm birth. Therefore, it is not very likely for lower birth weight with PFAS exposure to be caused by lower gestational age.

Most studies controlled for infant sex. However, if PFASs affect sex hormone homeostasis, it is possible that the potential effects of PFASs on birth weight differ between boys and girls. Only Washino et al. (2009) stratified data by the sex of the newborn. They found no association between PFOS and birth weight in boys, but a statistically significant decrease in birth weight was found in girls (adjusted beta = -269.4 -465.7, -7 3.0) per 10-fold increase in PFOS). Sex-stratified estimates were similar for the association between PFOA and birth weight. Maisonet et al. (2012) restricted their analysis to girls and found statistically significant lower birth weight of at least 130 g when comparing the highest with the lowest tertile of PFOA and PFOS. Girls may be more vulnerable to PFASs with respect to birth weight, which implies the need to consider effect modification by infant sex."

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Priestly (2016). Literature review and report on the potential health effects of perfluoroalkyl compounds, mainly perfluorooctane sulphonate (PFOS). Monash University

In the 'Executive Summary', Priestly commented on the limitations of the epidemiology studies. At the end of the section 'Health effects based on studies in humans'. Priestly included a section that considered the implications for fire fighters. Priestly's comments are reported below:

Limitations of epidemiology studies

"The evidence from epidemiological studies is still somewhat confusing despite the plethora of studies published over the past ten years. The epidemiological studies are suggesting, but not yet proving, a possible link between PFOS/PFOA and thyroid disease, blood lipid and uric acid disorders, foetal development and disturbances of normal birth characteristics, perinatal neurodevelopment, altered sperm function and disturbances of the immune system. The studies generally report 'associations' between measured serum levels of PFOS, PFOA and other PFAS and specified adverse health outcomes. However, associations are not necessarily causal, and the reported changes in serum biomarkers may be within normal limits and not be representative of disease. In some of the studies, the authors concede that any apparent association may have reverse causality, where the increased incidence of disease has caused PFAS to accumulate to a higher level in those parts of the cohort.

It would be reasonable to draw the conclusion that the evidence for all these effects needs further corroboration, since the evidence is somewhat inconsistent as to which specific PFAS are responsible and in some cases, the direction of the change attributed to specific PFAS is different across studies. Some of the studies that suggest an association between serum PFAS and changes in hormonal status, blood lipid regulation, foetal developmental effects and immune dysfunction are from 'normal' cohorts with no obvious sources of exposure other than 'background. That is, the blood PFAS concentrations are in the range for populations that have accumulated body burdens of PFAS mainly through food, water and household dusts. Studies on cohorts with occupational exposures, where serum PAS levels are orders of magnitude higher, provide much weaker evidence of an association with some of these adverse health effects. This may be because the industrial cohorts are generally smaller, and not all end points are assessed.

In some of the recent epidemiological studies with large cohorts, the standard practice is to compare the incidence of the disease or effect under study between the highest and lowest tertiles/quartiles of the range of PFAS concentrations measured. Where multiple PFAS have been measured, it is not always clear how the statistical methodology allows for the study to specify which PFAS is mainly responsible for the findings, or whether the effects are the result of combined exposures. This is particularly difficult when one PFAS (e.g. PFOS, PFOA or another fluorotelomer) is implicated in one study, but another is implicated in a different study for the same effect. It raises the question about whether some of the relatively small observed changes could occur by chance, or have little clinical significance. Given that there is conflicting and unresolved evidence for a precise mode of action to explain some of these effects (e.g. whether they can be linked to activation of the PPARα or receptor or not), and that the toxicological profiles of PFOS, PFOA appear to differ to some extent, with much less solid evidence relating to the toxicology of the shorter chain PFAS and PFOA/S precursors, metabolites or other fluorotelomers, it is probably still to early to place too much weight on the emerging epidemiological evidence. This is a view that is echoed in reviews by leading international authorities, such as the US EPA."

Implications for fire fighters

Priestly noted:

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"This report does not attempt to undertake a formal health risk assessment for fire fighters who may have been exposed to PFOS through past use of AFFFs, particularly the 3M Lightwater formulation that was known to contain a low concentration (1-5%) of PFOS.

There are only two studies (Rotander et al. 2015a, b) of which I am aware where levels of PFAS have been measured in the serum of Australian fire fighters. This study identified PFOS and PFHxS and their derivatives, as well several other fluoroalkyl compounds in serum, including 1-chloro-perflourooctane sulfonic acid as a fluorotelomer uniquely found in fire fighter serum. PFOS concentrations in serum were 92 – 343 ng/mL in a smaller cohort (n=20) and 3.4 – 391 ng/mL in a larger cohort (n=149) both recruited in 2013. PFHxS levels 49 – 326 and 0.7 – 277 ng/mL in these two cohorts. The results indicate that the occupational exposures of fire fighters results in serum PFOS levels around one order of magnitude higher than normal populations (controls in this study were PFOS 1 – 40 and PFHxS 0.2 – 22 ng/mL respectively). A history of blood donation was found to be associated with lower PFAS levels, but there were no discernible effects on serum cholesterol (Total, LDL or HDL), triglycerides or uric acid.

While PFOS blood levels in fire fighters are higher than that in the general population, they do not approach those measured in PFOS/PFOA manufacturing cohorts. This may be because the frequency of potential exposure scenarios would be more limited since fire fighters use PFOS-containing AFFFs mainly during training activities. A proviso to this assumption is that the potential for systemic PFOS absorption through the skin associated with work practices (such as cleaning out foam tanks) or inhalation of foam mists has not been grossly underestimated. There is little information on which one could assess the potential for gloves, overall and other personal protective equipment (PPE) to limit PFOS exposures in this group.

The potential for PFOS-contaminated clothing to be transferred to the domestic environment and result in an additional exposure pathway to the fire fighters and their families could be further investigated, although I am advised that contaminated clothing should not be taken off-site."

Ballesteros et al. (2017). Exposure to perfluoroalkyl substances and thyroid functions in pregnant women and children: a systematic review of epidemiologic studies

Ballesteros et al. raised several issues about the literature they reviewed on exposure to PFAS and thyroid function in children and pregnant women including statistical analysis of data, confounding, exposure to multiple chemicals and temporality of exposure. The issues raised by Ballesteros et al. are reported below.

Hormone assessment data and data analysis

"The studies do differ in the THs measured to assess effects, since, ex- cept for TSH, which was measured in all but one article, the rest of the hormones were determined in a lower number of studies. Therefore, not all studies had information available on free THs, which reflect the levels of biologically active hormones that are available to the tissues and might have yielded more comprehensive information concerning the thyroid regulatory system.

Differences in the methods used to analyze THs might also be impor- tant. Studies used different types of immunoassay methods for hor- mone determination. However, some animal studies have criticized the use of these techniques for the assessment of FT4. These researchers hypothesized that the reduction in FT4 in the presence of PFOS could have been due to negative bias in analog techniques, resulting from competitive displacement of FT4 and the labeled FT4 analog from serum and assay binding proteins in the presence of this contaminant (Chang et al. 2007; Luebker et al. 2005). This concern prompted a study of potential bias from the presence of PFAS in a human population with typical U.S. serum PFOS concentrations but higher PFOA concen- trations due to their proximity to a

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Teflon factory (Lopez-Espinosa et al. 2012a). Such bias from the use of an analog with respect to dialysis methods in experimental studies (Chang et al. 2007; Luebker et al. 2005) was not observed in this human population (Lopez-Espinosa et al. 2012a). According to the authors, possible differences in the re- sults between animal and human studies could be due to the differences in levels of exposure to PFAS (higher in rats than in humans) and also the inter-species differences in the principal proteins that bind T4 (in rats: albumin and transthyretin [TTR], and in humans: thyroxine- binding globulin [TBG], while albumin and TTR play comparatively less important roles), and their interaction with PFAS (Lopez-Espinosa et al. 2012a)."

Strength of the association, confounding and exposure to multiple chemicals

"Several aspects make it difficult to assess the strength of the association across studies. There is a substantial variation among the studies in the estimates of the association (regression coefficients, % change, estimated mean differences, Pearson correlation coefficient, and p for trend). Although PFAS concentrations were measured in the same units (ng/mL), contaminants were not treated in the same way (continuous or categorical) in the statistical analyses, which also hampers comparison among studies.

Another important issue when discussing the strength of association is the control for confounding variables, and there was heterogeneity across studies in this respect. Several variables known to influence thyroid status and PFAS, such as BMI, are not addressed in all the studies. Some studies adjusted models for this variable (Berg et al. 2015; de Cock et al. 2014; Kim et al. 2011; Lewis et al. 2015), others checked whether it was a possible confounder but finally it was not included (Lopez-Espinosa et al. 2012b; Wang et al. 2013, 2014) and the rest did not include it in the statistical analysis. The adjustment of models for BMI is under debate, since BMI might be causally "downstream" of both exposure (PFAS) and outcome (THs) variables (Webster et al. 2014). Most studies did not measure other important biomarkers which might affect TH levels, such as iodine status or thyroid antibodies. Some studies (Lopez-Espinosa et al. 2012b; Wang et al. 2013, 2014; Webster et al. 2014) excluded people with thyroid diseases or thyroid treatments, as they receive medication prescribed to adjust hormones to normal levels. However, some studies did not make such exclusions and the medications could thus obscure the association, if present, for those individuals.

Study populations are likely to be exposed to multiple chemical contaminants at the same time and, therefore, multipollutant analyses including other PFAS were conducted in some of the studies reviewed (Berg et al. 2015; Chan et al. 2011; Lin et al. 2013; Lopez-Espinosa et al. 2012b). The magnitude of the associations was similar across studies except for Berg et al. (2015), where the associations between PFHxS or PFOA and TSH were no longer significant after including PFOS, but results were not reported in the article and have not been discussed in this review. While the combined thyroid effects of chemical mixtures are certainly possible, since other chemical substances with endocrine-disrupting properties and with similar sources of exposure such as diet have been associated with alterations of TH levels in some previous studies (Boas et al. 2012), it is important to mention the low correlation between human serum levels of PFAS and other chemicals measured in blood or urine, such as polybrominated diphenyl ethers, organochlorine compounds, polychlorinated biphenyls, bisphenols, and phtalates (Fisher et al. 2016; Robinson et al. 2015). This low correlation gives some reassurance that PFAS, and not other TH disruptors, are likely to be driving the associations seen in these studies."

Temporality of exposure

"Studies varied in the epidemiological design: cross-sectional, case- control, or cohort. Cross-sectional studies are unable to establish a temporal sequence due to the simultaneous measurement of exposure

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and outcome. For example, there is some literature showing that THs can affect kidney function (Chonchol et al. 2008), and excretion rates and serum PFAS levels can depend on kidney function (Watkins et al. 2013). Therefore, this factor associated with THs could be affecting the cross-sectional relationship. In addition, effects may occur some time after exposure and thus they could not be observed at the time of the cross-sectional study."

Negri et al. (2017) Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data

In the 'Conclusions on epidemiological data' section, Negri et al. included a section on 'Risk of bias', and commented as follows.

Risk of bias

"We considered baby sex, gestational age, maternal age, prepregnancy BMI, education, parity, and smoking to be the most important potential confounders of the relationship between PFAA and BrthW, as they have been shown to be associated with both exposure and outcome. However, when we restricted the analysis to studies with full adjustment for these confounders, results were similar to those including all studies. On one hand, another important potential confounder, related to both exposure and outcome, is glomerular filtration rate (GFR). Some studies have shown that women whose GFR fails to rise sufficiently during pregnancy tend to have smaller babies (Verner et al. 2015). On the other hand, GFR is likely to influence the urinary excretion of xenobiotics like PFAA. Indeed, higher blood PFAA levels have been observed in people with lower GFR (Verner et al. 2015). As renal elimination in humans seems to be negligible and no study adjusted for GFR, the influence of GFR on the results remains undefined. On one hand, fish consumption is another potential confounder since fish contains considerable amounts of PFAAs (Brantsaeter et al. 2013). On the other hand, fish intake has been suggested to have a favorable role on fetal growth (Brantsaeter et al. 2013). One study only (Whitworth et al. 2012) included lean fish intake in the regression model, and its results were not statistically heterogeneous with the pooled estimates of the other studies (data not shown). Besides confounding, other sources of bias must be considered, and for systematic reviews publication bias specifically. Among studies that were excluded because they did not report the outcome of interest, it is possible that some found a null association. However, the statistical tests we applied did not suggest that publication bias did occur."

Rappazzo et al. (2017). Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature

In the 'Discussion' section, Rappazzo et al. raised the following limitations and issues with the literature they reviewed on exposure to PFAS and health outcomes in children.

Potential conflict of interest

"Some of the studies under review are funded by non-governmental sources, leading to concern over conflict of interest. As described by the Institute of Medicine Roundtable on Environmental Health Sciences, Research, and Medicine Institute of Medicine Roundtable on Environmental Health Sciences and Medicine [112], conflicts of interest do not inherently represent an inadequacy of a publication or signal any level of misconduct, but rather describe a set of circumstances under which researchers may rely on the judgment of an outside force, or be influenced by considerations for such parties. The failure to avoid being compromised by these dependencies and influences may lead to a number of biases, including publication bias or selective reporting of outcomes."

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Non-response or loss to follow-up

"Many studies had non-response issues or loss to follow-up. This is mitigated somewhat in that some studies demonstrate that the non-responders did not differ substantially from the included subjects. However, there are may be underlying, unknown differences leading to the non-response, which may in turn lead to bias in effect estimates if these differences are related to both PFAS exposure and the health outcome of interest."

Timing of exposure

"For exposure assessment, the largest concern is the issue of timing of exposure. Many of the findings are obtained from cross-sectional studies with associations reported between serum PFAS concentration and measured health outcome, which have the potential to be affected by bias due to reverse causality, unlike a longitudinal study with repeated measurements over time."

Cross-sectional studies, temporality of exposure and optimal study design

"With cross-sectional studies, temporality cannot be established. Therefore it is not possible to determine whether observed health effects are due to PFAS exposure, or if underlying health conditions lead to a buildup of PFAS. Optimal study design to address this challenge would be longitudinal studies with repeated measurements before and after disease onset, which would establish temporality between PFAS exposure and health effects.

Design of studies involving thyroid disease and thyroid hormone concentrations are complicated by disease status and medication use. Those with known thyroid disease should be separated from those who are disease-free for comparison. Patients with thyroid disease are likely monitored and medicated to have thyroid hormone levels in a therapeutic range and comparisons of T_4 or TSH with this population's PFAS concentration would not be informative. Autoimmune status and iodine sufficiency are also informative when included in models."

Potential for exposure misclassification

"More generally for studies of PFAS, their concentrations are likely to be affected by when, and in whom, they are measured, introducing the potential for exposure misclassification. Measures during pregnancy are taken in maternal serum or cord blood. However, not all PFAS are equally transferred across the placenta [113]. Therefore, concentrations of PFAS in maternal serum are likely to differentially represent fetal PFAS, and there will be differential representation based on specific PFAS and placental transferability [113]. As well, maternal blood volume expands during pregnancy, leading to the potential for different periods of pregnancy to have different maternal serum concentrations of PFAS due to blood volume changes. Blood samples may also be taken from either fasting or non-fasting participants, which may make between study comparison of cardiovascular and lipid-related makers difficult. These potential sources of exposure misclassification may attenuate health effect estimates, as they are likely non-differential by outcome."

Dose response

"In addition, the potential exists for non-monotonic dose response curves for PFAS, some of which are known endocrine disrupting compounds. It is possible that lower concentrations/exposures may have a more disruptive effect than high concentrations/exposures, in particular with outcomes connected to the endocrine system such as thyroid function or pubertal development."

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Mixtures of PFAS and toxicological effects

"Relatedly, the toxicological effects of PFASs as a mixture of potentially dozens to hundreds of compounds are generally unknown [114]. Only a handful of studies covered in this review examined PFASs using a mixture method, and this was either a summed value or a factor representing PFAS exposure [44, 52, 75, 83]. While these studies provide some context for effects of PFASs as a mixture, they are limited in number and not performed for all health outcomes. A summed or representative metric of PFAS exposure also cannot inform researchers about potential interactions between individual PFASs. A more through exploration of how PFASs interact with one another, both in a toxicological manner and in effect measure modification, would help the understanding of how these exposures may or may not lead to adverse health outcomes."

Small numbers of studies

"Within the published literature, there is an incomplete assessment of pubertal onset in girls. Epidemiologic publications for pubertal onset in girls across PFAS concentrations look at age at menarche, but lack information on thelarche or the onset of female breast development. Breast development has been shown to be sensitive to PFAS exposure in laboratory animals and the dearth of information on this end point in developing human populations is an area that could be expanded to allow for better cross-species comparison. Existing cohorts of US girls with data on these pubertal end points could be mined to understand these associations.

The small number of studies for particular health outcomes limits our ability to draw conclusions based on the body of literature. Even general categories of childhood health outcomes (e.g., cardiometabolic) included only a few studies, though the neurodevelopmental literature has grown substantially even in just the past year."

Outcomes measured differently in different studies

"In addition, some of the same outcomes were classified or measured differently in different studies, making direct comparisons across studies more difficult. A focus on particular outcomes of import could help define the literature and direct future research in directions of interest and utility, and prompt more direct mechanistic studies."

Kirk et al. (2018). The PFAS Health Study. Systematic Literature Review. Australian National University

Under the section 'Study quality', Kirk et al. made the following comments about the literature they reviewed including risk of bias, cross-sectional studies and confounding.

Risk of bias

"The quality of studies covered by this systematic review was assessed using a multi-domain risk-of-bias tool for each specific 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

"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 disease 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

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questionnaire at the time of PFAS measurement, which raises the possibility that knowledge of one influences recall of the other."

Confounding

"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 because of lack of prior studies 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 PFAS and low birth weight, due to the fact that kidney function is associated with both excretion of blood levels of PFAS chemicals from the body and low birth weight infants. (270) Few earlier studies would have measured GFR."

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7. OUTCOMES OF THE PUBLIC CONSULTATION

The public consultation showed that there is concern from the public about how they feel PFAS exposure has already affected their health, and that it may affect their health into the future. Respondents also clearly indicated that future research into the human health effects of PFAS exposure is extremely important to them.

Some of the key findings have been included below. The full submissions analysis report has been included as 'Appendix 1: Public consultation report'.

7.1. Exposure pathways

- Overall, respondents indicated that past exposure to PFAS, occupational exposure to PFAS especially in firefighters, and skin contact with PFAS were the most concerning exposure pathways to them.
- When considering the views of those respondents who were occupationally exposed to PFAS (e.g. from working or training as a firefighter and being regularly exposed to PFAS containing foam), these respondents ranked past exposure to PFAS, occupational exposure to PFAS and skin contact with PFAS containing products as of most concern to them.
- When considering the views of those respondents who reported that they lived, or previously lived, in an area under investigation for PFAS contamination, these respondents ranked drinking water, contaminated soil and homegrown produce as the exposure pathways of most concern to them.

7.2. Concerns about potential health impacts of PFAS exposure

- Over two thirds of respondents were "*concerned*" or "*very concerned*" about the following impacts of PFAS on their health:
  - that their future health, or their family's future health might be impacted by PFAS exposure;
  - that their health, or their family's health may have already been impacted by PFAS exposure;
  - avoiding exposure to PFAS;
  - that their health, or their family's health, was being indirectly affected by PFAS exposure e.g. by causing stress and anxiety.

- When given the opportunity to identify which potential health impacts of PFAS exposure concerned them most, over 55 percent (189 of the 339 respondents who responded to the question) noted that they were most concerned about a link between PFAS exposure and cancer(s).

7.3. Information and understanding

- Over half of respondents felt "*not at all informed*" or "*not informed*" about the Government's response to addressing health concerns of communities exposed to PFAS. Conversly, 21 percent of respondents reported feeling "*informed*" or "*very informed*" about the Government's response.

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- Approximately half of respondents felt "*not at all informed*" or "*not informed*" about research on the effects of PFAS exposure and levels of exposure to PFAS in specific communities. Conversly, 32 percent reported feeling "*informed*" and 37 percent felt "*very informed*" about research on PFAS, and levels of exposure to PFAS in specific communities respectively.
- Forty-five percent of respondents reported feeling "*not at all informed*" or "*not informed*" about different ways people and communities may be exposed to PFAS. Conversly, 40 percent of respondents reported feeling "*informed*" or "*very informed*" about PFAS exposure pathways.

7.4. Future health impact and exposure research priorities

- When asked about their views on what research on PFAS exposure should be prioritised, respondents reported that research on the health effects of occupational exposure to PFAS should be prioritised, along with further research into potential health impacts on communities that have experienced high exposure to PFAS due to contamination.
  - Respondents who identified as occupationally exposed to PFAS, mainly firefighters, prioritised future research on the health effects of occupational exposure to PFAS, and research on potential health effects on communities that have experienced high exposure to PFAS due to contamination.
  - Respondents who reported that they lived, or have lived, in an area currently being investigated for PFAS contamination prioritised research on the potential health effects on communities that have experienced high exposure to PFAS, and research into the potential health effects of PFAS exposure on vulnerable populations such as pregnant women, babies, young children and the elderly.

- Thirty-one of the 109 respondents who commented on other areas of human health research they want prioritised, commented on a need for blood testing for those who have been exposed through their work or who live in or near an investigations site.

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8. RESEARCH

8.1. Other research underway

8.1.1. Australian National University has been commissioned to undertake an epidemiological study

Australian National University has been commissioned by the Department of Health to examine the potential health effects resulting from PFAS exposure through an epidemiological study. This study will focus on the communities of Oakey in Queensland, Williamtown in New South Wales and Katherine in the Northern Territory. The first phase of this study produced the Kirk et al. draft systematic review that has been included in this analysis.

The epidemiological study will be comprised of the following four components:

- a focus group study to determine the concerns of individuals living in the vicinity of Williamtown and Oakey in relation to exposure to PFAS and their health;
- a blood serum study to define the serum concentrations (mean and range) of PFAS in Williamtown and Oakey residents living in the Investigation Areas and to compare the levels to those of people residing in non-contaminated areas in the townships and surrounding areas;
- a cross-sectional survey to investigate the exposure and risk factors for high serum PFAS levels, including sociodemographic (e.g. age, sex, location) and other factors (e.g. duration of residence in the area, water source), and associations of high serum PFAS levels with common symptoms, signs and diagnosed illnesses in the Williamtown and Oakey communities; and
- a data linkage study to examine whether sex-specific age-adjusted rates of diseases potentially associated with PFAS are higher among people who have lived in the Investigation Areas of Williamtown and Oakey, compared to those living outside the Investigation Areas and in the general Australian population.

8.1.2. Per- and Poly-Fluorinated Alkyl Substances – National Health Research Program

As part of the Australian Government's response to potential PFAS contamination on or near Commonwealth sites, the Per- and Poly-Fluorinated Alkyl Substances – National Health Research Program has been established. This measure will cost $12.5 million from 2017–18 to 2020–21. It is funded within existing resources in the Department of Defence and Department of Health, and will be administered by the National Health and Medical Research Council (NHMRC). Grant funding will be delivered to researchers through a call for proposals.

The program aims to increase the evidence and understanding of potential human health effects from prolonged exposure to PFAS. This increased understanding will also inform appropriate responses to PFAS contamination by the Government.

8.1.3. An organisation representing firefighters noted it will be conducting research into PFAS in 2018

In its submission during the public consultation period, this organisation indicated that it is in the process of commissioning a voluntary health study which will be conducted in collaboration with a

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major Victorian Hospital and an acknowledged research university. The study, which is currently in the early stages of development and anticipated to commence in 2018, will test potential methods of reducing PFAS levels in firefighters by drawing blood and plasma.

The name of the organisation has been withheld as submissions were provided anonymously.

8.1.4. Other research on environmental remediation and PFAS

A $13 million research program was launched in December 2017 to investigate PFAS remediation. The Australian Research Council will administer a range of research programs that investigate existing and emerging solutions for PFAS removal and disposal and to develop new technologies and processes that can be deployed across the country.

The PFAS Remediation Research Program will fund a range of research projects focused on:

- minimising PFAS in the environment;
- developing effective technologies that can be applied to remediate PFAS contaminated soil, waterways, waste, debris and/or large volumes of groundwater;
- developing options and mechanisms through which these effective technologies can be applied in the field.

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REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). (2015). Draft Toxicological Profile for Perfluoroalkyls. Public Review Draft.

Alexander BH, Olsen GW, Burris JM et al. (2003). Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. Occupational and Environmental Medicine 60:10,722-9.

Alexander BH, Olsen GW. (2007). Bladder cancer in perfluorooctancesulfonyl fluoride manufacturing workers. Annals of Epidemiology 17:6,471-8.

Alkhalawi E, Kasper-Sonnenberg M, Wilhelm M et al. (2016). Perfluoroalkyl acids (PFAAs) and anthropometric measures in the first year of life: Results from the Duisburg Birth Cohort. Journal of Toxicology and Environmental Health, Part A 79:22-23,1041-49.

Andersen CS, Fei C, Gamborg M et al. (2010). Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy. American journal of epidemiology 172:11,1230-7.

Andersen CS, Fei C, Gamborg M et al. (2013). Prenatal exposures to perfluorinated chemicals and anthropometry at 7 years of age. American journal of epidemiology 178:6,921-7.

Andersen ME, Butenhoff JL, Chang SC, et al. (2008). Perfluoroalkyl acids and related chemistries-toxicokinetics and modes of action. Toxicological Sciences 102:1,3-14.

Anderson-Mahoney P, Kotlerman J, Takhar H et al. (2008). Self-reported health effects among community residents exposed to perfluorooctanoate. New Solutions: A journal of environmental and occupational health policy 18:2,129-43.

Apelberg BJ, Witter FR, Herbstman JB et al. (2007). Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environmental Health Perspectives 115:11,1670-6.

Arbuckle TE, Kubwabo C, Walker M et al. (2013). Umbilical cord blood levels of perfluoroalkyl acids and polybrominated flame retardants. International journal of hygiene and environmental health 216:2,184-94.

Ashley-Martin J, Dodds L, Arbuckle TE et al. (2016). Maternal and Neonatal Levels of Perfluoroalkyl Substances in Relation to Gestational Weight Gain. International Journal Environmental Research and Public Health 13:1.

Ashley-Martin J, Dodds L, Levy AR et al. (2015). Prenatal exposure to phthalates, bisphenol A and perfluoroalkyl substances and cord blood levels of IgE, TSLP and IL-33. Environmental research 140, 360-8.

Bach CC, Bech BH, Brix N et al. (2015a). Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: a systematic review. Critical Reviews in Toxicology 45:1,53-67.

Bach CC, Bech BH, Nohr EA et al. (2015b) Serum perfluoroalkyl acids and time to pregnancy in nulliparous women. Environmental research 142,535-41.

Bach CC, Liew Z, Bech BH et al. (2015c). Perfluoroalkyl acids and time to pregnancy revisited: An update from the Danish National Birth Cohort. Environmental Health 14:1,59.

Bach CC, Bech BH, Nohr EA et al. (2016). Perfluoroalkyl Acids in Maternal Serum and Indices of Fetal Growth: The Aarhus Birth Cohort. Environmental Health Perspectives 124:6,848-54.

ED_002330_00119784-00384

Bae J, Kim S, Schisterman EF et al. (2015). Maternal and paternal serum concentrations of perfluoroalkyl and polyfluoroalkyl substances and the secondary sex ratio. Chemosphere 133,31-40.

Ballesteros V, Costa O, Iñiguez C et al. (2017). Exposure to perfluoroalkyl substances and thyroid function in pregnant women and children: A systematic review of epidemiologic studies. Environment International 99,15-28.

Barrett ES, Chen C, Thurston SW et al. (2015). Perfluoroalkyl substances and ovarian hormone concentrations in naturally cycling women. Fertility and sterility 103:5,1261-70.e3.

Barry V, Darrow LA, Klein M et al. (2014). Early life perfluorooctanoic acid (PFOA) exposure and overweight and obesity risk in adulthood in a community with elevated exposure. Environmental research 132,62-9.

Barry V, Winquist A, Steenland K (2013). Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environmental Health Perspectives 121,1313–1318.

Bellinger, DC. (2013). Prenatal Exposures to Environmental Chemicals and Children's Neurodevelopment: An Update. Safety and health at work 4:1,1–11.

Berg V, Nøst TH, Hansen S et al. (2015) Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. Environment International 77,63-9.

Berg V, Nøst TH, Pettersen RD et al. (2017). Persistent Organic Pollutants and the Association with Maternal and Infant Thyroid Homeostasis: A Multipollutant Assessment. Environmental Health Perspectives 125:1,127-33.

Berk M, Williams LJ, Andreazza AC et al. (2014). Pop, heavy metal and the blues: Secondary analysis of persistent organic pollutants (POP), heavy metals and depressive symptoms in the NHANES National Epidemiological Survey. BMJ Open 4:7,e005142.

Bloom MS, Kannan K, Spliethoff HM et al. (2010). Exploratory assessment of perfluorinated compounds and human thyroid function. Physiology and Behavior 99:2,240-5.

Bonefeld-Jørgensen EC, Long M, Bossi R et al. (2011). Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: A case control study. Environmental Health: A global access science source 10:1,88.

Bonefeld-Jørgensen EC, Long M, Fredslund SO et al. (2014). Breast cancer risk after exposure to perfluorinated compounds in Danish women: a case-control study nested in the Danish National Birth Cohort. Cancer Causes & Control 25:11,1439–48.

Braun JM, Chen A, Romano ME et al. (2016). Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: the HOME Study. Obesity 24:1,231-7.

Braun JM, Kalkbrenner AE, Just AC et al. (2014). Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. Environmental health perspectives 122:5,513-20.

Buck Louis GM, Chen Z, Schisterman EF et al. (2015). Perfluorochemicals and human semen quality: the LIFE study. Environmental Health Perspectives 123:1,57-63.

Buck Louis GM, Peterson CM, Chen Z et al. (2012). Perfluorochemicals and Endometriosis: the ENDO Study. Epidemiology 23:6,799-805.

ED_002330_00119784-00385

Buck Louis GM, Sapra KJ, Barr DB et al. (2016). Preconception perfluoroalkyl and polyfluoroalkyl substances and incident pregnancy loss, LIFE Study. Reproductive toxicology (Elmsford, NY) 65,11-17.

Buck Louis GM, Sundaram R, Schisterman EF et al. (2013). Persistent environmental pollutants and couple fecundity: the LIFE study. Environmental Health Perspectives 121:2,231-6.

Bull S, Burnett K, Vassaux K et al. (2014). Extensive literature search and provision of summaries of studies related to the oral toxicity of perfluoroalkylated substances (PFASs), their precursors and potential replacements in experimental animals and humans. Area 1: Data on toxicokinetics (absorption, distribution, metabolism, excretion) in in vitro studies, experimental animals and humans. Area 2: Data on toxicity in experimental animals. Area 3: Data on observations in humans. EFSA supporting publication 11:4,EN-572.

Buser MC, Scinicariello F. (2016). Perfluoroalkyl substances and food allergies in adolescents. Environment international 88,74-9.

Callan AC, Rotander A, Thompson K et al. (2016). Maternal exposure to perfluoroalkyl acids measured in whole blood and birth outcomes in offspring. Science of the Total Environment 569-570,1107-13.

Campbell S, Raza M, Pollack AZ. (2016). Perfluoroalkyl substances and endometriosis in US women in NHANES 2003-2006. Reproductive toxicology 65,230-5.

Chan E, Burstyn I, Cherry N et al. (2011). Perfluorinated acids and hypothyroxinemia in pregnant women. Environmental Research 111:4,559-64.

Chang ET, Adami H-O, Boffetta P et al. (2014). A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and cancer risk in humans. Critical Reviews of Toxicology 44:sup 1,1-81.

Chang ET, Adami H-O, Boffetta P et al. (2016). A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and immunological health conditions in humans. Critical Reviews in Toxicology 46:4,279-31.

Château-Degat M-L, Pereg D, Dallaire R et al. (2010). Effects of perfluoroocatnesulfonate exposure on plasma lipid levels in the Inuit population of Nunavik (Northern Quebec). Environmental Research 110:7, 710-17.

Chen J, Das SR, La Du J et al. (2013). Chronic PFOS exposures induce life stage-specific behavioral deficits in adult zebrafish and produce malformation and behavioral deficits in F1 offspring. Environmental Toxicology and Chemistry 32:1,201–6.

Chen MH, Ha EH, Liao HF et al. (2013). Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiology 24:6,800-8.

Chen MH, Ha EH, Wen TW et al. (2012). Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PloS one 7:8,e42474.

Christensen KY, Maisonet M, Rubin C et al. (2011). Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort. Environment international 37:1,129-35.

Christensen KY, Raymond M, Thompson BA et al. (2016). Perfluoroalkyl substances in older male anglers in Wisconsin. Environment International 91,312–18.

ED_002330_00119784-00386

Consonni D, Straif K, Symons JM et al. (2013). Cancer risk among tetrafl uoroethylene synthesis and polymerization workers. American Journal of Epidemiology 178:1,350-8.

Corsini E, Luebke RW, Germolec DR et al. (2014). Perfluorinated compounds: Emerging POPs with potential immunotoxicity. Toxicology Letters 230:2263-70.

Corsini E, Sangiovanni E, Avogadro A et al. (2012). In viro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). Toxicology and applied pharmacology 258:2,248-55.

Costa G. (2004). Report on the meeting held on Friday 20th and Saturday 21st 2004 at the Inn at Montchanin Village (Wilmington, USA) with 3M and DuPont delegations. DuPont. Submitted to the U.S. Environmental Protection Agency. AR226-1866.

Costa G, Sartori S, Consonni D. (2009). Thirty years of medical surveillance in perfluooctanoic acid production workers. Journal of Occupational and Environmental Medicine 51:3,364-72.

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT). (2010). Statement on the Tolerable Daily Intake for Perfluorooctane Sulfonate. Food Standards Agency.

Dallaire R, Ayotte P, Pereg D et al. (2009a). Determinants of plasma concentrations of perfluorooctanesulfonate and brominated organic compounds in Nunavik Inuit adults (Canada). Environmental Science and Technology 43:13,5130-6.

Dallaire R, Dewailly É, Pereg D et al. (2009b). Thyroid function and plasma concentrations of polyhalogenated compounds in Inuit adults. Environmental health perspectives *117*:9, 1380-6.

Dalsager L., Christensen N., Husby S et al. (2016). Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4 years among 359 children in the Odense Child cohort. Environment international 96,58-64.

Darrow LA, Groth AC, Winquist A et al. (2016). Modeled Perfluorooctanoic Acid (PFOA) Exposure and Liver Function in a Mid-Ohio Valley Community. Environmental Health Perspectives 124:8,1227-33.

Darrow LA, Howards PP, Winquist A et al. (2014). PFOA and PFOS serum levels and miscarriage risk. Epidemiology 25:4,505-12.

Darrow LA, Stein CR, Steenland K. (2013). Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010. Environmental Health Perspectives 121:10,1207-13.

de Cock M, de Boer MR, Lamoree M et al. (2014a). First year growth in relation to prenatal exposure to endocrine disruptors – a Dutch prospective cohort study. International Journal of Environmental Research and Public Health 11:7,7001-21.

de Cock M, de Boer MR, Lamoree M et al. (2014b). Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants – a Dutch prospective cohort study. Environmental health – a global access science resource 13:1,106.

De Witt JC, Peden-Adams MM, Keller JM et al. (2012). Immunotoxicity of perfluorinated compounds: Recent developments. Toxicologic Pathology 40:2;300-11.

Dhingra R, Darrow LA, Klein M et al. (2016). Perfluorooctanoic acid exposure and natural menopause: A longitudinal study in a community cohort. Environmental research 146,323-30.

ED_002330_00119784-00387

Ding G, Zhang J, Chen Y et al. (2013). Combined effects of PFOS and PFOA on zebrafish (Danio rerio) embryos. Archives of Environmental Contamination and Toxicology 64:4,668–675.

Donauer S, Chen A, Xu Y et al. (2015). Prenatal exposure to polybrominated diphenyl ethers and polyfluoroalkyl chemicals and infant neurobehavior. Journal of Pediatrics 166:3,736–42.

Dong GH, Tung KY, Tsai CH et al. (2013). Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. Environmental health perspectives 121:4,507-13,513e501-508.

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

Ducatman A, Zhang J, Fan H. (2015). Prostate-specific antigen and perfluoroalkyl acids in the C8 health study population. Journal of occupational and environmental medicine 57:1,111-4.

Emmett EA, Zhang H, Shofer F et al. (2006). Community exposure to perfluorooctanoate: relationships between serum levels and certain health parameters. Journal of Occupational and Environmental Medicine 48:8,771-9.

Eriksen KT, Raaschou-Nielsen O, McLaughlin JK et al. (2013). Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population. PLoS ONE 8:2,e56969.

Eriksen KT, Sørensen M, McLaughlin JK et al. (2009). Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general Danish population. Journal of the National Cancer Institute 101:8,605–609.

Fan H, Ducatman A, Zhang J. (2014). Perfluorocarbons and Gilbert syndrome (phenotype) in the C8 Health Study Population. Environmental research 135:70-5.

Fei C, McLaughlin JK, Lipworth L et al. (2008b). Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environmental health perspectives 116:10,1391–5.

Fei C, McLaughlin JK, Lipworth L et al. (2009). Maternal levels of perfluorinated chemicals and subfecundity. Human reproduction 24:5,1200-5.

Fei C, McLaughlin JK, Lipworth L et al. (2010). Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environmental research 110:8,773-7.

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

Fei C, McLaughlin JK, Tarone RE et al. (2008a). Fetal growth indicators and perfluorinated chemicals: a study in the Danish National Birth Cohort. American journal of epidemiology 168:1,66-72.

Fei C, Olsen J. (2011). Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at age 7 years. Environmental Health Perspectives 119:4,573–8.

Fei C, Weinberg CR, Olsen J. (2012). Commentary: Perfluorinated chemicals and time to pregnancy: A link based on reverse causation? Epidemiology (Cambridge, Mass) 23:2,264-266.

Fisher M, Arbuckle TE, Wade M et al. (2013). Do perfluoroalkyl substances affect metabolic function and plasma lipids? Analysis of the 2007-2009, Canadian Health Measures Survey (CHMS) Cycle 1. Environmental Research 121,95-103.

ED_002330_00119784-00388

Fitz-Simon N, Fletcher T, Luster MI et al. (2013). Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid. Epidemiology 24:4,569- 76.

Fleisch AF, Rifas-Shiman SL, Mora AM et al. (2016). Early Life Exposure to Perfluoroalkyl Substances and Childhood Metabolic Function. Environmental Health Perspectives 125:3,481.

Fletcher T, Galloway TS, Melzer D et al. (2013). Associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans. Environment International 57-58,2–10.

Food Standards Australia New Zealand (FSANZ). (2017). Hazard assessment report – Perfluorooctane Sulfonate (PFOS), Perfluorooctanoic Acid (PFOA), Perfluorohexane Sulfonate (PFHxS). Food Standards Australia New Zealand.

Forns J, Iszatt N, White RA et al. (2015). Perfluoroalkyl substances measured in breast milk and child neuropsychological development in a Norwegian birth cohort study. Environment International 83,176–82.

Frisbee SJ, Shankar A, Knox SS et al. (2010). Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: Results from the C8 health project. Archives of Pediatrics & Adolescent Medicine 164:9,860–9.

Fromme H, Mosch C, Morovitz M et al. (2010). Pre- and postnatal exposure to perfluorinated compounds (PFCs). Environmental Science & Technology 44:18,7123– 9.

Fu Y, Wang T, Fu Q et al. (2014). Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population. Ecotoxicology and Environmental Safety 106,246-252.

Gallo V, Leonardi G, Genser B et al. (2012). Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure. Environmental Health Perspectives 120:5,655-60.

Geiger SD, Xiao J, Ducatman A et al. (2014a). The association between PFOA, PFOS and serum lipid levels in adolescents. Chemosphere 98,78–83.

Geiger SD, Xiao J, Shankar A. (2013). Positive association between perfluoroalkyl chemicals and hyperuricemia in children. American Journal of Epidemiology 177:11,1255-62.

Geiger SD, Xiao J, Shankar A. (2014b). No association between perfluoroalkyl chemicals and hypertension in children. Integrated blood pressure control 7,1-7.

Ghisari M, Eiberg H, Long M et al. (2014). Polymorphisms in Phase I and Phase II genes and breast cancer risk and relations to persistent organic pollutant exposure: a case-control study in Inuit women. Environmental Health: a global access science source 13:1,19.

Gilliland FD, Mandel JS. (1993). Mortality among employees of a perfluorooctanoic acid production plant. Journal of occupational medicine: official publication of the Industrial Medical Association 35:9,950-4.

Gilliland FD, Mandel JS. (1996). Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins, and cholesterol: a study of occupationally exposed men. American Journal of Industrial Medicine 29:5,560-568.

ED_002330_00119784-00389

Gleason JA, Post GB, Fagliano JA. (2015). Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010. Environmental Research 136,8-14.

Goudarzi H, Araki A, Itoh S et al. (2016a). The Association of Prenatal Exposure to Perfluorinated Chemicals with Glucocorticoid and Androgenic Hormones in Cord Blood Samples: The Hokkaido Study. Environmental Health Perspectives Online doi: 10.1289/EHP142.

Goudarzi H, Miyashita C, Okada A et al. (2016b). Effects of prenatal exposure to perfluoroalkyl acids on prevalence of allergic diseases among 4-year-old children. Environment International 94,124-32.

Goudarzi H, Nakajima S, Ikeno T et al. (2016c). Prenatal exposure to perfluorinated chemicals and neurodevelopment in early infancy: The Hokkaido Study. Science of the Total Environment 541,1002-1010.

Govarts E, Remy S, Bruckers L et al. (2016). Combined Effects of Prenatal Exposures to Environmental Chemicals on Birth Weight. International Journal of Environmental Research and Public Health 13:5,495.

Grandjean P, Andersen EW, Budtz-Jorgensen E et al. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. Journal of American Medical Association 307:4,391-7.

Grandjean P, Budtz-Jorgensen E. (2013). Immunotoxicity of perfluorinated alkylates: Calculation of benchmark doses based on serum concentrations in children. Environmental Health 12:1,35.

Grandjean P, Heilmann C, Weihe P et al. (2016). Serum vaccine antibody concentrations in adolescents exposed to perfluorinated compounds. Environmental Health Perspectives doi:10.1289/EHP275.

Grandjean P, Poulsen LK, Heilmenn C et al. (2010). Allergy and sensitization during childhood associated with prenatal and lactational exposure to marine pollutants. Environmental Health Perspectives 118:10,1429-33.

Granum B, Haug LS, Namork E et al. (2013). Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. Journal of Immunotoxicology 10:4,373-9.

Grice MM, Alexander BH, Hoffbeck R et al. (2007). Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. Journal of occupational and environmental medicine 49:7,722-9.

Gump BB, Wu Q, Dumas AK et al. (2011). Perfluorochemical (PFC) exposure in children: Associations with impaired response inhibition. Environmental science & technology 45:19,8151-59.

Halldorsson TI, Rytter D, Haug LS et al. (2012). Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. Environmental Health Perspectives 120:5,668-73.

Hamm MP, Cherry N, Chan E et al. (2010). Maternal exposure to perfluorinated acids and fetal growth. Journal of Exposure Science and Environmental Epidemiology 20:7,589-97.

Hansmeier N, Chao T-C, Herbstman JB et al. (2015). Elucidating the molecular basis of adverse health effects from exposure to anthropogenic polyfluorinated compounds using toxicoproteomic approaches. Journal of proteome research 14:1,51-8.

ED_002330_00119784-00390

Hardell E, Kärrman A, van Bavel B et al. (2014). Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. Environment International 63,35–39.

Heilmann C, Budtz-Jørgensen E, Nielsen F et al. (2010). Serum concentrations of antibodies against vaccine toxoids in children exposed perinatally to immunotoxicants. Environmental Health Perspectives 118:10,1434-8.

Heilmann C, Grandjean P, Weihe P et al. (2006). Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. PLoS Med 3:8,1352-8.

Hoffman K, Webster TF, Weisskopf MG et al. (2010). Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12–15 years of age. Environmental Health Perspectives 118:12,1762–7.

Høyer BB, Ramlau-Hansen CH, Vrijheid M et al. (2015b). Anthropomorphy in 5- to 9-year-old Greenlandic and Ukrainian children in relation to prenatal exposure to perfluorinated alky substances. Environmental Health Perspectives 123:8,841-6.

Høyer BB, Ramlau-Hansen CH, Obel C et al. (2015a). Pregnancy serum concentrations of perfluorinated alkyl substances and offspring behaviour and motor development at age 5–9 years—A prospective study. Environment Health 14:1,2.

Hua JY, Smith SJ. (2004). Neural activity and the dynamics of central nervous system development. Nature Neuroscience 7:4,327–32.

Humblet O, Diaz-Ramirez LG, Balmes JR et al. (2014). Perfluoroalkyl Chemicals and Asthma among Children 12-19 Years of Age: NHANES (1999-2008). Environmental Health Perspectives 122:10,1129-33.

Innes KE, Ducatman AM, Luster MI et al. (2011). Association of osteoarthritis with serum levels of the environmental contaminants perfluorooctanoate and perfluorooctane sulfonate in a large Appalachian population. American Journal of Epidemiology 174:4,440-50.

Innes KE, Wimsatt JH, Frisbee S et al. (2014). Inverse association of colorectal cancer prevalence to serum levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in a large Appalachian population. BMC Cancer 14:1,45.

Inoue K, Okada F, Ito R et al. (2004). Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: Assessment of PFOS exposure in a susceptible population during pregnancy. Environmental Health Perspectives 112:11,1204-07.

International Agency for Research on Cancer (IARC). (2017). IARC monographs on the evaluation of carcinogenic risks to humans. Lyon, France.

Itoh S, Araki A, Mitsui T et al. (2016). Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido Study on Environment and Children's Health. Environment international 94,51-9.

Jaacks LM, Boyd Barr D, Sundaram R et al. (2016). Pre-Pregnancy Maternal Exposure to Persistent Organic Pollutants and Gestational Weight Gain: A Prospective Cohort Study. International Journal of Environmental Research and Public Health 13:9,905.

Jain RB. (2013). Association between thyroid profile and perfluoroalkyl acids: data from NHNAES 2007-2008. Environmental research 126,51-9.

ED_002330_00119784-00391

Jensen DV, Christensen J, Virtanen HE et al. (2014). No association between exposure to perfluorinated compounds and congenital cryptorchidism: A nested case-control study Among 215 Boys from Denmark and Finland. Reproduction 147:4,411-7.

Jensen TK, Andersen LB, Kyhl HB et al. (2015). Association between perfluorinated compound exposure and miscarriage in Danish pregnant women. PloS one 10:4,e0123496.

Ji K, Kim S, Kho Y et al. (2012). Serum concentrations of major perfluorinated compounds among the general population in Korea: dietary sources and potential impact on thyroid hormones. Environment International 45: 78-85.

Jiang W, Zhang Y, Zhu L et al. (2014). Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women. Environment International 64,40-47.

Joensen UN, Bossi R, Leffers H et al. (2009). Do perfluoroalkyl compounds impair human semen quality? Environmental Health Perspectives 117:6,923-7.

Joensen UN, Veyrand B, Antignac JP et al. (2013). PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. Human reproduction (Oxford, England) 28:3,599-608.

Johansson N, Fredriksson A, Eriksson P. (2008). Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. Neurotoxicology 29:1,160–9.

Johnson PI, Sutton P, Atchley DS et al. (2014). The navigation guide – evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth. Environmental health perspectives 122:10,1028-39.

Jorgensen KT, Specht IO, Lenters V et al. (2014). Perfluoroalkyl substances and time to pregnancy in couples from Greenland, Poland and Ukraine. Environmental health 13:1,116.

Jusko TA, De Roos AJ, Lee SY et al. (2016). A birth cohort study of maternal and infant serum PCB-153 and DDE concentrations and responses to infant tuberculosis vaccination. Environmental health perspectives 124:6,813-21.

Karlsen M, Grandjean P, Weihe P et al. (2016). Early-life exposure to persistent organic pollutants in relation to overweight in preschool children. Reproductive Toxicology.

Karnes C, Winquist A, Steenland K. (2014). Incidence of type II diabetes in a cohort with substantial exposure to perfluorooctanoic acid. Environmental research 128,78-83.

Kataria A, Trachtman H, Malaga-Dieguez L et al. (2015). Association between perfluoroalky acids and kidney function in a s crosssectional study of adolescents. Environmental Health 14:89.

Kato S, Itoh S, Yuasa M et al. (2016). Association of perfluorinated chemical exposure in utero with maternal and infant thyroid hormone levels in the Sapporo cohort of Hokkaido Study on the Environment and Children's Health. Environmental Health and Preventive Medicine 21:5,334-44.

Kerger BD, Copeland TL, DeCaprio AP. (2011). Tenuous dose-response correlations for common disease states: case study of cholesterol and perfluorooctanoate/sulfonate (PFOS/PFOS) in the C8 Health Project. Drug and Chemical Toxicology 34:4,396-404.

ED_002330_00119784-00392

Khalil N, Chen A, Lee M et al. (2016). Association of Perfluoroalkyl Substances, Bone Mineral Density, and Osteoporosis in the U.S. Population in NHANES 2009-2010. Environmental health perspectives 124:1,81-7.

Kielsen K, Shamim Z, Ryder LP et al. (2016). Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. Journal of immunotoxicology 13:2,270-3.

Kim DH, Kim UJ, Kim HY et al. (2016). Perfluoroalkyl substances in serum from South Korean infants with congenital hypothyroidism and healthy infants--Its relationship with thyroid hormones. Environmental Research 147,399-404.

Kim JH, Park HY, Jeon JD et al. (2015). The modifying effect of Vitamin C on the association between perfluorinated compounds and insulin resistance in the Korean elderly: A double blind, randomized, placebo-controlled crossover trial. European Journal of Nutrition 55:3,1011-1020.

Kim S, Choi K, Ji K et al. (2011). Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environmental science and technology 45:17,7465-72.

Kirk M, Smurthwaite K, Bräunig J et al. (2018). The PFAS Health Study: Systematic Literature Review. Canberra: The Australian National University.

Kishi R, Nakajima T, Goudarzi H et al. (2015). The association of prenatal exposure to perfluorinated chemicals with maternal essential and long-chain polyunsaturated fatty acids during pregnancy and the birth weight of their offspring: The Hokkaido Study. Environmental health perspectives 123:10,1038-45.

Knox SS, Jackson T, Frisbee SJ et al. (2011a). Perfluorocarbon exposure, gender and thyroid function in the C8 Health Project. Journal of Toxicological Sciences 36:4,403-10.

Knox SS, Jackson T, Javins B et al. (2011b). Implications of early menopause in women exposed to perfluorocarbons. The Journal of clinical endocrinology and metabolism 96:6,1747-53.

Koustas E, Lam J, Sutton P et al. (2014). The Navigation Guide—evidence-based medicine meets environmental health: systematic review of nonhuman evidence for PFOA effects on fetal growth. Environmental Health Perspectives 122:10,1015–1027.

Kristensen SL, Ramlau-Hansen CH, Ernst E et al. (2013). Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction. Human reproduction (Oxford, England) 28:12,3337-48.

Kvist L, Giwercman YL, Jonsson BA et al. (2012). Serum levels of perfluorinated compounds and sperm Y:X chromosome ratio in two European populations and in Inuit from Greenland. Reproductive Toxicology (Elmsford, N.Y.) 34:4,644-50.

Kwon EJ, Shin JS, Kim BM et al. (2016). Prenatal exposure to perfluorinated compounds affects birth weight through GSTM1 polymorphism. Journal of Occupational and Environmental Medicine 58:6,e198-e205.

Lam J, Koustas E, Sutton P et al. (2014). The navigation guide – evidence-based medicine meets environmental health: integration of animal and human evidence for PFOA effects on fetal growth. Environmental health perspectives 122:10,1040-51.

Lauritzen HB, Larose TL, Olen T et al. (2016). Maternal serum levels of perfluoroalkyl substances and organochlorines and indices of fetal growth: a Scandinavian case-cohort study. Pediatric Research doi: 10.1038/pr.2016.187.

ED_002330_00119784-00393

Lee ES, Han S, Oh JE. (2016). Association between perfluorinated compound concentrations in cord serum and birth weight using multiple regression models. Reproductive toxicology (Elmsford, NY) 59,53-9.

Lee YJ, Kim MK, Bae J et al. (2013). Concentrations of perfluoroalkyl compounds in maternal and umbilical cord sera and birth outcomes in Korea. Chemosphere 90:5,1603-9.

Lenters V, Portengen L, Rignell-Hydbom A et al. (2016). Prenatal Phthalate, Perfluoroalkyl Acid, and Organochlorine Exposures and Term Birth Weight in Three Birth Cohorts: Multi-Pollutant Models Based on Elastic Net Regression. Environmental health perspectives 124:3,365-72.

Lenters V, Portengen L, Smit LA et al. (2015). Phthalates, perfluoroalkyl acids, metals and organochlorines and reproductive function: a multipollutant assessment in Greenlandic, Polish and Ukrainian men. Occupational and environmental medicine 72:6,385-93.

Leonard RC. (2006). Ammonium perfluorooctanoate: Phase II. Retrospective cohort mortality analyses related to a serum biomarker of exposure in a polymer production plant. Wilmington, DE: E.I. du pont de Nemours and Company. (Unpublished).

Leonard RC, Kreckmann KH, Sakr CJ et al. (2008). Retrospective cohort mortality study of workers in a polymer production plant including a reference population of regional workers. Annals of Epidemiology 18:1,15-22.

Leter G, Consale C, Eleuteri P et al. (2014). Exposure perfluoroalkyl substances and sperm DNA global methylation in Arctic and European populations. Environment and Molecular Mutagenesis 55:7,591-600.

Lewis RC, Johns LE, Meeker JD. (2015). Serum biomarkers of exposure to perfluoroalkyl substances in relation to serum testosterone and measures of thyroid function among adults and adolescents from NHANES 2011-2012. International journal of environmental research and public health 12:6,6098-114.

Lien GW, Huang CC, Shiu JS et al. (2016). Perfluoroalkyl substances in cord blood and attention deficit/hyperactivity disorder symptoms in seven-year-old children. Chemosphere 156,118–27.

Lien GW, Huang CC, Wu KY et al. (2013). Neonatal-maternal factors and perfluoroalkyl substances in cord blood. Chemosphere 92:7,843-50.

Liew Z, Ritz B, Bonefeld-Jorgensen EC et al. (2014). Prenatal exposure to perfluoroalkyl substances and the risk of congenital cerebral palsy in children. American journal of epidemiology 180:6,574-81.

Liew Z, Ritz B, von Ehrenstein OS et al. (2015). Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: A nested case-control study in the Danish National Birth Cohort. Environmental health perspectives 123:4,367–73.

Lin CY, Chen PC, Lin YC et al. (2009). Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults. Diabetes Care 32:4,702-7.

Lin CY, Lin LY, Chiang CK et al. (2010). Investigation of the associations between low-dose serum perfluorinated chemicals and liver enzymes in US adults. Am J Gastroenterol 105:6,1354-63.

Lin CY, Lin LY, Wen TW et al. (2013a) Association between levels of serum perfluorooctane sulfate and carotid artery intima-media thickness in adolescents and young adults. International Journal of Cardiology 168:4,3309-16

ED_002330_00119784-00394

Lin CY, Wen LL, Lin LY et al. (2011). Associations between levels of serum perfluorinated chemicals and adiponectin in a young hypertension cohort in Taiwan. Environmental Science and Technology 45:24,10691-8.

Lin CY, Wen LL, Lin LY et al. (2013b). The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults. Journal of Hazardous Materials 244–245,637–44.

Lin LY, Wen LL, Su TC et al. (2014). Negative association between serum perfluorooctane sulfate concentration and bone mineral density in US premenopausal women: NHANES, 2005-2008. The Journal of clinical endocrinology and metabolism 99:6,2173-80.

Lind DV, Priskor L, Lassen TH et al. (2016). Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age as a marker of endocrine disruption. Reproductive Toxicology Online doi: 10.1016/j.reprotox.2016.08.019.

Lind L, Zethelius B, Salihovic S et al. (2014). Circulating levels of perfluoroalkyl substances and prevalent diabetes in the elderly. Diabetologia 57:3,473-9.

Loccisano AE, Longnecker MP, Campbell JL Jr et al. (2013). Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. Journal of Toxicology and Environmental Health Part A 76:1,25–57.

Looker C, Luster MI, Calafat AM et al. (2014). Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicological Sciences 138:1,76-88.

Lopez-Espinosa MJ, Fletcher T, Armstrong B et al. (2011). Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) with age of puberty among children living near a chemical plant. Environmental Science and Technology 45:19,8160-6.

Lopez-Espinosa MJ, Mondal D, Armstrong B et al. (2012). Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environmental Health Perspectives 120:7,1036-41.

Lopez-Espinosa MJ, Mondal D, Armstrong B et al. (2016). Perfluoroalkyl Substances, Sex Hormones, and Insulin-like Growth Factor-1 at 6-9 Years of Age: A Cross-Sectional Analysis within the C8 Health Project. Environmental Health Perspectives 124:8,1269-75.

Lum KJ, Sundaram R, Barr DB et al. (2017). Perfluoroalkyl Chemicals, Menstrual Cycle Length, and Fecundity: Findings from a Prospective Pregnancy Study. Epidemiology 28:1,90-8.

Lundin JI, Alexander BH, Olsen GW et al. (2009). Ammonium Perfluorooctanoate Production and Occupational Mortality. Epidemiology 20:6,921-8.

Lyngso J, Ramlau-Hansen CH, Høyer BB et al. (2014). Menstrual cycle characteristics in fertile women from Greenland, Poland and Ukraine exposed to perfluorinated chemicals: a cross-sectional study. Human reproduction (Oxford, England) 29:2,359-67.

MacNeil J, Steenland NK, Shankar A et al. (2009). A cross-sectional analysis of type II diabetes in a community with exposure to perfluorooctanoic acid (PFOA). Environ Research 109:8,997-1003.

Maisonet M, Calafat AM, Marcus M et al. (2015a). Prenatal Exposure to Perfluoroalkyl Acids and Serum Testosterone Concentrations at 15 Years of Age in Female ALSPAC Study Participants. Environmental Health Perspectives 123:12,1325-30.

Maisonet M, Näyhä S, Lawlor DA et al. (2015b). Prenatal exposures to perfluoroalkyl acids and serum lipids at ages 7 and 15 in females. Environment International 82,49–60.

ED_002330_00119784-00395

Maisonet M, Terrell ML, McGeehin MA et al. (2012). Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environmental Health Perspectives 120:10,1432-7.

Mandel J, Johnson R. (1995). Mortality Study of Employees at 3M Plant in Decatur, Alabama. University of Minnesota, School of Public Health, Division of Environmental and Occupational Health, Minneapolis, MN.

March LM, Schwarz JM, Carfrae BH et al. (1998). Clinical validation of self-reported osteoarthritis. Osteoarthritis and Cartilage 6:2,87–93.

Martin H. (2010). Role of PPAR-gamma in inflammation. Prospects for therapeutic intervention by food components. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 690:1-2,57-63.

Mattsson K, Rignell-Hydbom A, Holmberg S et al. (2015). Levels of perfluoroalkyl substances and risk of coronary heart disease: Findings from a population-based longitudinal study. Environmental research 142,148-54.

Melzer D, Rice N, Depledge MH et al. (2010). Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. Environmental Health Perspectives 118:5,686-92.

Min JY, Lee KJ, Park JB et al. (2012). Perfluorooctanoic acid exposure is associated with elevated homocysteine and hypertension in US adults. Occupational and environmental medicine 69:9,658-62.

Minnesota Department of Health (MDH). 2007. Groundwater health risk limits. St. Paul, MN: MDH. http://www.health.state.mn.us/divs/eh/groundwater/hrltable.html.

Mogensen UB, Grandjean P, Heilmann C. (2015). Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. Environmental Health: a global access science source 14:1,47.

Monroy R, Morrison K, Teo K et al. (2008). Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environmental Research 108:1,56-62.

Morken, N-H, Travlos GS, Wilson RE et al. (2014). Maternal glomerular filtration rate in pregnancy and fetal size. PLOS One 9:7,e101897.

Mundt DJ, Mundt KA, Luippold RS et al. (2007). Clinical epidemiological study of employees exposed to surfactant blend containing perfluorononanoic acid. Occupational and Environmental Medicine 64:9,589-94.

National Toxicology Program. (2016). NTP Monograph: Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid and Perfluorooctane Sulfonate. US Department of Health and Human Services.

Negri E, Metruccio F, Guercio V et al. (2017). Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data. Critical Reviews in Toxicology 47:6,482-508.

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

ED_002330_00119784-00396

New Jersey Drinking Water Quality Institute. (2016). Health-based maximum contaminant level support document: Perfluorooctanoic Acid (PFOA). Public Review draft.

Nissen S, Wolski K. (2007). Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. The New England journal of medicine 356:24,2457-71.

Nolan LA, Nolan JM, Shofer FS. (2009). The relationship between birth weight, gestational age and perfluorooctanoic acid (PFOA)-contaminated public drinking water. Reproductive toxicology (Elmsford, NY) 27:3,231-8.

Nolan LA, Nolan JM, Shofer FS et al. (2010). Congenital anomalies, labor/delivery complications, maternal risk factors and their relationship with perfluorooctanoic acid (PFOA)-contaminated public drinking water. Reproductive Toxicology 29:2,147-55.

Ode A, Kallen K, Gustafsson P et al. (2014). Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. PLoS ONE 9:4,e95891.

Okada E, Sasaki S, Kashino I et al. (2014). Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. Environment international 65,127-34.

Okada E, Sasaki S, Saijo Y et al. (2012). Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. Environmental research 112,118-25.

Olsen GW, Burlew MM, Burris JM et al. (2001a). A Cross-Sectional Analysis of Serum Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoate (PFOA) in Relation to Clinical Chemistry, Thyroid Hormone, Hematology and Urinalysis Results from Male and Female Employee Participants of the 2000 Antwerp and Decatur Fluorochemical Medical Surveillance Program. Final Report. 3M Company, St. Paul, MN.

Olsen GW, Burlew MM, Burris JM et al. (2001b). A Longitudinal Analysis of Serum Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoate (PFOA) Levels in Relation to Lipid and Hepatic Clinical Chemistry Test Results from Male Employee Participants of the 1994/95, 1997 and 2000 Fluorochemical Medical Surveillance Program. Final Report. 3M Company, St. Paul, MN.

Olsen GW, Burlew MM, Marshall JC et al. (2004). Analysis of episodes of care in a perfluorooctanesulfonyl fluoride production facility. Journal of Occupational and Environmental Medicine 46:8,837-46.

Olsen GW, Burris JM, Burlew MM et al. (1998a). 3M Final report: an epidemiologic investigation of plasma cholecystokinin, hepatic function and serum perfluorooctanoic acid levels in production workers. 3M Company. September 4, 1998. U.S. Environmental Protection Agency Administrative Record 226-0476.

Olsen GW, Burris JM, Burlew MM et al. (2000). Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. Drug and chemical toxicology 23:4,603-20.

Olsen GW, Burris JM, Burlew MM et al. (2003a). Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. Journal of occupational and environmental medicine 45:3,260-70.

Olsen GW, Burris JM, Mandel JH et al. (1999). Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. Journal of Occupational and Environmental Medicine 41:9,799-806.

ED_002330_00119784-00397

Olsen GW, Butenhoff JL, Zobel LR. (2009). Perfluoroalkyl chemicals and human fetal development: an epidemiologic review with clinical and toxicological perspectives. Reproductive Toxicology 27,3-4,212-30.

Olsen GW, Ehresman DJ, Bueher BD et al. (2012). Longitudinal assessment of lipid and hepatic clinical parameters in workers involved with the demolition of perfluoroalkyl manufacturing facilities. Journal of occupational and environmental medicine 54:8,974-83.

Olsen GW, Gilliland FD, Burlew MM et al. (1998b). An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. Journal of occupational and environmental medicine 40:7,614-622.

Olsen GW, Logan PW, Hansen KJ et al. (2003b). An occupational exposure assessment of a perfluorooctanesulfonyl fluoride production site: biomonitoring. AIHA Journal 64:5,651-9.

Olsen GW, Zobel LR. (2007). Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. International archives of occupational and environmental health 81:2,231-46.

Osuna CE, Grandjean P, Weihe P et al. (2014). Autoantibodies Associated with Prenatal and Childhood Exposure to Environmental Chemicals in Faroese Children. Toxicological Sciences 142:1,158-66.

Oulhote Y, Shamim Z, Kielsen K et al. (2016a). Children's white blood cell counts in relation to developmental exposure to methylmercury and persistent organic pollutants. Reproductive Toxicology.

Oulhote Y, Steuerwald U, Debes F et al. (2016b). Behavioural difficulties in 7-year old children in relation to developmental exposure to perfluorinated alkyl substances. Environment International 97,237-45.

Pennings JL, Jennen DG, Nygaard UC et al. (2015). Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. Journal of Immunotoxicology 13:2,173-80.

Pirali B, Negri S, Chytiris S et al. (2009). Perfluorooctane sulfonate and perfluorooctanoic acid in surgical thyroid specimens of patients with thyroid diseases. Thyroid 19:12,1407-12.

Polańska K, Jurewicz J, Hanke W. (2012). Exposure to environmental and lifestyle factors and attention-deficit/hyperactivity disorder in children – A review of epidemiological studies. International Journal of Occupational Medicine and Environmental Health 25:4,330-355.

Potera C. (2012). Perfluorinated compounds may lower vaccine protection in children. Environmental Health Perspectives 120:4,A150-A151.

Power MC, Webster TF, Baccarelli AA et al. (2013) Cross-sectional association between polyfluoroalkyl chemicals and cognitive limitation in the National Health and Nutrition Examination Survey. Neuroepidemiology 40:2,125-32.

Predieri B, Iughetti L, Guerranti C et al. (2015). High Levels of Perfluorooctane Sulfonate in Children at the Onset of Diabetes. International Journal of Endocrinology 2015,1-7.

Priestly B. (2016). Literature Review and Report on the Potential Health Effects of Perfluoroalkyl Compounds, Mainly Perfluorooctane Sulfonate (PFOS). Monash University.

ED_002330_00119784-00398

Psaty BM, Furberg CD. (2007). Rosiglitazone and Cardiovascular risk. The New England Journal of Medicine 356:24,2522-24.

Qin XD, Qian Z, Vaughn MG et al. (2016). Positive associations of serum perfluoroalkyl substances with uric acid and hyperuricemia in children from Taiwan. Environmental Pollution 212,519–24.

Quaak I, de Cock M, de Boer M et al. (2016). Prenatal Exposure to Perfluoroalkyl Substances and Behavioral Development in Children. International journal of environmental research and public health 13:5,511.

Raleigh KK, Alexander BH, Olsen GW et al. (2014). Mortality and cancer incidence in ammonium perfluorooctanoate production workers. Occupational and environmental medicine 71:7,500-6.

Rantakokko P, Mannisto V, Airaksinen R et al. (2015). Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a cohort study. Environmental health: a global access science resource 14:1,79.

Rappazzo KM, Coffman E, Hines EP. (2017). Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature. International journal of environmental research and public health 14:7,691.

Raymer JH, Michael LC, Studabaker WB et al. (2012). Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and their associations with human semen quality measurements. Reproductive toxicology (Elmsford, NY) 33:4,419-27.

Rijs KJ, Bogers RP. (2017). PFOA exposure and health: a review of scientific literature. RIVM Report 2017-0086. National Institute of Public Health and the Environment, The Netherlands.

Robledo CA, Yeung E, Mendola P et al. (2015). Preconception maternal and paternal exposure to persistent organic pollutants and birth size: The LIFE study. Environmental Health Perspectives 123:1,88-94.

Rotander A, Toms LM, Aylward L et al. (2015). Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF). Environment international. 82,28-34.

Roth N, Wilks MF. (2014). Neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals: a systematic review of the epidemiological literature using a quality assessment scheme. Toxicology Letters 230:2,271-81.

Rylander C, Duong TP, Odland JO et al. (2009). Perfluorinated compounds in delivering women from south central Vietnam. Journal of Environmental Monitoring 11:11,2002-8.

Saikat S, Krels I, Davies B et al. (2013). The impact of PFOS on health in the general population: a review. Environmental science, processes and impacts 15:2,329-35.

Sakr CJ, Leonard RC, Kreckmann KH et al. (2007a). Longitudinal study of serum lipids and liver enzymes in workers with occupational exposure to ammonium perfluorooctanoate. Journal of occupational and environmental medicine 49:8,872-879.

Sakr CJ, Kreckmann KH, Green JW et al. (2007b). Cross-sectional study of lipids and liver enzymes related to a serum biomarker of exposure (ammonium perfluorooctanoate or APFO) as part of a general health survey in a cohort of occupationally exposed workers. Journal of occupational and environmental medicine 49:10,1086-1096.

ED_002330_00119784-00399

Sakr CJ, Symons JM, Kreckmann KH et al. (2009). Ischaemic heart disease mortality study among workers with occupational exposure to ammonium perfluorooctanoate. Occupational and environmental medicine 66:10,699-703.

Savitz DA. (2007). Biomarkers of perfluorinated chemicals and birth weight (Guest Editorial). Environmental Health Perspectives 115:11,A528–A529.

Savitz DA, Stein CR, Bartell SM et al. (2012a). Perfluorooctanoic acid exposure and pregnancy outcome in a highly exposed community. Epidemiology 23:3,386-92.

Savitz DA, Stein CR, Elston B et al. (2012b). Relationship of perfluorooctanoic acid exposure to pregnancy outcome based on birth records in the mid-Ohio Valley. Environmental health perspectives 120:8,1201-7.

Shah-Kulkarni S, Kim BM, Hong YC et al. (2016). Prenatal exposure to perfluorinated compounds affects thyroid hormone levels in newborn girls. Environment international 94,607-13.

Shankar A, Xiao J, Ducatman A. (2011a). Perfluoroalkyl chemicals and chronic kidney disease in US adults. American Journal of Epidemiology 174:8,893-900.

Shankar A, Xiao J, Ducatman A. (2011b). Perfluoroalkyl chemicals and elevated serum uric acid in US adults. Clinical Epidemiology 3,251-258.

Shankar A, Xiao J, Ducatman A. (2012) Perfluorooctanoic acid and cardiovascular disease in US adults. Archives of internal medicine 172:18,1397-403.

Shapiro GD, Dodds L, Arbuckle TE et al. (2016). Exposure to organophosphorus and organochlorine pesticides, perfluoroalkyl substances, and polychlorinated biphenyls in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: The MIREC Study. Environmental research 147,71-81.

Shi Y, Yang L, Li J et al. (2017). Occurrence of perfluoroalkyl substances in cord serum and association with growth indicators in newborns from Beijing. Chemosphere 169,396-402.

Shiue I. (2017). Urinary arsenic, pesticides, heavy metals, phthalates, polyaromatic hydrocarbons, and polyfluoroalkyl compounds are associated with sleep troubles in adults: USA NHANES, 2005-2006. Environmental Science and Pollution Research 24:3,3108-16.

Shrestha S, Bloom MS, Yucel R et al. (2015). Perfluoroalkyl substances and thyroid function in older adults. Environment international 75,206-14.

Skuladottir M, Ramel A, Rytter D et al. (2015). Examining confounding by diet in the association between perfluoroalkyl acids and serum cholesterol in pregnancy. Environ Research 143:Pt A,33-8.

Smit LA, Lenters V, Høyer BB et al. (2015). Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. Allergy 70:6,653-60.

Specht IO, Hougaard KS, Spano D et al. (2012). Sperm DNA integrity in relation to exposure to environmental perfluoralkyl substances. A study of spouses of pregnant women in three geographical regions. Reproductive Toxicology (Elmsford, N.Y.) 33:4,577-83.

Spulber S, Kilian P, Wan Ibrahim WN et al. (2014). PFOS induces behavioral alterations, including spontaneous hyperactivity that is corrected by dexamfetamine in zebrafish larvae. PLoS ONE 9:4, e94227.

ED_002330_00119784-00400

Starling AP, Engel SM, Richardson DB et al. (2014a). Perfluoroalkyl substances during pregnancy and validated preeclampsia among nulliparous women in the Norwegian Mother and Child Cohort Study. American journal of epidemiology 179:7,824-33.

Starling AP, Engel SM, Whitworth KW et al. (2014b). Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort study. Environment international 62,104-12.

Steenland K, Tinker S, Frisbee S. et al. (2009). Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. American journal of epidemiology 170:10,1268–78.

Steenland K, Tinker S, Shankar A et al. (2010). Association of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with uric acid among adults with elevated community exposure to PFOA. Environmental health perspectives 118:2,229-33.

Steenland K, Woskie S. (2012). Cohort mortality study of workers exposed to perfluorooctanoic acid. American Journal of Epidemiology 176:10,909–17.

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

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

Stein CR, McGovern KJ, Pajak AM et al. (2016). Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. Pediatric Research 79:2,348-57.

Stein CR, Savitz DA. (2011). Serum perfluorinated compound concentration and attention deficit/hyperactivity disorder in children 5–18 years of age. Environmental health perspectives 119:10, 1466–71.

Stein CR, Savitz DA, Bellinger DC. (2013) Perfluorooctanoate and neuropsychological outcomes in children. Epidemiology (Cambridge, Mass) 24:4,590–9.

Stein CR, Savitz DA, Bellinger DC. (2014a). Perfluorooctanoate exposure in a highly exposed community and parent and teacher reports of behaviour in 6–12-year-old children. Paediatric and perinatal epidemiology 28:2,146–56.

Stein CR, Savitz DA, Dougan M. (2009). Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. American journal of epidemiology 170:7,837-46.

Stein CR, Savitz DA, Elston B et al. (2014b). Perfluorooctanoate exposure and major birth defects. Reproductive toxicology (Elmsford, NY) 47,15-20.

Stiles J, Jernigan TL. (2010). The basics of brain development. Neuropsychology review 20:4,327–48.

Strøm BB, Wu Q, Dumas AK et al. (2011). Perfluorochemical (PFC) exposure in children: Associations with impaired response inhibition. Environmental Science and Technology 45:19,8151–9.

Strøm M, Hansen S, Olsen SF et al. (2014). Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomes—A prospective study with long-term follow-up. Environment international 68,41–8.

ED_002330_00119784-00401

Su TC, Kuo CC, Hwang JJ et al. (2016). Serum perfluorinated chemicals, glucose homeostasis and the risk of diabetes in working-aged Taiwanese adults. Environment international 88,15-22.

Taylor KW, Hoffman K, Thayer KA et al. (2014). Polyfluoroalkyl chemicals and menopause among women 20-65 years of age (NHANES). Environmental health perspectives 122:2,145-50.

Tillett T. (2010). PFCs and cholesterol. A sticky connection. Environmental Health Perspectives 118:2, A81.

Timmermann CAG, Budz-Jørgensen E, Petersen MS et al. (2016). Shorter duration of breastfeeding at elevated exposures to perfluoroalkyl substances. Reproductive Toxicology Online doi: 10.1016/j.reprotox.2016.07.01.

Timmermann CAG, Rossing LI, Grøntved A et al. (2014). Adiposity and glycemic control in children exposed to perfluorinated compounds. Journal of clinical endocrinology and metabolism 99:4,E608-E614.

Toft G, Joensson BAG, Lindh CH et al. (2012). Exposure to perfluorinated compounds and human semen quality in arctic and European populations. Human Reproduction 27:8,2532-40.

Toft G, Jönsson BAG, Bonde JP et al. (2016). Perfluorooctane sulfonate concentrations in amniotic fluid, biomarkers of fetal Leydig cell function and cryptorchidism and hypospadias in Danish boys (1980-1996). Environmental Health Perspectives 124:1,151-6.

Tsai MS, Lin CC, Chen MH et al. (2017). Perfluoroalkyl substances and thyroid hormones in cord blood. Environmental Pollution 222,543-8.

Tsai MS, Lin CY, Lin CC et al. (2015). Association between perfluoroalkyl substances and reproductive hormones in adolescents and young adults. International journal of hygiene and environmental health 218:5,437-43.

Ubel FA, Sorenson SD, Roach DE. (1980). Health status of plant workers exposed to fluorochemicals – a preliminary report. American Industrial Hygiene Association Journal 41:8,584-9.

Uhl SA, James-Todd T, Bell ML. (2013). Association of osteoarthritis with perfluorooctanoate and perfluorooctane sulfonate in NHANES 2003-2008. Environmental health perspectives 121:4,447-52.

US EPA. (2016a). Health Effects Support Document for Perfluorooctanoic Acid (PFOA). US Environmental Protection Agency, Washington.

US EPA. (2016b). Health Effects Support Document for Perfluorooctane Sulfonate (PFOS). US Environmental Protection Agency, Washington.

Vagi SJ, Azziz-Baumgartner E, Sjödin A et al. (2014). Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds phthalates and bisphenol a in polycystic ovary syndrome: a case-control study. BMC Endocrine Disorders 14:1,86-98.

Vassiliadou I, Costopoulou D, Ferderigou A et al. (2010). Levels of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in blood samples from different groups of adults living in Greece. Chemosphere 80:10,1199-206.

Velez MP, Arbuckle TE, Fraser WD. (2015). Maternal exposure to perfluorinated chemicals and reduced fecundity: the MIREC study. Human reproduction (Oxford, England) 30:3,701-9.

Verner MA, Loccisano AE, Morken NH et al. (2015). Associations of perfluoroalkyl substances (PFASs) with lower birth weight: an evaluation of potential confounding by glomerular filtration rate

ED_002330_00119784-00402

using a physiologically based pharmacokinetic model (PBPK). Environmental Health Perspectives 123:12,1317–24.

Verner M, Loccisano A, Yoon M et al. (2014). The association between prenatal exposure to perfluoroalkyl substances (PFAS) and reduced birth weight: is glomerular filtration rate the underlying cause? (Abstract). Abstract 89. Toxicologist 138:1,18.

Vested A, Ramlau-Hansen CH, Olsen SF et al. (2013). Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. Environmental health perspectives 121:4,453-8.

Vestergaard S, Nielsen F, Andersson AM et al. (2012). Association between perfluorinated compounds and time to pregnancy in a prospective cohort of Danish couples attempting to conceive. Human reproduction (Oxford, England) 27:3,873-80.

Vesterinen HM, Johnson PI, Atchley DS et al. (2014). Fetal growth and maternal glomerular filtration rate: a systematic review. Journal of Maternal-Fetal & Neonatal Medicine 28:18,2176–81.

Vieira VM, Hoffman K, Shin H-M et al. (2013). Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. Environmental health perspectives 121:3,318-23.

Vuong AM, Yolton K, Webster GM et al. (2016). Prenatal polybrominated diphenyl ether and perfluoroalkyl substance exposures and executive function in school-age children. Environmental Research 147,556–64.

Wang IJ, Hsieh WS, Chen CY et al. (2011). The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. Environmental research 111:6,785-91.

Wang Y, Adgent M, Su PH et al. (2016). Prenatal exposure to perfluorocarboxylic acids (PFCAs) and fetal and postnatal growth in the Taiwan maternal and infant cohort study. Environmental health perspectives 124:11,1794-800.

Wang Y, Rogan WJ, Chen H-Y et al. (2015). Prenatal exposure to perfluoroalkyl substances and children's IQ: The Taiwan maternal and infant cohort study. International journal of hygiene and environmental health 218:7,639-44.

Wang Y, Rogan WJ, Chen PC et al. (2014). Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environmental health perspectives 122:5,529-34.

Wang Y, Starling AP, Haug LS et al. (2013). Association between perfluoroalkyl substances and thyroid stimulating hormone among pregnant women: a cross-sectional study. Environmental Health: a global access science source 12:1,76.

Wang J, Zhang Y, Zhang W et al. (2012). Association of perfluorooctanoic acid with HDL cholesterol and circulating miR-26b and miR-199-3p in workers of a fluorochemical plant and nearby residents. Environment science and technology 46:17,9274-81.

Washino N, Saijo Y, Sasaki S et al. (2009). Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environmental health perspectives 117:4,660-7.

Watkins DJ, Josson J, Elston B et al. (2013). Exposure to perfluoroalkyl acids and markers of kidney function among children and adolescents living near a chemical plant. Environmental Health Perspectives 121:5,625-30.

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Webster GM, Rauch SA, Marie NS et al. (2016). Cross-sectional associations of serum perfluoroalkyl acids and thyroid hormones in U.S. Adults: Variation according to TPOAb and iodine status (NHANES 2007-2008). Environmental health perspectives 124:7,935-42.

Webster GM, Venners SA, Mattman A et al. (2014). Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. Environmental research 133,338-47.

Wen LL, Lin LY, Su TC et al. (2013). Association between serum perfluorinated chemicals and thyroid function in U.S. adults: the National Health and Nutrition Examination Survey 2007-2010. The Journal of clinical endocrinology and metabolism 98:9,E1456-64.

Whittaker PG, Macphail S, Lind T. (1996). Serial hermatologic changes and pregnancy outcome. Obstetrices & Gynecology 88:1,33–39.

Whitworth KW, Haug LS, Baird DD et al. (2012a). Perfluorinated compounds and subfecundity in pregnant women. Epidemiology 23:2,257-63.

Whitworth KW, Haug LS, Baird DD et al. (2012b). Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study. American journal of epidemiology 175:12,1209-16.

Whitworth KW, Haug LS, Sabaredzovic A et al. (2016). Brief Report: Plasma Concentrations of Perfluorooctane Sulfonamide and Time-to-pregnancy Among Primiparous Women. Epidemiology 27:5,712-5.

Winquist A, Steenland K. (2014a). Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. Environmental health perspectives 122:12,1299-1305.

Winquist A, Steenland K. (2014b). Perfluorooctanoic acid exposure and thyroid disease in community and worker cohorts. Epidemiology 25:2,255-64.

Wu K, Xu X, Peng L et al. (2012). Association between maternal exposure to perfluorooctanoic acid (PFOA) from electronic waste recycling and neonatal health outcomes. Environment international 48,1-8.

Wu H, Yoon M, Verner M-A et al. (2015). Can the observed association between serum perfluoroalky substances and delayed menarche be explained on the basis of puberty-related changes in physiology and pharmacokinetics? Environment international 82,61-8.

Yamaguchi M, Arisawa K, Uemura H et al. (2013). Consumption of seafood, serum liver enzymes, and blood levels of PFOS and PFOA in the Japanese population. Journal of occupational health 55:3,184-94.

Yang L, Li J, Lai J et al. (2016). Placental Transfer of Perfluoroalkyl Substances and Associations with Thyroid Hormones: Beijing Prenatal Exposure Study. Scientific reports 6,21699.

Yeung LW, Guruge KS, Taniyasu S et al. (2013). Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicology and environmental safety 96,139-46.

Zeng XW, Qian Z, Emo B. (2015). Association of polyfluoroalkyl chemical exposure with serum lipids in children. The Science of the total environment 512–513,364–70.

ED_002330_00119784-00404

Zhang C, Sundaram R, Maisog J et al. (2015). A prospective study of prepregnancy serum concentrations of perfluorochemicals and the risk of gestational diabetes. Fertility Sterility 103:1,184-189.

Zhou Y, Hu LW, Qian Z et al. (2016). Association of perfluoroalkyl substances exposure with reproductive hormone levels in adolescents: By sex status. Environment International 94,189-95.

Zhu Y, Qin XD, Zeng XW et al. (2016). Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: By gender and asthma status. Science for the Total Environment 559,166-73.

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Expert Health Panel

for per- and poly-fluorinated substances (PFAS)

Appendix One

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PART A – INTRODUCTION

1. INTRODUCTION

Background

Per- and poly-fluoroalkyl substances (PFAS) used in fire-fighting foams on Defence bases, civilian airports and firefighting training grounds, have migrated through the groundwater into adjoining areas.

These chemicals can persist in humans, animals and the environment. In particular, they appear to accumulate in humans and are then very slowly eliminated from the body. The Environmental Health Standing Committee (enHealth) currently advises that 'there is currently no consistent evidence that exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes adverse human health effects' (enHealth, 2016); and recent reviews conducted by regulatory bodies have concluded that there is no compelling evidence that PFAS at the concentrations found in these areas are harmful to health. However, as a precaution it is generally recommended that exposure be minimised wherever possible.

Investigation of health effects and research priorities

To further investigate the potential health effects of PFAS, the Australian Government has set aside funds to commission further research into the potential health effects of PFAS exposure.

The Expert Health Panel for PFAS (the Panel) has been established to review the current literature on potential health effects of PFAS exposure and identify priority areas for research. *Allen + Clarke* is an independent organisation that is assisting the Panel with this work.

The Panel has two main priorities:

1. to provide advice to the Government on the health impacts of PFAS, and
2. to advise the National Health and Medical Research Council on priority research areas for future research into the human health impacts of PFAS.

It is expected that the Panel will provide its advice to the Minister for Health, the Hon Greg Hunt MP, in the form of an interim report by 22 December 2017. To inform this advice, the Panel will:

- take into account the evidence available from both Australian and international scientific research into the potential human health effects of PFAS exposure; and
- consider the views of the public and other stakeholders through an invitation for public written submissions.

The public consultation process that informed this report was the mechanism for the public and other stakeholders to provide their views to the Panel on the potential health impacts of PFAS, and their views on priorities for further research.

Structure of this report

Part A of this document provides a high level introduction to the Panel and this public consultation process (section 1), a summary of the key themes from the public consultation

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(section 2), and the methodology (section 3). Part A also provides high level information about the respondents, including:

- Respondent numbers and type (section 4)
- Demographic information (section 5).

Part B of this document sets out the findings of the public consultation. The findings from the public consultation are presented in an order consistent with the sections of the public consultation document, specifically:

- Exposure pathways (section 6)
- Concern for potential health effects (section 7)
- Informational and understanding (section 8)
- Future research priorities (section 9)
- Other comments (section 10).

The questions used in the consultation document have been included in green boxes for context. They are accompanied by a short paragraph on the design of the question and what information each question was designed to collect.

Sections discussing the free text responses provided by respondents are broken down into sub-themes that emerged through an analysis of the text of the responses.

2. METHODOLOGY

Allen + Clarke provided support to the Panel to develop a consultation document and undertake the public consultation. The purpose of the public consultation document was to allow the public to provide information to the Panel on their health concerns regarding PFAS exposure and contamination, the exposure pathways that concern them, and the extent to which they feel they have been informed on various aspects of PFAS contamination.

The consultation document also collated respondents' views on which areas of human health research relating to PFAS they felt should be prioritised as part of the Government's further research into the potential health effects of PFAS exposure. The consultation document is included as Appendix One.

As the Panel is required to provide an interim report to the Minister of Health by 22 December 2017, the survey predominantly contained closed-ended questions, supplemented by four open-ended questions to give respondents the opportunity to include any further comments. The reporting timeframes meant that the public consultation period could only be open for 19 days (1 November to 19 November 2017).

Respondents could complete the survey either electronically via the Survey Monkey website, by downloading a PDF version of the survey and emailing it to a dedicated email address (PFAS@allenandclarke.com), or by printing a hard copy and mailing it to a Department of Health postal address. All responses submitted were via either Survey Monkey or email; no postal submissions were received within the consultation period.

Once received by *Allen + Clarke*, all submissions were anonymised and given numerical identifiers. All questions were voluntary, and many respondents chose only to answer some of

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the questions. Submissions where respondents had not answered any questions except for the demographic information were deemed incomplete and were removed. Of the 497 submissions received through Survey Monkey, six submissions were removed due to the respondent only answering some demographic questions.

In addition, eight submissions were submitted via email, leading to a total of 499 complete submissions: 491 submissions received through Survey Monkey, and eight email submissions via email to the consultation's dedicated email address.

Respondents were asked questions across five areas:

1. **General information** on the respondent including demographic data (age, sex), and which sector best represented them as either an individual or a group.
2. **Exposure pathways** including questions on why PFAS exposure is relevant to the respondent, and which exposure pathways concerned respondents the most.
3. **Concerns about potential health impacts** of PFAS exposure, including questions on which potential health impacts from PFAS exposure concerned respondents the most.
4. **Information and understanding** including questions on how informed respondents feel about research on PFAS and the government response to address health concerns.
5. **Future research priorities** including questions on which topics related to human health should be prioritised for future research.
6. **Other comments**, providing an opportunity for respondents to discuss other issues relevant to health concerns relating to PFAS exposure or future research priorities.

Multiple questions were asked in each of these sections.

Ranking questions were used to ask respondents to prioritise information where multiple options exist. Questions that used ranking questions related to exposure pathways and research priorities, as this allowed respondents to indicate the relative importance of each potential option. This data was analysed through Survey Monkey to generate weighted averages that indicate the relative priority respondents gave to each exposure pathway or research priority.

Likert scale questions were used to seek respondents' views on the extent to which they agree or disagree with a particular statement, or the strength of their views on a particular subject. Likert scales were used to gauge the level of concern people held that PFAS had affected respondents' health, and their understanding of research relating to PFAS.

Responses under these questions were analysed through Survey Monkey.

Open ended questions were provided to allow respondents to:

1. provide their views without prompting them or providing a list that may prejudice their answers (for example, an open question was used to ask respondents about which potential health impacts on human health resulting from PFAS exposure they were concerned about), and
2. provide additional or supporting information following structured, closed questions (for example, an open question was provided to allow respondents to indicate additional potential exposure sources that were not included in the closed question).

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Responses to open questions were entered into an NVivo database and analysed for themes using NVivo software. *Allen + Clarke* set up a coding framework that grouped answers to similar questions together into themes, for example, all responses relating to potential exposure pathways were grouped together. Responses coded to these themes were further coded into sub-themes, for example, responses relating to occupational exposure to PFAS were grouped together, and responses relating to drinking water exposure pathways were grouped together. Coding responses in this way gave an indication of how many respondents gave answers under different themes to inform analysis of which issues respondents discussed the most.

Eight submissions were received through the PFAS consultation inbox, and where possible, the information in the submissions were included within the relevant sections of this analysis. A number contained a substantive amount of supplementary information that did not relate to the questions asked in the public consultation document. The supplementary information included providing research papers on PFAS, and operational policies regarding historical use of PFAS. All submissions were also provided to the Panel in full for consideration, including any supplementary information.

Where relevant, quotes from the responses have been used to provide depth to the analysis. Quotes can give emphasis to people's experiences and concerns in a more personal and direct manner, however it is important to note that individual quotes do not necessarily represent a widely held view or even the views of a majority of submitters. Quotes have only been used where there is no identifying information that could link the quote to the respondent to ensure that respondents remain anonymous. Where quotes have been used from submissions provided on behalf of organisations, an indication of the type of organisation has been provided. Organisations have not been named as the public consultation document specified that all submissions would remain anonymous.

Using sub-groups for more detailed analysis

Demographic information gathered under the *General Information* and *Exposure Pathways* question areas was used to classify respondents into sub-groups to allow for more detailed comparisons between groups of respondents. Based on the responses received, two sub-groups were created to allow for the responses from each group to be compared and analysed independently of the other responses. The two sub-groups created based on the number of respondents were:

- **Occupationally exposed**: respondents who reported that they were occupationally exposed to PFAS containing chemicals at some point in their lives (n=249), and
- **Living in an investigation area:** respondents who reported living, or having lived, in an area being investigated for PFAS contamination (n=224).

These two sub-groups provided different perspectives regarding the health impacts and exposure pathways they were concerned about, and the research priorities they thought were most important.

Because respondents were allowed to indicate multiple reasons for why PFAS exposure was of interest to them, there is some cross over between these groups. For example, of the 249 respondents who reported being occupationally exposed to PFAS, 40 of these respondents (16%) also reported that they live, or have lived, in an area being investigated for PFAS contamination. Their answers were therefore included in the responses of both sub-groups. A

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small number of respondents did not fit into either of these subgroups, however their responses were considered as part of the wider analysis of responses as a whole.

Number and types of submissions

491 complete submissions were submitted through the online survey and an additional eight submissions were received through the PFAS Consultation inbox.

In total, 499 complete submissions were received from a range of individuals and organisations.

3. RESPONDENTS

Are you making a submission as:

Of the total 499 completed submissions, 455 were on behalf of individual/s and 34 were on behalf of groups or organisations. Ten respondents skipped this question.

Not every respondent reported demographic information or answered every question.

4. DEMOGRAPHICS

Sex:

Respondents were asked to specify their sex. 482 respondents chose to specify their gender. Of these respondents, 332 identified as male, 144 as female, one as X (indeterminate/Intersex/unspecified), and five preferred not to say.

Overall, 332 of the 482 respondents who chose to specify their gender were male. However, when looking at subgroups, respondents who reported that they were occupationally exposed to PFAS were 96 percent male (237 respondents out of the 248 who reported that they were occupationally exposed).

There was a more even split in gender amongst those who reported that they lived, or previously lived, in an area being investigated for PFAS contamination. Of the 222 respondents who reported that they live, or previously lived, in an area being investigated for PFAS contamination – 123 identified as female (55%), 95 identified as male (43%), and four preferred not to say (2%).

Age

Respondents were asked to indicate their age as best represented by the following range:

- aged under 25 (6 respondents);
- aged 25 to 44 (168 respondents);
- aged 45 to 64 (244 respondents);
- aged 65 to 84, or (61 respondents);

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- prefer not to say (4 respondents).

483 respondents chose to answer this question.

PART B: FINDINGS

This part presents the findings of the analysis of submissions. The findings are presented in relation to the following areas:

- Exposure pathways (section 6);
- Concern for potential health effects (section 7);
- Informational an understanding (section 8);
- Future research priorities (section 9);
- Other comments (section 10).

5. EXPOSURE PATHWAYS

Respondents were asked to indicate which PFAS exposure pathways concerned them the most, and if there were any other exposure pathways not listed in the consultation document that concerned them.

These questions were designed to inform the Panel of which aspects of PFAS contamination were of most concerned to respondents.

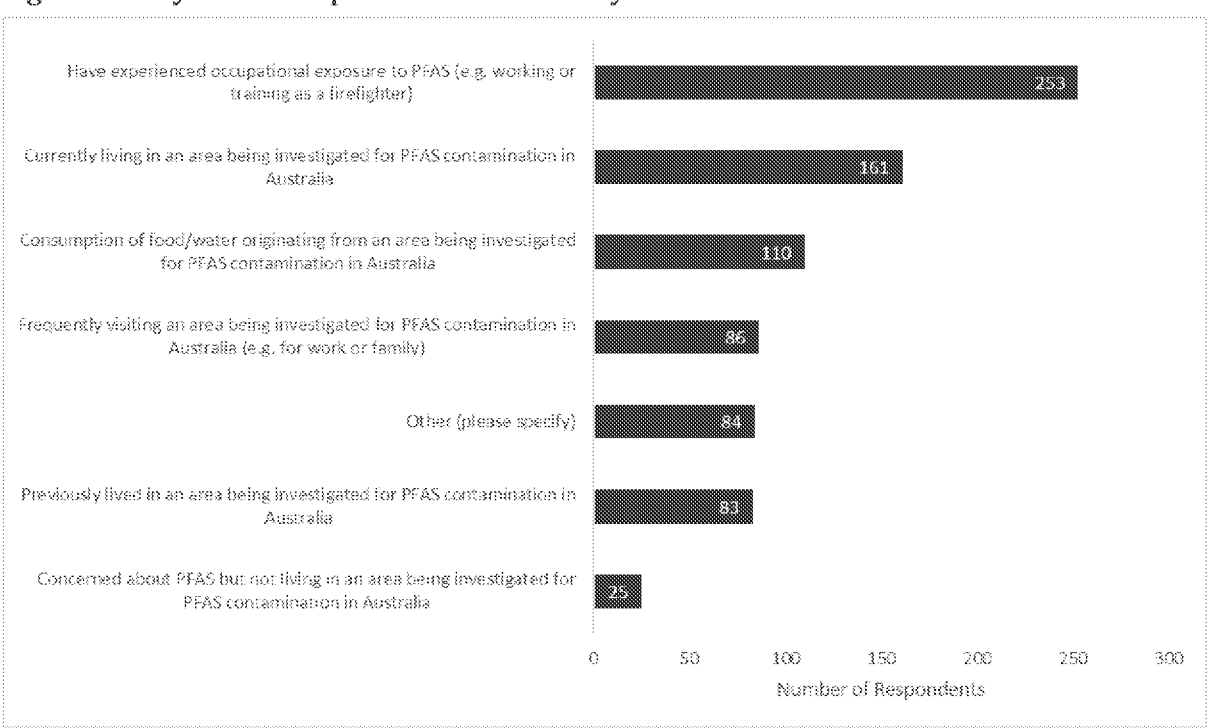
5.1. Why PFAS exposure is of concern to respondents

Question: Why is PFAS exposure of interest to you? (Check as many that apply)

Respondents were asked to use check-boxes to indicate why PFAS exposure was of interest to them from a list of pre-populated reasons. Respondents could select as many reasons for their interest in PFAS exposure as applied to their or their organisation's circumstances.

Figure 1 displays the results under this question.

Figure 1: Why is PFAS exposure of interest to you?



5.2. What sources or potential exposure to PFAS concern respondents the most?

Question: What sources of potential exposure to PFAS concern you the most? Please rank in order of what concerns you most: from 1 (most concern) to 10 (least concern).

Respondents were asked to rank the listed exposure pathways in order of what sources of exposure to PFAS they were the most concerned about. The weighted averages in the analysis below have been calculated so that a higher weighted average indicates that respondents were relatively more concerned about that particular exposure pathway compared to others.

Figure 2 displays the weighted average of these answers.

442 respondents answered this question. However, eight respondents noted in the following free text question that it was impossible to rank exposure pathways, and all should be of concern.

Figure 2: Exposure pathways ranked by level of concern

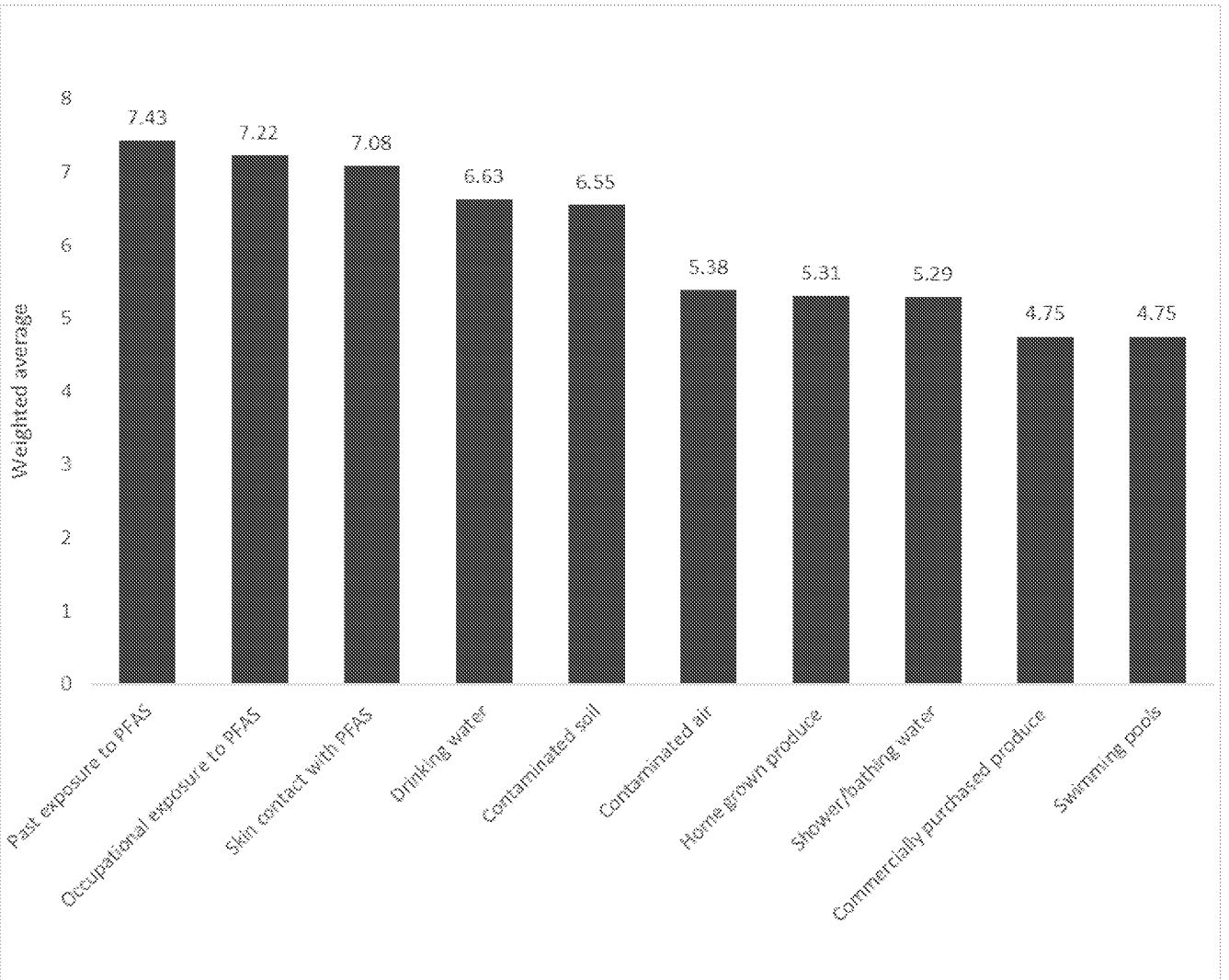


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Figure 3 compares the different weighting between respondents who reported that they were occupationally exposed to PFAS, versus those who reported they lived, or have lived, in an area being investigated for PFAS contamination.

Figure 3: Exposure pathways occupationally exposed respondents vs. respondents who lived in a PFAS contaminated area were concerned about

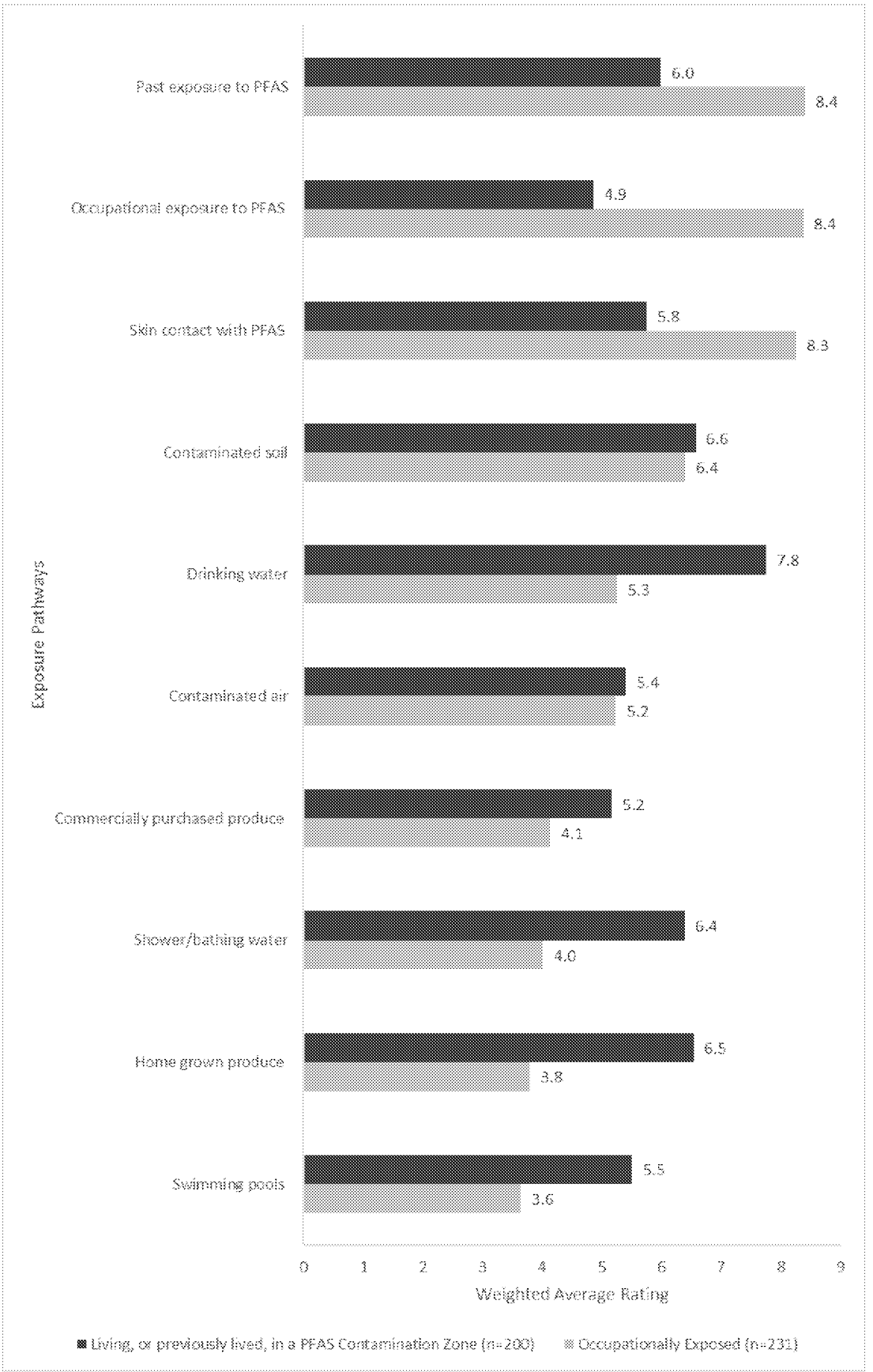

Figure 3 indicates that the exposure pathways that most concerned those who were occupationally exposed to PFAS were:

- past exposure to PFAS;
- working in an industry using PFAS chemicals; and
- skin contact with PFAS containing chemicals.

The exposure pathways that most concerned respondents who reported that they live, or have previously lived, in an area currently being investigated for PFAS exposure were:

- drinking water;
- contaminated soil; and
- home grown produce.

5.3. Other source of potential exposure causing respondents concern

Question: Is there a potential source of exposure to PFAS not listed in the table that you are more concerned about?

Respondents were asked if there were any other exposure pathways that were not listed in the previous question. Ninety-three respondents provided further detail on exposure pathways that they were concerned about.

While the question asked for comments on exposure pathways not listed in the previous question, 47 respondents provided additional information on occupational exposure pathways that they had selected from the list in the previous question, and 13 respondents commented on aspects of bore water contamination that concerned them. Their answers have been included in the analysis to give additional detail on why respondents were concerned about these exposure pathways.

Responses under this question provided on behalf of organisations have also been included and do not deviate significantly from those provided by individuals.

Respondents reported more detail the different kinds of occupational exposure pathways that concerned them

Forty-seven of the 93 respondents who answered this question reiterated their concerns regarding occupational exposure to PFAS, or provided more detail on the different exposure pathways they were occupationally exposed to PFAS. All of these respondents reported that this occupational exposure was in relation to training or working as a firefighter.

Some of the various occupational exposure pathways related to firefighting that respondents reported being concerned about include:

- while refilling firefighting trucks (7 respondents);
- using it as dishwashing liquid at work (6 respondents);
- accidentally ingesting foam (4 respondents);
- contaminated training grounds or dust inhalation (7 respondents).

One respondent noted:

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> *"During training and operation duties it was not unusual to be soaked to the skins with foam also it was often in your face and was ingested"*

When discussing occupational exposure, four respondents also reported concern that their families were exposed to PFAS at home when washing their work clothing or when they went home after wearing contaminated clothing.

PFAS contaminated bore and surface water

Thirteen of the 93 respondents reported that PFAS contaminated bore water or surface water was an exposure pathway that concerned them. This included concerns from respondents about coming into contact with PFAS through irrigation systems or rivers/streams that were contaminated with PFAS.

One respondent, a state Government department, noted PFAS contamination in drinking water from unreticulated water or town supply, or unlicensed ground water bores was of concern:

> *The exposure pathways that ... is most concerned about are drinking water from sources which are not on a reticulated water or town water supply. Where there is use of surface water or groundwater used for drinking water or for irrigation of crops and livestock. There is a high potential in some areas for unlicensed groundwater bores to exist and for this bore water to be used for drinking water. Due to the chemical characteristics of PFAS, highly mobile persistent surfactant, the contamination can find its way into unlicensed bore water supplies.*

Exposure to PFAS in utero or through breastmilk

Seven of the 93 respondents reported that they were concerned about babies being exposed to PFAS *in utero*, with three of these respondents also noting concern that babies may be exposed to PFAS through breastmilk.

Other concerns respondents noted

Respondents provided a range of other PFAS exposure pathways they were concerned about including:

- home grown product or fish (5 respondents);
- contaminated dust (4 respondents);
- other products such as Teflon (3 respondents);
- commercially purchased food (2 respondents);
- contaminated atmosphere and rain (2 respondents);
- drinking water (2 respondents);and
- playing in AFFF foam as a child at public parades (1 respondent).

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6. CONCERNS ABOUT POTENTIAL HEALTH IMPACTS OF PFAS EXPOSURE

Respondents were asked to report how concerned they felt about various aspects of PFAS exposure and any potential effects on their own health, or their family's health. They were also asked to report any health issues they were concerned about relating to PFAS exposure.

These questions were designed to inform the Panel of what health issues potentially related to PFAS exposure were of concern to the public. This information will be used to inform the Panel's deliberations regarding future research priorities.

6.1. How concerned respondents felt about aspects of PFAS exposure on their health

How concerned are you that you about the following? Please use the scale below ranging from 1 (not at all concerned) to 5 (very concerned).

Respondents were asked to report how concerned they felt that four different aspects of potential PFAS contamination was having an effect on their health, or their family's health. The four aspects of potential PFAS contamination were the extent to which the respondent was concerned:

- that they or their family's future health might be affected by PFAS;
- that they or their family's health had already been affected by PFAS;
- about avoiding exposure to PFAS; and
- about indirect health impacts of living inside an investigation area.

Respondents were asked to report how concerned they felt about each aspect using the following scale:

1	2	3	4	5	N/A
Not at all concerned	Not concerned	Neutral	Concerned	Very Concerned	

Figure 4 details how respondents answered this question.

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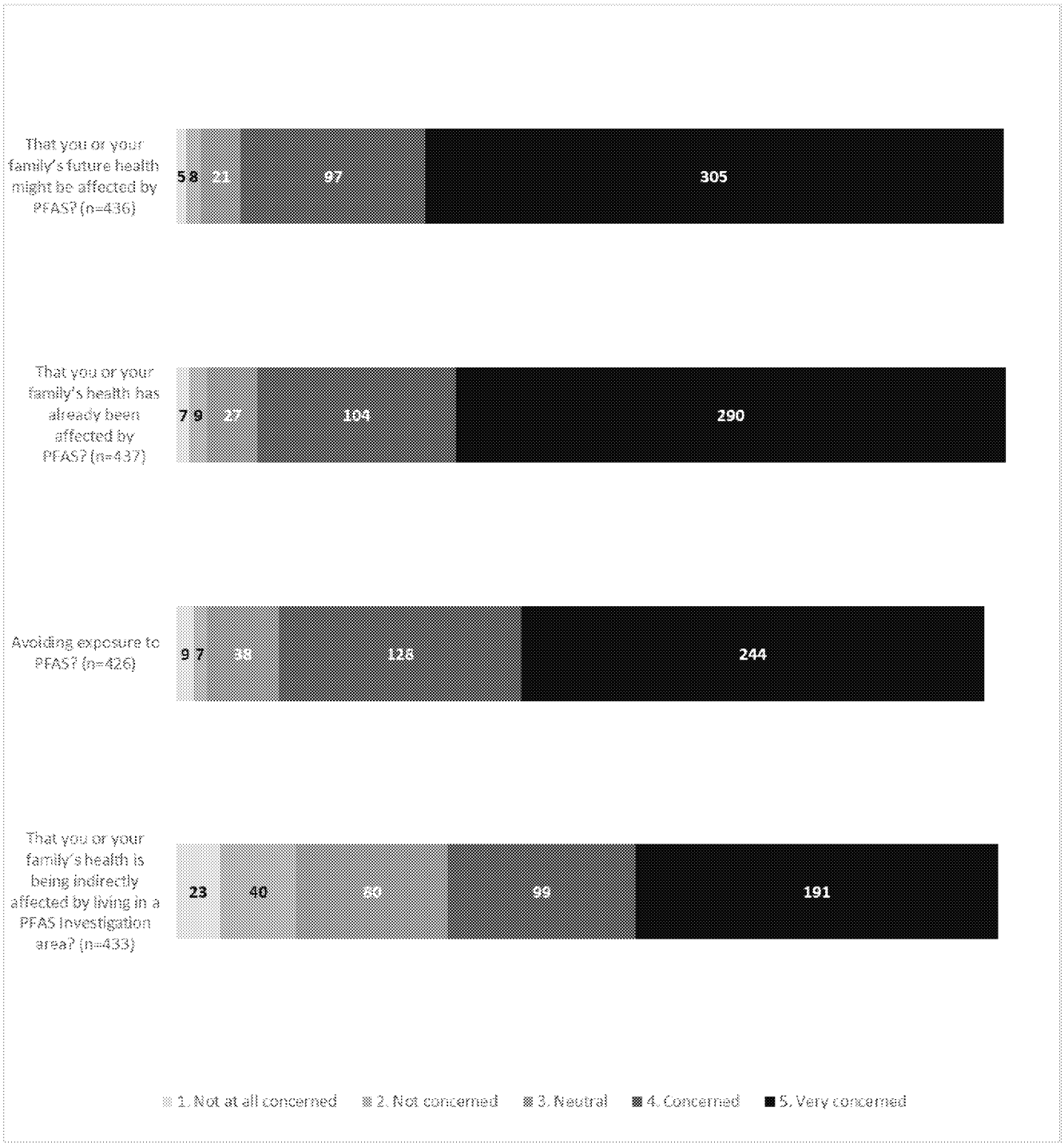
Figure 4 : How concerned are you about the following health impacts of PFAS? 1 (not at all concerned) to 5 (very concerned)



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Question: How concerned are you that you or your family's future health might be affected by PFAS?

436 respondents chose to answer this question.

Seventy percent of respondents who answered the question reported being "very concerned" that PFAS might affect their own future health or their family's future health, and a further 22 percent reported they were "concerned."

Question: How concerned are you that you or your family's health has already been affected by PFAS?

437 respondents chose to answer this question.

Sixty-seven percent of respondents who answered this question reported feeling "very concerned" that their own health, or their family's health, had already been affect by PFAS exposure, and a further 24 percent reported they were "concerned."

Question: How concerned are you about avoiding exposure to PFAS?

426 respondents chose to answer this question.

Fifty-three percent of respondents who answered this question reported feeling "very concerned" about avoiding exposure to PFAS, a further 30 percent reported that they were "concerned."

Question: How concerned are you that you or your family's health is being indirectly affected by living in a PFAS investigation area (e.g. stress and anxiety due to financial impacts, publicity or media attention?)

433 respondents chose to answer this question.

Forty-four percent of the respondents who answered this question reported feeling "very concerned" that their health, or their family's health, was being indirectly affected by PFAS exposure, and a further 23 percent reported that they were "concerned."

Under this question, respondents were asked whether their health was being indirectly affected by *living in a PFAS investigation area*. 433 respondents answered this question, however only 161 of these respondents reported that they live in a PFAS investigation area. Responses under this question have not been filtered by those who reported themselves as currently living in an investigation area. Instead, these responses could possibly be interpreted to represent wider concern that respondents were being indirectly affected by PFAS exposure more broadly, or an expression of concern for those who were living in such areas, or a mix of both.

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6.2. Potential health impacts respondents were concerned about

Question: If you are concerned about exposure to PFAS, what potential impacts on human health from PFAS exposure are you concerned about?

Respondents were asked how concerned they were about the effect of PFAS on their health, or their family's health. 339 respondents specifically mentioned potential health impacts on their own health, or on their families, that they were concerned about.

This question was in a free text form so that responses were unprompted.

Responses under this question provided on behalf of organisations have also been included and do not deviate significantly from those provided by individuals.

The most common response was concern that PFAS exposure may cause cancer

189 respondents reported they were concerned about a link between PFAS exposure and cancer(s).

Sixteen of the 189 respondents that expressed concern about cancer being caused by PFAS exposure noted that they, their families, or their former colleagues, had received a cancer diagnosis and they were concerned that this may be linked to PFAS exposure.

178 of the 189 respondents expressed general concern about cancer being caused by PFAS exposure without providing further detail. The remaining 11 respondents specified the types of cancers that they were concerned about in particular. These included testicular, prostate, kidney, liver, pancreatic, stomach, bladder and thyroid cancers.

One respondent, representing a community support group for residents affected by PFAS exposure, expressed concern that there is a high incidence of cancer within their community, noting that:

> *"39 residents within one 5km stretch of road were reported to have suffered some form of cancer in the last 15 years alone; that number has now increased to 50 upon further investigation."*

Respondents noted that they were concerned about any potential health effects that could arise from PFAS exposure

Eighty-two of the 339 respondents who answered this question noted that they were concerned about *any* possible health effects that could arise from PFAS exposure. These included comments noting that it was difficult to formulate a response to this question when people were not clear on the actual effects of exposure to PFAS. Examples of comments under this theme include "*All 'probable links'*" and "*all or any – we do not know which is the problem*".

Fifteen of the 82 respondents who noted they were concerned about any or all health impacts from PFAS, also noted that they were concerned that PFAS exposure would be linked to other health conditions in the future. One of these respondents, an individual who reported currently living in an area being investigated for PFAS contamination, noted that they were worried that PFAS exposure may cause cancers and thyroid conditions but also that these conditions:

> *"are just the things they have evidence for – I am most concerned about the unknown that is yet to be researched."*

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Many respondents were concerned about the long-term health effects on adults, workers and children

A total of 69 respondents commented that they were concerned that PFAS exposure may cause or lead to long-term health effects. Of these 69 respondents:

- 33 respondents discussed their concern for potential long-term health effects for children who have been exposed to PFAS,
- ten respondents discussed their concerns with regard to workers who were occupationally exposed to PFAS over a long period, and
- seven respondents noted concern that their own PFAS exposure may cause epigenetic changes that may affect their children and grandchildren.

The 33 respondents who noted concern for potential long-term health effects on children's health from PFAS exposure provided a range of comments on their concerns. Some respondents shared their experience regarding their concern for their children's health and noted that their children had been exposed from a young age, many since they were toddlers or *in utero*.

Mental health impacts including stress and anxiety

Twenty-six of the 189 respondents that answered this question reported that they were concerned about the effect PFAS exposure or contamination was having on their mental health. When discussing factors that were affecting their mental health, five respondents noted that their mental health was being affected by the uncertainty over their future health and not knowing what PFAS exposure might mean for the health of their families. Four respondents noted that financial pressures from living in a contaminated area, such as not being able to access bank loans or sell their houses, was impacting their mental health.

There were a number of personal stories or anecdotes provided illustrating the impact PFAS contamination and exposure has had on people's mental health.

One respondent who reported being occupationally exposed to PFAS suggested that the mental health aspect of PFAS exposure had not received prominence in the response when they noted that:

> *"there is level of mental anguish that sits at the centre of this that seems to have slipped by unnoticed."*

Another respondent who also reported being occupationally exposed to PFAS, discussed feeling like a *"testing opportunity"* for Australia while their mental health was significantly compromised knowing that they had limited options to move away from the contamination zone.

> *"Stress due to not being listened to in regards to wanting a basic test and to stop being told you don't fall into the investigation group. Please treat me as a person with worries and stress and not as a nobody".*

Respondents were concerned that PFAS exposure may cause or aggravate thyroid issues or hormonal imbalances

Thirty-two respondents commented that they were concerned about thyroid issues or a range of other hormonal imbalances including disruption to their endocrine system or other hormonal imbalance.

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Concern that PFAS exposure may cause developmental effects in babies

Twenty-one respondents commented that they were concerned that PFAS exposure may adversely affect babies *in utero* or via exposure from breastmilk. Concerns included that PFAS may cause birth defects, developmental delays, low birth weight, neurological conditions or stillbirth.

Other health concerns that respondents wrote about

Respondents provided a range of other health issues that they were concerned may be linked to PFAS exposure including:

- Immune system response (17 respondents);
- High cholesterol and associated heart issues (15 respondents);
- Premature death (14 respondents);
- Kidney disorders (13 respondents);
- Liver issues (11 respondents);
- Fertility issues (10 respondents);
- Respiratory issues (6 respondents);
- Skin conditions (6 respondents);
- Neurological uses such as stroke or Parkinson's (6 respondents);
- Digestive issues (4 respondents);
- Dental issues (3 respondents);
- Autism (3 respondents);
- Blood poisoning (2 respondents);
- ADHD (1 respondent); and
- Compromised vision (1 respondent).

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7. INFORMATION AND UNDERSTANDING

Question: How informed do you feel on the following topics? Please use the scale below ranging from 1 (not at all informed) to 5 (very informed).

Respondents were asked how informed they felt about four different aspects of information and understanding relating to PFAS exposure and contamination:

- the Government's response to address the health concerns of communities exposed to PFAS;
- research on the effects of PFAS exposure;
- levels of exposure to PFAS in specific communities; and
- different ways people and communities may be exposed to PFAS.

Respondents were asked to report how informed they felt about each aspect using the following scale:

1	2	3	4	5	N/A
Not at all informed	Not informed	Neutral	Informed	Very Informed	

Figure 5 details how respondents answered this question.

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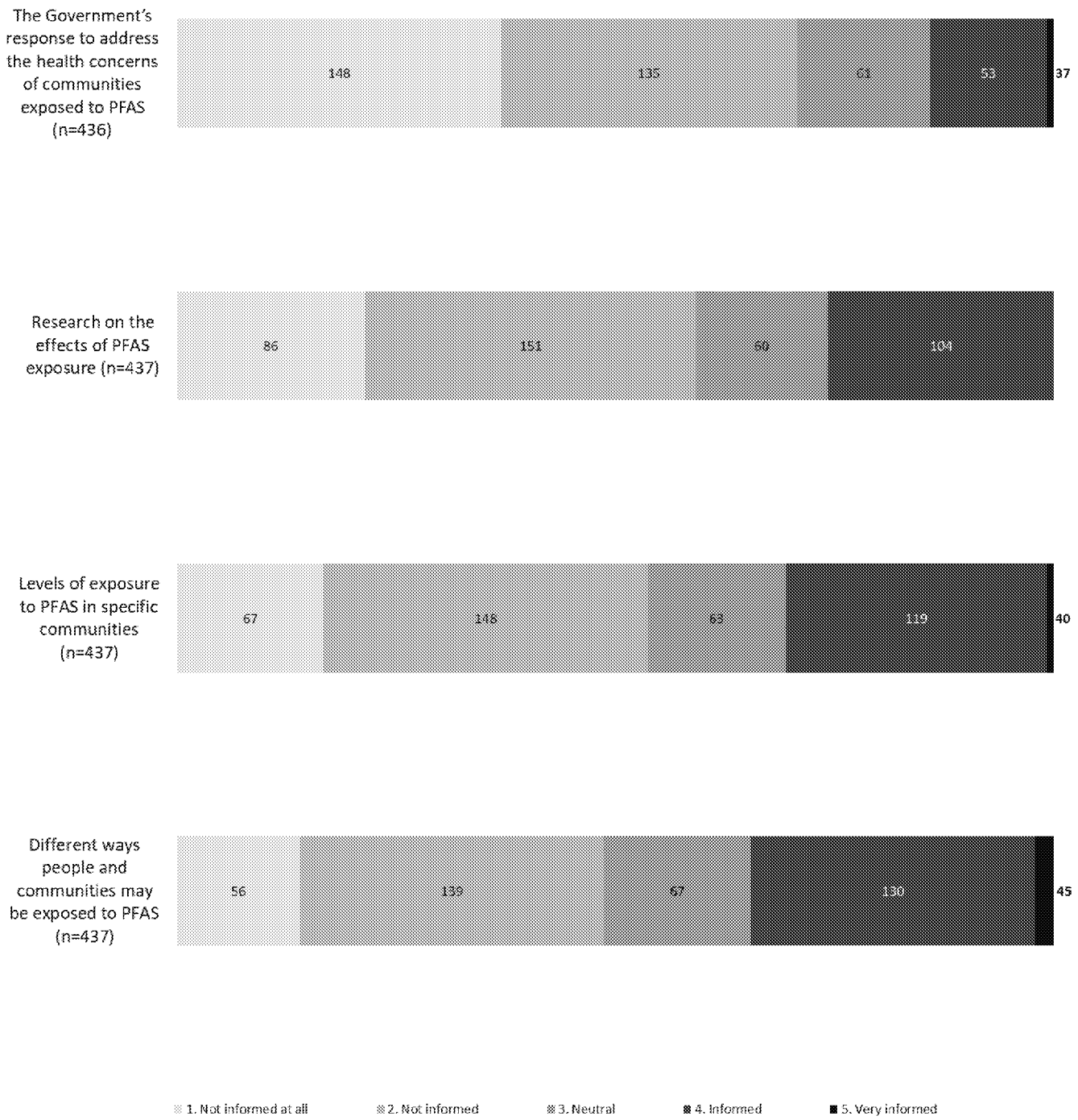
Figure 5: How informed do you feel on the following topics? Please use the scale below ranging from 1 (not at all informed) to 5 (very informed).



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Question: The Government's response to address the health concerns of communities exposed to PFAS

436 respondents answered this question.

Sixty-five percent of respondents who answered this question reported that the felt "not at all informed" or "not informed" about the Government's response to addressing health concerns of communities exposed to PFAS.

Twenty-one percent of respondents who answered this question reported that they felt "informed" or "very informed" about the Government's response to addressing health concerns of communities exposed to PFAS.

Question: Research on the effects of PFAS exposure

437 respondents answered this question.

Fifty-four percent of respondents who answered this question reported that they felt "not at all informed" or "not informed" about research on the effects of PFAS exposure.

Thirty-two percent of respondents who answered this question reported that they felt "informed" or "very informed" about research on the effects of PFAS exposure.

Question: Levels of exposure to PFAS in specific communities

437 respondents answered this question.

Forty-nine percent of respondents who answered this question reported that they felt "not at all informed" or "not informed" about levels of PFAS exposure in specific communities.

Thirty-six percent of respondents who answered this question reported that they felt "informed" or "very informed" about levels of PFAS exposure in specific communities.

Question: Different ways people and communities may be exposed to PFAS

437 respondents answered this question.

Forty-five percent of respondents who answered this question reported that they felt "not at all informed" or "not informed" about different ways people and communities may be exposed to PFAS.

Forty percent of respondents who answered this question reported that they felt "informed" or "very informed" about different ways people and communities may be exposed to PFAS.

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8. FUTURE HEALTH IMPACT AND EXPOSURE RESEARCH PRIORITIES

Respondents were asked to indicate which research areas regarding PFAS and human health they thought were most important. These questions were designed to inform the Panel's deliberations regarding the recommended setting of future research priorities.

8.1. Importance of conducting further research on long-term health effects of PFAS exposure

Question: How important is it that the Australian Government undertakes more research to understand the long-term health impacts of exposure to PFAS? 1 (not important at all) to 5 (extremely important)

428 respondents chose to answer this question

Respondents were asked to use a sliding scale from one to five to indicate how important they thought it was that the Australian Government undertakes further research into the long-term health impacts of exposure to PFAS.

The average of these responses was 4.88, and the median was five.

An average close to five and a median of five indicates that respondents thought that more research into the long-term health impacts of exposure to PFAS was very important to respondents.

8.2. Prioritising human health research areas

Question: Do you have a preference for research on preventing further PFAS contamination, or for methods to monitor and treat already exposed communities?

433 respondents chose to answer this question.

Respondents were asked whether they had a preference for research on preventing further PFAS contamination, or monitoring and treating existing PFAS contamination.

Ninety-one percent of respondents reported that both of these research areas were important to them.

Eight percent of respondents that answered this question thought that monitoring and treating existing PFAS contamination should be given priority. One percent of respondents who answered this question thought that research into preventing further PFAS contamination was more important, and one percent of respondents who answered this question said neither was important to them.

Question: What areas of human health research do you think should be prioritised?

Respondents were asked to rank the listed research priorities in order of research topics they thought should be prioritised.

The potential areas of human health research that respondents could select from were:

- the potential health effects on workers exposed to high levels of PFAS at work (occupational exposure);

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- the potential health effects on communities that have experienced high exposure to PFAS due to contamination;
- the potential health effects of PFAS exposure on vulnerable populations such as pregnant women, babies, young children and the elderly;
- the best methods to minimise exposure to PFAS in individuals and communities; and
- the potential health effects on communities that have experienced lower background exposure to PFAS chemicals.

Figure 6 displays the weighted average of these answers. A higher weighted average indicates that respondents thought that research area was more important to them relative to the other areas.

Figure 7 compares the different weighting between respondents who reported that they were occupationally exposed to PFAS, versus those who reported that they lived, or have lived, in an are being investigated for PFAS contamination.

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Figure 6: What areas of human health research do you think should be prioritised?

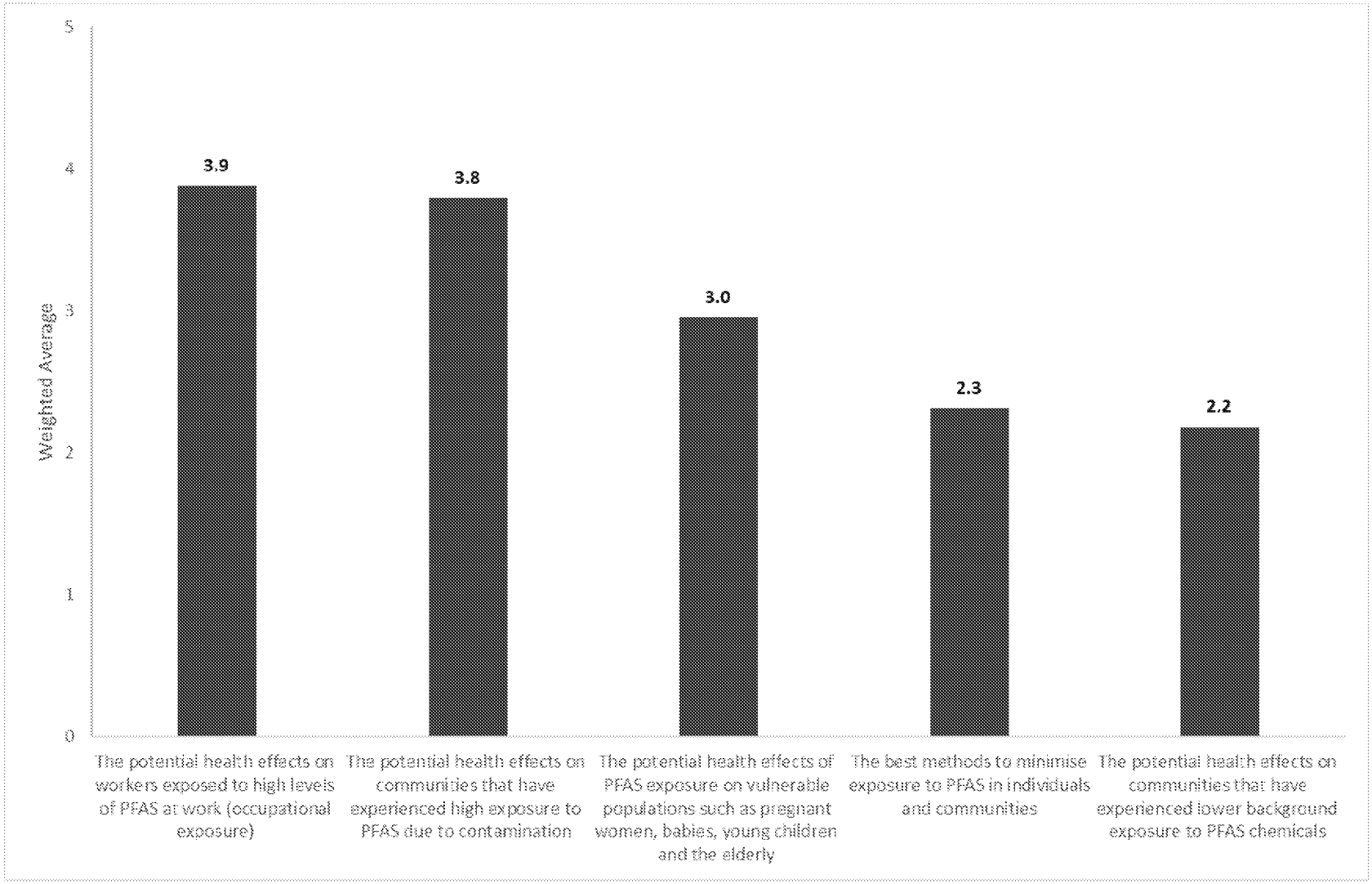

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Figure 7: Research priorities areas occupationally exposed respondents vs respondents who lived, or previously lived in an area being investigated for PFAS contamination wanted prioritised

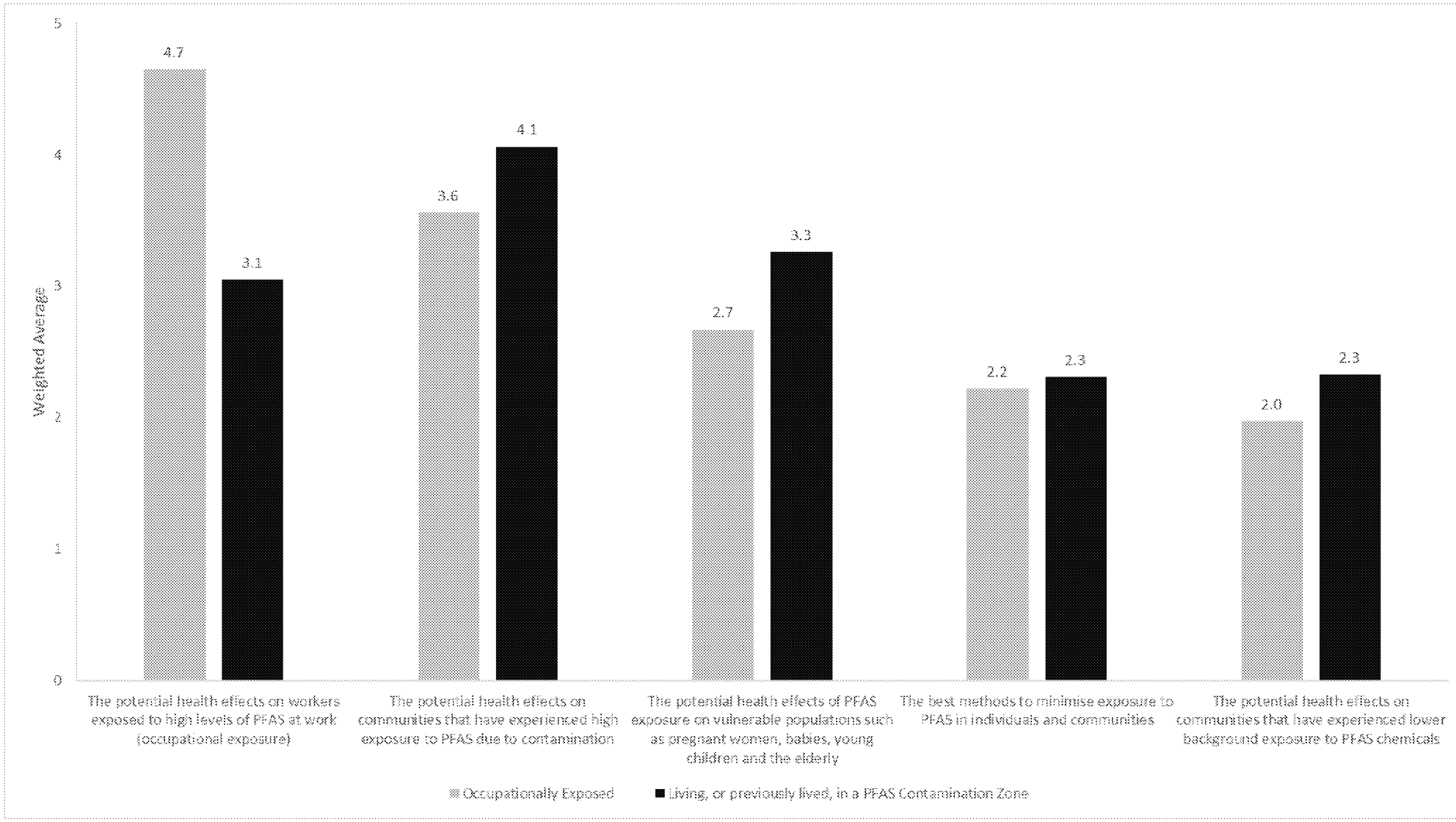

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Figure 7 indicates that the occupationally exposed respondents sub-group prioritised further research into occupational exposure relative to other research. The sub-group of respondents that reported living in an investigation area prioritised further research on potential health impacts on communities that have experienced high exposure to PFAS due to contamination.

8.3. Other areas of human health research respondents want prioritised

Question: Is there an area of human health research not listed here that you would like to see prioritised for further research?

109 respondents answered this question specifically, and 147 respondents commented on research priorities including during their comments made in the general comments section at the end of the consultation document where there was a free text box for general comments (section 9 in this document provides a summary of these responses). This question was a free-text response question to allow respondents to write about areas that had not been specified in the previous question that asked them to rank research priorities.

Responses under this question provided on behalf of organisations have also been included and do not deviate significantly from those provided by individuals.

Blood testing for those affected by PFAS contamination

Thirty-one respondents reported that they wanted more widely available blood testing for those affected by PFAS contamination. Of these 31 respondents, eight respondents specifically wanted testing for firefighters who had been exposed to PFAS containing foam. Some respondents explained why they wanted regular blood testing, for example one respondent who reported being occupationally exposed to PFAS noted:

> *"I want regular testing for persons past and present who were exposed to PFAS and PFOA due to their occupation. We were exposed to these chemicals without knowing the damage they can possibly have. At the very least we can monitor our own levels and this can be documented for future research."*

In describing why blood testing services should be offered more widely, respondents mainly reported two reasons:

- six respondents wanted to check their own levels for health reasons to understand their own exposure history; and
- seven respondents thought that it should be used to inform research or epidemiological studies.

One of the respondents who reported currently living in an area being investigated for PFAS contamination and wanted extended blood testing services noted that:

> *"More regular repeated blood tests would prove that we are not still being contaminated while they sort all the rest of this debacle and give us great peace of mind."*

One organisation that represents firefighters recommended extending blood testing to "every Federal, State and Territory career firefighter, including appropriate support and analysis at no cost.

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Further research on the health effects on firefighters of occupational exposure to PFAS

Thirty-one respondents requested research specifically on firefighters who have been exposed to PFAS containing foam and how this may affect their health. One of these respondents who reported that he was occupationally exposed to PFAS noted that:

> *"The aviation rescue firefighters are the canaries in the coal mine on this issue"*

Two respondents discussed the fact that firefighters often have "*combined multiplier effect of occupational exposure*", and they are exposed to PFAS at the fire pits which were filled with aviation fuels and AFFF that was accidentally ingested, and they also handled PFAS containing chemicals in raw and undiluted forms.

One organisation that represents firefighters requested research on the *"best practice methods for measurement of exposure levels of firefighters to historical PFAS."*

Specific research on cancer

Nineteen respondents wanted research specific health conditions that they thought may be linked to PFAS exposure. Fourteen of these respondents requested that research be undertaken into whether PFAS exposure caused cancer. Other health conditions that people wanted further research on included: effects on the human immune system, links to kidney problems, and heart problems or other conditions that may be linked to PFAS.

Long term health effects of PFAS exposure

Seventeen respondents who commented on research priorities requested long term monitoring, or research into the long-term effects of PFAS exposure on health. One respondent who reported concern for PFAS exposure but did not live in an investigation area, noted that this should include intergenerational effects:

> *"We include in this concern for intergenerational equity - how will today's exposure impact on descendants of those exposed and future environments?"*

Another respondent who reported having previously lived in an investigation area commented on the need for this research to include those who have moved out of areas currently being investigated for PFAS contamination:

> *"[What about] people who have moved out of the area but lived there for a substantial time i.e. 5+ years? Or does the effects happen in less years?"*

One organisation that represents firefighters requested further research on:

> *"the evidence and understanding of potential human health effects from prolonged exposure to PFAS but also on potential methods of reducing PFAS levels."*

Health effects of PFAS exposure on vulnerable populations

Fifteen respondents who commented on research priorities requested studies on health effects on vulnerable populations including children (9 respondents), babies (5 respondents), people with autoimmune deficiencies or existing health issues (2 respondents) veterans (1 respondent), and the elderly (1 respondent).

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Reducing levels of PFAS in blood

Twelve respondents requested studies on how to reduce blood levels of PFAS.

Other research priorities

Respondents provided a range of other topics for research that they though should be prioritised including:

- research into the mental health effects from PFAS exposure (8 respondents);
- effects on indigenous health (2 respondents).

Ten respondents also noted that it was difficult to prioritise research areas, as research into the health effects for different exposed communities (for example, vulnerable populations and occupationally exposed workers) are all equally important.

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9. OTHER COMMENTS

Question: Do you have any other comments or views on potential health impacts associated with PFAS exposure or priority areas for further research that you would like the Panel to consider?

Respondents were given the opportunity to provide other comments on potential health impacts associated with PFAS exposure or priority areas for further research. These comments have been included for analysis in the sections they are relevant to. A range of other comments were made by respondents under this question that are outside of the scope of work for the Panel, which is to advise the Australian Government on the potential health impacts associated with PFAS exposure and identify priority areas for further research. However, these have been summarised below.

Comments on communication by the Australian government

Forty-eight respondents commented on a perceived failure of communication regarding PFAS contamination by the Australian Government. This includes the following perceptions from respondents:

- that the Government is deliberately ignoring the concerns of those affected by PFAS contamination;
- that the Government perceives PFAS contamination as a low priority; and
- that the Government is slow in responding to PFAS contamination.

Comments regarding a need for more financial support for affected communities

Twenty-five respondents commented that there was a need for more financial assistance for communities affected by PFAS contamination. Examples of the kind of assistance requested by respondents includes:

- annual health checks;
- compensation for lost property value or other financial compensation; and
- mental health support.

Need for clarity regarding potential health effects of PFAS and exposure guidance values

Eighteen respondents commented on the need for more clarity regarding guidance on the potential health effects of PFAS exposure. One respondent who reported being occupationally exposed to PFAS noted:

> *"There has been a lot of testing and no one has told us anything other than it's very contaminated. I want to know if it is going to effect my health."*

Twelve respondents commented on the need for clarity regarding safe exposure levels for PFAS.

One organisation requested further research on the need for established maximum thresholds for food and water in Australia.

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10. KEY THEMES

The public consultation process has delivered a clear statement of concern that respondents feel that PFAS exposure has already affected their health, and that it may affect their health into the future. Respondents reported that future research into the human health effects of PFAS exposure is extremely important to them.

Some of the key findings have been included below.

Exposure pathways

- Overall, respondents indicated that past exposure to PFAS, occupational exposure to PFAS, and skin contact with PFAS were the most concerning exposure pathways to them.
- When considering the views of those respondents who were occupationally exposed to PFAS, for example, from working or training as a firefighter and being regularly exposed to PFAS containing foam, these respondents ranked past exposure to PFAS, occupational exposure to PFAS and skin contact with PFAS containing products as of the most concern to them.
- When considering the views of those respondents who reported that they lived, or previously lived, in an area under investigation for PFAS contamination, these respondents ranked drinking water, contaminated soil and homegrown produce as the exposure pathways of the most concern to them.

Concerns about potential health impacts of PFAS exposure

- Over two thirds of respondents were "concerned" or "very concerned" about the following impacts of PFAS on their health:
  - that their future health, or their family's future health might be impacted by PFAS exposure;
  - that their health, or their family's health may have already been impacted by PFAS exposure;
  - avoiding exposure to PFAS;
  - that their health, or their family's health, was being indirectly affected by PFAS exposure, for example, by causing stress and anxiety.
- When given the opportunity to identify which potential health impacts of PFAS exposure concerned them most, over 55 percent (189 of the 339 respondents that responded to the question) noted that they were concerned about a link between PFAS exposure and cancer(s).

Information and understanding

- Responses under information and understanding were more mixed. Over half of respondents felt "not at all informed" or "not informed" about the Government's response to addressing health concerns of communities exposed to PFAS. Twenty-one percent of respondents reported feeling "informed" or "very informed" about the Government's response.

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- Approximately half of respondents felt "not at all informed" or "not informed" about research on the effects of PFAS exposure and levels of exposure to PFAS in specific communities. Thirty-two percent and 37 percent reported feeling "informed" or "very informed" about research on PFAS, and levels of exposure to PFAS in specific communities respectively.
- Forty-five percent of respondents reported feeling "not at all informed" or "not informed" about different ways people and communities may be exposed to PFAS. Forty percent of respondents reported feeling "informed" or "very informed" about PFAS exposure pathways.

Future health impact and exposure research priorities

- When asked about their views on what research on PFAS exposure should be prioritised, respondents reported that research on the health effects of occupational exposure to PFAS should be prioritised, along with further research into potential health impacts on communities that have experienced high exposure to PFAS due to contamination.
- Respondents who identified as occupationally exposed to PFAS prioritised future research on the health effects of occupational exposure to PFAS, and research on potential health effects on communities that have experienced high exposure to PFAS due to contamination.
- Respondents who reported that they lived, or have lived, in an area currently being investigated for PFAS contamination prioritised research on the potential health effects on communities that have experienced high exposure to PFAS, and research into the potential health effects of PFAS exposure on vulnerable populations such as pregnant women, babies, young children and the elderly.
- Thirty-one of the 109 respondents who commented on other areas of human health research they want prioritised, commented on a need for blood testing for those affected by PFAS contamination.

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APPENDIX ONE – COPY OF PUBLIC CONSULTATION DOCUMENT

EXPERT HEALTH PANEL FOR PFAS – PUBLIC CONSULTATION PROCESS

PFAS chemicals

Per- and poly-fluoroalkyl substances (PFAS) used in fire-fighting foams on Defence bases, civilian airports and firefighting training grounds have migrated through the groundwater into adjoining areas.

These chemicals can persist in humans, animals and the environment. In particular they appear to accumulate in humans and are then very slowly eliminated from the body. The Environmental Health Standing Committee (enHealth) currently advises that 'there is currently no consistent evidence that exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes adverse human health effects' (enHealth, 2016); and recent reviews conducted by regulatory bodies have concluded that there is no compelling evidence that PFAS at the concentrations found in these areas are harmful to health. However, as a precaution it is generally recommended that exposure be minimised wherever possible.

Investigation of health effects and research priorities

To further investigate the potential health effects of PFAS, the Australian Government has set aside funds to do further research into the potential health effects of PFAS exposure.

The Expert Health Panel for PFAS (the Panel) has been established to provide updated and independent advice to the Australian Government on the potential health impacts associated with PFAS exposure and identify priority areas for further research. Allen + Clarke is an independent organisation that is assisting the Panel with this work.

To provide its advice to Government, the Panel is reviewing the current health and other scientific evidence available in addition to seeking the views of the public who are concerned by PFAS contamination. To do this, the Panel, assisted by Allen + Clarke, is currently undertaking a public consultation process.

This document

This document poses questions to the public to help the Panel to better understand the range of public views on the potential health impacts of PFAS contamination and what the focus of future research should be. It poses the same questions as the online survey that can be found at www.allenandclarke.com/PFAS.

You don't have to answer all of the questions if you don't want to. The questionnaire should take you about 20 minutes and there is an opportunity to provide extra comments at the end. It may take longer than this depending on the length of written responses to questions.

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HOW TO COMPLETE THE CONSULTATION QUESTIONS

Web

Complete the survey online at **www.allenandclarke.com/PFAS**

Email

Email your completed responses to **PFAS@allenandclarke.com**

Post

Send your completed survey to:

Expert Health Panel for PFAS
MDP 5
Department of Health
GPO Box 9848
Canberra ACT 2601

Please ensure your responses are returned by 11:59pm on **19 November 2017.**

HOW WILL YOUR RESPONSES BE MANAGED?

Your responses will be used to collate a report that analyses the submissions to identify the key themes, areas of concern and areas of focus for future research. *Allen + Clarke* is collecting the consultation information into a report, which will be provided to the Panel to help inform their advice to the Australian Government. Once completed, a short summary report explaining the findings will be made available at the Department of Health website.

Your response is voluntary. Information you provide will be held by Allen + Clarke but anonymised individual responses may be made available to the Panel. Personal information will be handled in accordance with applicable privacy and information law

There will be an opportunity for you to provide additional comments or concerns that you would like the Panel to consider at the end of this questionnaire.

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GENERAL

Are you making a submission as:

- ☐ An individual
- ☐ On behalf of a group/organisation
- ☐ Other (please specify): ……..

If you are making a submission on behalf of a group/organisation, please provide the name of the group/organisation and your position in that group or organisation:

Please indicate which sector best represents you:

- ☐ Individual/family
- ☐ Academic/research
- ☐ Non-government association
- ☐ Medical/public health professional
- ☐ Local/State government
- ☐ National government
- ☐ Commercial fishery/farmer
- ☐ Other (please specify): ……..

Sex:

- ☐ Female
- ☐ Male
- ☐ X (Indeterminate/Intersex/Unspecified)
- ☐ Prefer not to say

Age:

- ☐ Under 25
- ☐ 25 - 44
- ☐ 45 - 64
- ☐ 65 - 84
- ☐ 85+
- ☐ Prefer not to say

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EXPOSURE

1. **Why is PFAS exposure of interest to you? (Check as many that apply)**

- ☐ Currently living in an area being investigated for PFAS contamination in Australia
- ☐ Previously lived in an area being investigated for PFAS contamination in Australia
- ☐ Frequently visiting an area being investigated for PFAS contamination in Australia (e.g. for work or family)
- ☐ Consumption of food/water originating from an area being investigated for PFAS contamination in Australia
- ☐ Concerned about PFAS but not living in an area being investigated for PFAS contamination in Australia
- ☐ Other: please specify……..

2. **What sources of potential exposure to PFAS concern you the most? Please rank in order of what concerns you most: from 1 (most concern) to 10 (least concern).**

Exposure	Ranking (1 – 10)
Drinking water	
Shower/bathing water	
Swimming pools or while swimming in rivers and watering holes	
Commercially purchased produce (fruit, vegetables, meat, eggs, dairy products or seafood)	
Home grown produce (fruit, vegetables, meat, eggs, dairy products or seafood)	
Contaminated air	
Contaminated soil	
Working in an industry using PFAS chemicals	
Skin contact with PFAS containing products	
Past exposure to PFAS	

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Is there a potential source of exposure to PFAS not listed in the table that you are more concerned about?

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CONCERNS ABOUT HEALTH IMPACTS

3. **How concerned are you that you about the following? Please use the scale below ranging from 1 (not at all concerned) to 5 (very concerned).**

	1 Not at all concerned	2 Not concerned	3 Neutral	4 Concerned	5 Very concerned	N/A
That you or your family's health has already been affected by PFAS?	○	○	○	○	○	○
That you or your family's future health might be affected by PFAS?	○	○	○	○	○	○
About avoiding exposure to PFAS?	○	○	○	○	○	○
That you or your family's health is being indirectly affected by living in a PFAS Investigation area (e.g. stress and anxiety due to financial impacts, publicity or media attention?)	○	○	○	○	○	○

4. **If you are concerned about exposure to PFAS, what potential impacts on human health from PFAS exposure are you concerned about?**

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INFORMATION AND UNDERSTANDING

5. **How informed do you feel on the following topics? Please use the scale below ranging from 1 (not at all informed) to 5 (very informed).**

	1 Not at all informed	2 Not informed	3 Neutral	4 Informed	5 Very informed	N/A
Levels of exposure to PFAS in specific communities	○	○	○	○	○	○
Research on the effects of PFAS exposure	○	○	○	○	○	○
Different ways people and communities may be exposed to PFAS	○	○	○	○	○	○
The Government's response to address the health concerns of communities exposed to PFAS	○	○	○	○	○	○

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FUTURE HEALTH IMPACT AND EXPOSURE RESEARCH PRIORITIES

6. **How important is it that the Australian Government undertakes more research to understand the long-term health impacts of exposure to PFAS?**

Not important at all 1 2 3 4 5 Extremely important

7. **Do you have a preference for research on preventing further PFAS contamination, or for methods to monitor and treat already exposed communities?**

- ☐ Preventing more PFAS contamination
- ☐ Monitoring and treating existing PFAS contamination
- ☐ Both are important to me
- ☐ Neither are important to me

8. **What areas of human health research do you think should be prioritised?**

Research area	Ranking (1 – 5)
The potential health effects on workers exposed to high levels of PFAS at work (occupational exposure)	
The potential health effects on communities that have experienced high exposure to PFAS due to contamination	
The potential health effects on communities that have experienced lower background exposure to PFAS chemicals	
The potential health effects of PFAS exposure on vulnerable populations such as pregnant women, babies, young children and the elderly	
The best methods to minimise exposure to PFAS in individuals and communities	

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Is there an area of human health research not listed here that you would like to see prioritised for further research?

OTHER COMMENTS

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9. **Do you have any other comments or views on potential health impacts associated with PFAS exposure or priority areas for further research that you would like the Panel to consider?**

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