Addendum to PFAS Evidence Evaluation for Australian Drinking Water Guidelines Chemical Fact Sheets

Addendum / Work Expansion for 2024 NHMRC PFAS Review of Australian Health-based Guideline Values

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Basis of Report

This report has been prepared by SLR Consulting Australia (SLR) with all reasonable skill, care and diligence, and taking account of the timescale and resources allocated to it by agreement with the National Health and Medical Research Council (the Client). Information reported herein is based on the interpretation of data collected, which has been accepted in good faith as being accurate and valid.

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Executive Summary

An Australian drinking water guideline (DWG) and existing Fact Sheet are available for three per- and polyfluoroalkyl substances (PFAS): for perfluorooctane sulfonic acid + perfluorohexane sulfonic acid (PFOS+PFHxS) and for perfluorooctanoic acid (PFOA). The DWGs are based on guidance values derived in a comprehensive review by Food Standards Australia New Zealand (FSANZ) in 2017.

In response to new advisories as well as overseas and growing community concerns, the National Health and Medical Research Council (NHMRC) prioritised the review of existing health-based guideline values for PFAS in drinking water to determine whether they are suitable to adopt or adapt for the Australian context. For the review, SLR Consulting Australia Pty Ltd (SLR) was contracted by NHMRC in April 2023 to undertake an evidence review of selected PFAS in drinking water (including PFOS, PFHxS, PFOA, perfluorobutane sulfonic acid and its potassium salt (PFBS) and hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt ("GenX chemicals")) from recent guidance and reviews from national / international jurisdictions. Selected underpinning studies of available guideline values were assessed for their suitability to be adopted / adapted in the Australian context. The final version of the evidence review, termed the '2024 PFAS Review', from SLR was completed in February 2024, and used to inform a draft update to the PFAS fact sheet within the Guidelines.

Since the finalisation of the 2024 PFAS Review, the United States Environmental Protection Agency (US EPA), in April 2024, published final health effects documentation for a number of PFAS, including PFOS and PFOA. These reports included several key and candidate studies for PFOS and PFOA that had not previously been evaluated in the SLR 2024 PFAS Review nor by FSANZ (2017).

NHMRC commissioned SLR to undertake an updated evidence evaluation and prepare this Addendum Report to the 2024 PFAS Review, which considers the April 2024 health effects documentation for PFOS and PFOA (US EPA 2024a, b), as well as a recently published peer-reviewed scientific paper by an international collaboration of scientists deriving guidance values for PFOA (Burgoon et al. 2023). SLR was also requested by NHMRC to undertake an assessment of methods / rationale / guidance used to derive a total / sum of PFAS guideline value from key international jurisdictions that currently have a total / sum of PFAS guideline value as identified in the 2024 PFAS Review (i.e. a review of approaches for PFAS mixtures assessment in drinking water).

The updated evidence evaluation has been undertaken in line with the same methodological framework as used in the 2024 PFAS Review which is intended to implement best practice methods for evidence evaluations as per the NHMRC Standards for Guidelines. Critical evaluation of 19 additional studies in scope of this expanded review was also undertaken using the same approach as in the 2024 PFAS Review.

This Addendum Report summarises the updated evaluation undertaken for PFOS and PFOA and concludes by identifying potential drinking water guideline values for adoption/adaption in the Australian context, as well as the results of the review of mixtures assessment approaches.

The candidate DWGs for potential adoption/adaption of suitable information for PFOS and PFOA are provided in **Section 5.0** and **6.0** of this report, with the conclusions presented in **Section 7.0**. As relevant identified guidance values have utilised different critical studies, critical effects and points of departure along with different uncertainty factors for guidance value determination, this has resulted in ranges being provided. In summary, the following options for guideline values were proposed.

- PFOS – guideline values of 3.4 or 77 ng/L (based on a high confidence toxicology study in rats), or guideline values of 27 or 95 ng/L (based on a medium confidence developmental toxicology study in mice) were considered to be potentially suitable, as is the current Australian guideline value of 70 ng/L. The candidate guideline values of 3.4 ng/L (from a high confidence study) and 27 ng/L (from a medium confidence study) are based on the same critical endpoints as the candidate guideline values of 77 ng/L and 95 ng/L, respectively, but the former were derived using serum points of departure modelled by the US EPA whereas the latter using serum points of departure measured in the experimental studies. The difference between modelled and measured values could not be readily reconciled, therefore the use of the measured values from the studies is associated with less uncertainty.
- PFOA – guideline values ranging from 63 to 554 ng/L were considered to be potentially suitable, as is the current Australian guideline value of 560 ng/L. The values of 227 ng/L and 402 ng/L were derived from a study with high confidence, whereas other values were derived from studies of medium or low confidence. Nevertheless, it is recognised that the candidate guideline value of 227 ng/L is based on the development of acinar pancreatic neoplastic lesions in rats, which are unlikely to be relevant to humans based on currently available information. The value of 402 ng/L is based on non-neoplastic hepatic necrosis in rats. Although there is also uncertainty with respect to the dose at which non-neoplastic hepatic necrosis may occur in humans and it is recognised by SLR that rats are likely more sensitive to this effect than humans, SLR considers there is insufficient information to rule out human relevancy of this effect based on currently available information.

Based on concentrations identified in existing water quality data in the Australian context as part of the 2024 PFAS Review, it is unlikely that PFOS and PFOA will present a human health risk from drinking water in uncontaminated regions of Australia.

A review of different approaches used currently or in the past by international jurisdictions to evaluate / assess PFAS mixtures in drinking water revealed the approaches can be grouped into the following five categories; i) hazard index (HI) approaches, ii) Relative Potency Factor (RPF) approaches, iii) Mixtures-Benchmark Dose (M-BMD) approaches, iv) practical (non-health) based approaches, and v) surrogate approaches. Each has its own pros and cons, and some are more data-intensive than others. Based on the review of these approaches, a PFAS mixture options assessment was presented which outlines four possible options for developing a PFAS mixture DWG in Australia, noting the options provided are not necessarily exhaustive.

As a potential way forward, the HI approach is most amenable for use in Australia. However, to establish an approach that is applicable to more than just a select number of PFAS for which there are DWGs, the HI approach is suggested to be combined with the surrogate approach as it is still health-based, does not require marked amounts of data, and can be readily explained and applied. A technical document would be required to derive the additional DWG and justify the approach (including explaining how it should be used). Alternatively, a simple surrogate approach could be easily applied to a large number of measurable PFAS, noting this approach is not data-driven and would be highly conservative.

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Acronyms and Abbreviations

Acronym	Definition
∑	Sum
6:2 FTS	6:2 Fluorotelomer Sulfonic Acid (CAS No. 27619-97-2)
AIC	Akaike's Information Criterion
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AON	Adverse Outcomes Network
AOP	Adverse Outcomes Pathway
AOR	Adjusted Odds Ratio
APFO	Ammonium Perfluorooctanoate
AST	Aspartate Aminotransferase
ATSDR	US Agency for Toxic Substances and Disease Registry
BMC	Benchmark Concentration
BMDL	Lower Benchmark Dose
$BMDL_{0.5SD}$	Lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in response equal to 0.5 SD from the control mean
$BMDL_{1SD}$	Lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in response equal to 1 SD from the control mean
$BMDL_{5RD}$	Lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in response
BMR	Benchmark Response
BW	Body Weight
CAR	Constitutive Androgen Receptor
CD-4	Cluster of differentiation 4
CD-8	Cluster of differentiation 8
CDC	US Centre for Disease Control
ChE	Cholinesterase
CI	Confidence Interval
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
CSF	Cancer Slope Factor
DNA	Deoxyribonucleic Acid
DWG	Drinking Water Guideline
EFSA	European Food Safety Authority
EC	European Committee
EU	European Union
F1	First Filial Generation

Acronym	Definition
FSANZ	Food Standards Australia New Zealand
FGR	Foetal Growth Restriction
FTS	Fluorotelomer Sulphonate
GAC	Granular Activated Carbon
GD	Gestation Day
GenX	Hexafluoropropylene Oxide Ammonium Salt (CAS No 62037-80-3)
GenX chemicals	Hexafluoropropylene Oxide Ammonium Salt (CAS No 62037-80-3) and Hexafluoropropylene Oxide Dimer Acid (CAS No 13252-13-6)
GGT	γ-Glutamyltransferase
GLP	Good Laboratory Practice
HC	Health Canada
HED	Human Equivalent Dose
HDLC	High Density Lipoprotein Cholesterol
HET	Heterozygous
HFPO	Hexafluoropropylene oxide
HI	Hazard Index
HQ	Hazard Quotient
IC	Index Chemical
IgM	Immunoglobulin M
IL-1	Interleukin 1
IL-4	Interleukin 4
IU/L	International Units per Litre
KO	Knockout
LD	Lactation Day
LBW	Low Birth Weight
LDLC	Low-density Lipoprotein Cholesterol
LH	Luteinising Hormone
LOAEL	Lowest Observed Adverse Effect Level (i.e. the lowest dose tested at which an adverse effect was observed).
LOD	Limit of Detection
LOEL	Lowest Observed Effect Level (i.e. the lowest dose tested at which an effect was observed, the degree of which is not necessarily adverse).
Maine DHHS	Maine Department of Human Health Services
Mass DEP	Massachusetts Department of Environmental Protection
M-BMD	Mixture Benchmark Dose
MCL	Maximum Contaminant Level
MMR	Measles-mumps-rubella

Acronym	Definition
MIE	Molecular Initiating Event
NAMs	New Approach Methodologies
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council
NRMMC	Natural Resource Management Ministerial Council
NTP	National Toxicology Program
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
OEHHA	Californian Office of Environmental Health and Hazard Assessment
OR	Odds Ratio
PA	Prealbumin
PBPK	Physiologically Based Pharmacokinetic
PCB	Polychlorinated Biphenyl
PFAS	Per- and Poly-fluoroalkylated Substances
PFBA	Perflorobutanoic Acid (CAS No. 375-22-4)
PFBS	Perfluorobutane Sulfonic Acid (CAS No. 375-73-5).
PFCA	Perfluoro Carboxylic Acids
PFDA	Perfluorodecanoic Acid (CAS No. 335-76-2)
PFDoDA	Perfluorododecanoic Acid (CAS No. 307-55-1)
PFPeA	Perfluoropentanoic Acid (CAS No. 2706-90-3)
PFSA	Perfluoro Sulfonic Acids
PFHpA	Perfluoroheptanoic Acid (CAS No. 375-85-9)
PFHxS	Perfluorohexane Sulfonic Acid (CAS No. 355-46-4)
PFOA	Perfluorooctanoic Acid (CAS No. 335-67-1)
PFOS	Perfluorooctane Sulfonic Acid (CAS No. 1763-23-1)
PFOSA	Perfluorooctanesulfonamide
PFNA	Perfluorononanoic Acid (CAS No. 375-95-1)
PFUnDA	Pefluoroundecanoic Acid (CAS No. 2058-94-8)
PND	Postnatal Day
POD	Point of Departure
PPARα	Peroxisome Proliferator-Activated Receptor Alpha
PPARγ	Peroxisome Proliferator-Activated Receptor Gamma
PQL	Practical Quantitation Limit
PXR	Pregnane-X Receptor
QGC	Quantile G-computation
RBC	Red Blood Cell

Acronym	Definition
RCC	Renal Cell Carcinoma
RfD	Reference Dose
RPF	Relative Potency Factor
RSC	Relative Source Contribution
SD	Standard Deviation
SDH	Sorbitol Dehydrogenase
SGA	Small for Gestational Age
SLR	SLR Consulting Australia Pty Ltd
SRBC	Sheep Red Blood Cell
T3	Triiodothyronine
T4	Thyroxine
TB	Total Bilirubin
TC	Total Cholesterol
TOSHI	Target Organ Specific Hazard Index
TP	Total Protein
TRV	Toxicological Reference Value
TSH	Thyroid Stimulating Hormone
The Committee	NHMRC Water Quality Advisory Committee
The Guidelines	NHMRC and NRMMC (2011). Australian Drinking Water Guidelines 6 2011; Version 3.8 updated September 2022, National Health and Medical Research Council and Natural Resource Management Ministerial Council, Commonwealth of Australia, Canberra.
TRV	Toxicity Reference Value
TSH	Thyroid Stimulating Hormone
UF	Uncertainty Factor
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
WT	Wild Type

1.0 Introduction and Background

The National Health and Medical Research Council (NHMRC) undertakes a rolling review of the *Australian Drinking Water Guidelines* (2011) (the Guidelines) to ensure they reflect the best available evidence and are current and relevant to the Australian context.

Per- and polyfluoroalkyl substances (PFAS) are a group of over four thousand manufactured chemicals that do not occur naturally in the environment. Some PFAS are very effective at resisting heat, stains, grease and water, making them useful chemicals for a range of applications. Unfortunately, these properties can also make them problematic in the environment. Most people are likely to have had some exposure to PFAS. As these chemicals persist in humans and the environment, it is recommended that human exposure is minimised as a precaution.

In June 2017, the Department of Health commissioned NHMRC to develop health-based guideline values for perfluorooctane sulfonate (PFOS) + perfluorohexane sulfonate (PFHxS) and perfluorooctanoic acid (PFOA) in drinking water and recreational water. NHMRC developed advice in consultation with the Environmental Health Standing Committee (enHealth). In August 2018, a PFAS Chemical Fact Sheet was published in the Guidelines outlining derivation of the guideline values. The health-based guideline values are based on the tolerable daily intakes calculated in the Food Standards Australia New Zealand (FSANZ) Perfluorinated Chemicals in Food report (FSANZ 2017). In 2020, NHMRC updated its guideline values for recreational water.

In June 2020, the European Food Safety Authority (EFSA) updated its Tolerable Weekly Intake to include a new safety threshold for PFAS that is lower than the Australian values. In June 2022, the United States Environmental Protection Agency (US EPA) issued revised interim drinking water health advisories for two types of PFAS; PFOS and PFOA, that were lower than the Australian health-based guideline values for drinking water. Two new PFAS drinking water health advisories were also issued for perfluorobutane sulfonic acid and its potassium salt (PFBS) and for hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt (“GenX chemicals"). There is currently no advice for PFBS or GenX chemicals in the Guidelines.

In response to these new advisories as well as overseas and growing community concerns, NHMRC prioritised the review of existing health-based guideline values for PFAS in drinking water to determine whether they are suitable to adopt or adapt for the Australian context.

For the review, SLR Consulting Australia Pty Ltd (SLR) was contracted by NHMRC in April 2023 to undertake an evidence review of PFAS in drinking water from recent guidance and reviews from national/international jurisdictions. Selected underpinning studies of available guideline values were assessed for their suitability to be adopted/adapted in the Australian context. The final version of the evidence review, Review of Australian health-based Guideline Values for Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water (termed the 2024 PFAS Review)[1], from SLR was completed in February 2024.

[1] The results of the previous evaluation (termed the 2024 PFAS Review) were provided in the form of the following Technical Report and an Evaluation Report:

SLR (2024a). Evidence Evaluations for Australian Drinking Water Guidelines Chemical Fact Sheets – PFOS, PFHxS, PFOA, PFBS, and GenX Chemicals. PFOS, PFHxS, PFOA, PFBS, and GenX Chemicals Technical Report. Prepared for the National Health and Medical Research Council by SLR Consulting Australia Pty Ltd. SLR project No: 640.V30693.20000, dated 1 February 2024. Revision 4.0.

SLR (2024b). Evidence Evaluations for Australian Drinking Water Guidelines Chemical Fact Sheets – PFOS, PFHxS, PFOA, PFBS, and GenX Chemicals. PFOS, PFHxS, PFOA, PFBS, and GenX Chemicals Evaluation

In April 2024, the US EPA released the final PFAS National Drinking Water Standard, which superseded its previous interim drinking water health advisories for PFAS. As the timing of this release was after the 2024 PFAS Review by SLR was completed, the 2024 PFAS Review did not consider the US EPA final PFAS National Drinking Water Standard. As a result, NHMRC's Water Quality Advisory Committee (the Committee) advised that the US EPA final PFAS National Drinking Water Standard, including selected studies that had not been previously reviewed by either Food Standards Australia New Zealand (FSANZ) or SLR, should be evaluated in a consistent manner, as an expansion of work as part of the 2024 PFAS Review.

The Committee also discussed the concept of providing a total/sum of PFAS guideline value in the updated PFAS fact sheet. Other international jurisdictions, such as the European Union and the World Health Organization, have included a total/sum of PFAS guideline value in their drinking water health advisories. The Committee suggested that the rationale from key international jurisdictions to derive a total/ sum of PFAS guideline value should be evaluated for consideration to adopt/adapt in the updated PFAS fact sheet.

NHMRC have now contracted SLR to conduct an additional evidence evaluation for an expansion to the 2024 PFAS Review. The expansion of work is delivered in the Addendum herein and is intended to assist NHMRC and the Committee to determine whether further updates are required to the PFAS fact sheet within the Guidelines.

The scope of the expanded review herein is as follows.

Part 1

a) Consider the April 2024 US EPA *National Drinking Water Standard* for PFOA and PFOS, by reviewing the Final Human Health Toxicity Assessments for PFOA and PFOS (US EPA 2024a, b) and creating summary tables and information similar to those supplied for other international agency reviews in the Technical Report of the 2024 PFAS Review; only research questions relating to advice in the fact sheet on health-based guideline values[2] need to be answered.
b) Using the same methodological approach used for appraising studies in the 2024 PFAS Review, critically review selected studies that have not been previously assessed by either FSANZ (2017) or SLR (in the 2024 PFAS Review) that underpin the April 2024 US EPA final drinking water standards for PFOS and PFOA (US EPA 2024a, b) (three studies)[3] or the range of PFOA safe doses for human health as per Burgoon et al. (2023) (two studies)[4]. The studies are to be assessed for their suitability to be adopted/adapted for the Australian context.

Part 2

a) Undertake an assessment of methods / rationale / guidance used to derive a total / sum of PFAS guideline value from key international jurisdictions that currently have a total / sum of PFAS guideline value as identified in the 2024 PFAS Review (i.e. World

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[2] A guidance value is the same as a Toxicity Reference Value (TRV) and refers to a health-based intake of a chemical which can be ingested daily over a lifetime without adverse health effects. A guideline value for various environmental media (including drinking water) uses the health-based guidance value in its derivation but may only apportion a certain percentage of the guidance value to the intake from that particular medium.

[3] The three studies from the April 2024 US EPA (2024a, b) guidance values requiring evaluation are Wikström et al. (2020) (**PFOA and PFOS**), Dong et al. (2019) (**PFOA and PFOS**) and Shearer et al. (2021) (**PFOA only**).

[4] The two studies from the Burgoon et al. (2023) range of safe PFOA doses requiring evaluation are Abbott et al. (2007) and Dewitt et al. (2016).

Health Organization, European Union, Health Canada). Review the underpinning studies and methods used by these agencies to come to their conclusions.

Part 3

a) Using the same methodological approach used for appraising studies in the 2024 PFAS Review, critically review an additional 14 studies[5] which are candidate studies in US EPA (2024a, b) that have not been either reviewed by SLR previously as part of the 2024 PFAS Review or by FSANZ (2017). The studies are to be assessed for their suitability to be adopted/adapted for the Australian context.

These tasks were performed in consultation with the Committee and NHMRC.

The report herein includes relevant information, specific to this expanded review, that would normally be included in an Evidence Evaluation Report and Technical Report that captures the details and methods used to undertake the expanded review and its results.

1.1 Objectives

The overarching objective of this review is to expand the 2024 PFAS Review with relevant health-based information from the sources specified in the preceding section and consider the potential impact of exposure to PFAS in drinking water on human health outcomes and potential impact on the conclusions made in the 2024 PFAS Review.

2.0 Research Questions

Research questions for this expanded review were adapted from the final research protocol in the 2024 PFAS Review to render them specific to the two PFAS for which additional studies were evaluated in the addendum herein (i.e. PFOA and PFOS) and only relate to questions relevant to health-related advice. The research questions were previously reviewed and agreed upon by the Committee and NHMRC. The research questions guiding the expanded review are provided in **Table 2-1**.

Table 2-1 Research Questions for Expanded Review of Health-Related Advice in Fact Sheets for Select PFAS

#	Research Questions
Health-Related Advice	
Health-based guideline value	
1	What level of PFOA and PFOS in drinking water causes, or is likely to cause, adverse health effects?
2	What is the critical human health endpoint that determines this value?
3	What are the justifications for choosing this endpoint?
4	How was the guideline value derived and what are the uncertainties and/or limitations with the key studies or approaches used?
5	What are the justifications for choosing the study/ies in the guideline value derivation?
6	Are the guideline values / guidance relevant to the Australian context?

[5] The 14 additional studies requiring review are Timmermann et al. (2022), Dewitt et al. (2008), Sagiv et al. (2018), Song et al. (2018), Gallo et al. (2012), Darrow et al. (2016), Nian et a. (2019), NTP (2023), Zhang et al. (2023), NTP (2022), Zhong et al. (2016), Darrow et al. (2013), Vieira et al. (2013), and Butenhoff et al. (2012a).

#	Research Questions
7	Is there evidence of any emerging risks (from publications/studies in scope of the expanded review) that have not already been mentioned in the 2024 PFAS Review?

3.0 Methodology Overview

No literature searches were conducted for the expanded review herein as the review scope identified specific existing health-based guidance documentation and underpinning or candidate studies requiring evaluation.

The following tasks were undertaken as part of this expanded review.

Literature sourcing

The relevant health-based guidance documentation in scope for the expanded review (US EPA 2024a, b; Burgoon et al. 2023) and relevant individual papers listed in **Section 1.0** were sourced in full.

In addition, documentation with respect to a total or sum of PFAS guideline previously sourced as part of the 2024 PFAS Review were collated into a separate sub-folder (i.e. EU 2020, EC 2022, HC 2018a, b; HC 2023, Maine DHHS 2021, Mass DEP 2022, WHO 2022). Where derivation of the guideline was unclear from these documents, targeted literature searches[6] on the relevant agency websites were undertaken where necessary in an attempt to find additional details with respect to the derivation of the guidelines.

Relevant results were recorded in an Endnote library and soft copies of files saved into a designated folder on the SLR server. The server is backed up on a daily basis.

Data Collection, Quality Assessment, and Summary/Synthesis

Relevant data from the health-based guidance documentation (i.e. US EPA 2024a, b; Burgoon et al. 2023) and the sum of (or total) PFAS guidelines were extracted by populating various tables which focused on data needed to answer the research questions in this expanded review. The individual data extraction tables are provided in **Appendix A**.

Synthesis of the information was conducted by presenting summarised extracted data in tabular format for each individual research question. Expert judgement was used to highlight areas of uncertainty or areas where an organisation's methods/interpretation differs from Australian science policy. Quality of existing guidance/guidelines from agency sources (i.e. US EPA 2024a, b; Burgoon et al. 2023) was assessed using the Assessment Tool (Appendix C in the Research Protocol used for the 2024 PFAS Review). The individual completed Assessment tool tables for each of the three guideline/guidance documents in scope for this expanded review are provided in **Appendix B**.

Critical evaluation of studies in scope of this expanded review was also undertaken using the same approach as in the 2024 PFAS Review. As described in **Section 1.0**, discussions / critical evaluations of studies were limited to those studies that had not been previously reviewed and/or considered by an Australian agency for guidance/guideline value development (i.e. FSANZ 2017, 2021) or by SLR in the 2024 PFAS Review.

[6] Search terms on the agency websites included: "(sum of PFAS)" and/or "(total PFAS)".

This report provides the summary of the findings (**Section 4.0**), a discussion of the results for PFOS and PFOA along with an evaluation of the additional studies included in this expanded review (**Sections 5.0** and **6.0**), and conclusions (**Section 7.0**). Where health-based information was considered reasonable for potential derivation of a guideline value, calculations of prospective drinking water guidelines (DWGs) were undertaken using the methodology and default assumptions outlined in the Guidelines (NHMRC and NRMMC 2011) unless otherwise advised by the Committee.

The default equation is outlined in NHMRC and NRMMC (2011, Section 6.3.3) and has been adapted below as Equation 1. In this instance, units have been added in to show how they cancel out and the 'animal dose' in the equation can in fact be an animal or human dose, since both data types may be used to derive DWGs. In some instances, if adaption of existing guidance values was considered, these guidance values may already incorporate the safety factor shown in the denominator of Equation 1.

Equation 1:

Guideline value (ng/L) =

$$\frac{animal\ or\ human\ dose\ (ng/kg\ bw/d)\ x\ human\ weight\ (kg\ bw)\ x\ proportion\ of\ intake\ from\ water\ (fraction)}{volume\ of\ water\ consumed\ (L/d)\ x\ safety\ factor\ (unitless)}$$

Default assumptions typically used in the Guidelines are 70 kg bw for adult human body weight (or 13 kg bw for 2-year old child or 5 kg for an infant), 10% (0.1) for the proportion of intake from drinking water (apart from bottle-fed infants, where 100% is used), and 2 L/day of water consumption by an adult (1 L/day by a child, 0.75 L/day by a bottle-fed infant).

4.0 Results

4.1 Summary of responses to research questions

Responses to research questions were informed by the data extractions from the guidance/guideline documents included in this expanded review (i.e. US EPA 2024a, b; Burgoon et al. 2023). Refer to **Appendix A** for detailed data extraction tables.

Table 4-1 provides a synthesis of the results.

Table 4-1 Summary of findings from data extraction for health-based guidance/guideline values in scope for this review

#	Research Questions	Response	
1	What level of PFOA and PFOS in drinking water causes, or is likely to cause, adverse health effects?	PFOS	Overt adverse health effects from drinking water exposure to PFOS in humans have not been explicitly recorded in the US EPA (2024b) review. However, DWGs were not derived for PFOS in this review. Nonetheless, US EPA released the following Maximum Contaminant Level (MCL) for PFOS: • **4 ng/L** based on a Practical Quantitation Limit (PQL), i.e. a minimum reporting level (US EPA 2024c, d, g).
		PFOA	Overt adverse health effects from drinking water exposure to PFOA in humans have not been explicitly recorded in the US EPA (2024a) or Burgoon et al. (2023) reviews. However, DWGs were not derived in either review. Nonetheless, US EPA released the following MCL for PFOA: • **4 ng/L** based on a PQL, i.e. a minimum reporting level (US EPA 2024c, d, g).
2	What is the critical human health endpoint that determines this value?	PFOS	A health-based DWG was not derived for PFOS in the US EPA review (US EPA 2024b). A MCL of 4 ng/L was set based on a PQL. Nevertheless, US EPA (2024b) derived a health-based guidance value of 0.1 ng/kg/day based on a number of co-critical effects (see below). This guidance value was ultimately not used to set a DWG, as the DWG would have been too low to practically measure.
		PFOA	A health-based DWG was not derived for PFOA in either review (US EPA 2024a, Burgoon et al. 2023). A MCL of 4 ng/L was set based on a PQL. Nevertheless, US EPA (2024a) derived a health-based guidance value of 0.03 ng/kg/day based on a number of co-critical effects (see below). This guidance value was ultimately not used to set a DWG, as the DWG would have been too low to practically measure.
3	What are the justifications for choosing this endpoint?	PFOS	Detailed justification for each endpoint considered for derivation of guidance values for the studies in scope of this expanded evaluation is provided in **Section 5.0**. The US EPA (2024b) justified the use of critical (epidemiological) studies that serve as the basis of the guidance value for PFOS as follows (see also **Appendix A**): • They are all medium or high confidence epidemiological studies. • They are supported by multiple other medium or high confidence studies in both humans and animal models. • Oral PFOS exposure is associated with adverse effects. • They can lead to clinical outcomes in a sensitive life-stage (children). US EPA (2024b) also state that "*the available evidence indicates there are effects across immune, developmental, cardiovascular, and hepatic organ systems at the same or approximately the same level of PFOS exposure*" and

#	Research Questions	Response	
			the "*candidate RfDs* [reference doses] *within the developmental and cardiovascular outcomes are the same value (i.e. 1 × 10^{-7} mg/kg/day)*".
		PFOA	Detailed justification for each endpoint considered for derivation of guidance values for the studies in scope of this expanded evaluation is provided in **Section 5.2** and **6.2**. The following justifications were provided for the critical (and/or candidate) studies that serve as the basis of the guidance values for PFOA (see also **Appendix A**): • The US EPA (2024a) provide the same justifications for the critical (epidemiological) studies that serve as the basis of the PFOA guidance value as provided above for PFOS, i.e. medium and high confidence studies were used, they were supported by multiple other studies, PFOA exposure is associated with the adverse effect, etc. US EPA (2024a) also state that "*the available evidence indicates there are effects across immune, developmental, cardiovascular, and hepatic organ systems at the same or approximately the same level of PFOA exposure"* and *"the candidate RfDs within the immune, developmental, and cardiovascular outcomes are the same value (i.e., 3 × 10^{-8} mg/kg/day)*". • Burgoon et al. (2023) found that the overall uncertainty in both epidemiology and experimental animal studies is sufficient to give pause to the development of a credible critical effect for PFOA (noting they only reviewed studies pertaining to PFOA). However, recognising the importance of managing PFOA potential health risks, a provisional approach was developed based on several experimental animal studies, i.e. the review authors developed a range of safe doses based on liver effects in monkeys and developmental and immunological effects in mice. The existing human observational studies were not considered to be reliable for developing the critical effect in the absence of mechanistic data relevant to humans at serum concentrations seen in the general public.
4	How was the guideline value derived and what are the uncertainties and/or limitations with the key studies or approaches used?	PFOS	The guidance value, termed a Reference Dose (RfD), of **0.1 ng/kg/day** for PFOS was derived by the US EPA (2024b) using two underlying critical studies in humans (Wikström et al. 2020 and Dong et al. 2019) as follows. • Wikström et al. 2020: A lower bound on the benchmark dose level corresponding to the 95% lower confidence limit for a 5% change in response ($BMDL_{5RD}$) of 7.7 ng/mL was identified for decreased infant birth weight corresponding to a point of departure human equivalent dose (POD_{HED}) of 1.13 x 10^{-6} mg/kg/day. An uncertainty factor of 10 was applied (for variability in the human population) to give an RfD of 1.13 x 10^{-7} mg/kg/day (0.1 ng/kg/d). • Dong et al. 2019: A. $BMDL_{5RD}$ of 9.34 ng/mL was identified for increased total cholesterol in adults corresponding to a POD_{HED} of 1.20 x 10^{-6} mg/kg/day. An uncertainty factor of 10 was applied (for variability in the human population) to give an RfD of 1.2 x 10^{-7} mg/kg/day (0.1 ng/kg/d).

#	Research Questions	Response	
			A Cancer Slope Factor (CSF) of **39.5 (mg/kg/d)$^{-1}$** was also derived by the US EPA (2024b) for combined hepatocellular adenomas and carcinomas and in critical studies in female rats (Butenhoff et al. 2012b / Thomford 2002b).
		PFOA	The guidance value, termed RfD, of **0.03 ng/kg/day** for PFOA was derived by the US EPA (2024a) using four underlying critical studies in humans (Budtz-Jørgensen and Grandjean 2018, Timmerman et al. 2022, Wikström et al. 2020 and Dong et al. 2019) as follows. • Budtz-Jørgensen and Grandjean 2018: A $BMDL_{0.5SD}$ of 3.47 ng/mL was identified for decreased serum anti-tetanus & anti-diphtheria antibodies in children corresponding to a POD_{HED} of 3.05 x 10^{-7} mg/kg/day. An uncertainty factor of 10 was applied (for variability in the human population) to give an RfD of 3.05 x10^{-8} mg/kg/day (0.03 ng/kg/d). • Timmerman et al. (2022): A $BMDL_{0.5SD}$ of 2.26 ng/mL was identified for decreased serum anti-tetanus & anti-diphtheria antibodies in children corresponding to a POD_{HED} of 3.34 x 10^{-7} mg/kg/day. An uncertainty factor of 10 was applied (for variability in the human population) to give an RfD of 3.34 x10^{-8} mg/kg/day (0.03 ng/kg/d). • Wikström et al. 2020: A $BMDL_{5RD}$ of 2.2 ng/mL was identified for decreased infant birth weight corresponding to a POD_{HED} of 2.92 x 10^{-7} mg/kg/day. An uncertainty factor of 10 was applied (for variability in the human population) to give an RfD of 2.92 x 10^{-8} mg/kg/day (0.03 ng/kg/d). • Dong et al. 2019: A. $BMDL_{5RD}$ of 2.29 ng/mL was identified for increased total cholesterol in adults corresponding to a POD_{HED} of 2.75 x 10^{-7} mg/kg/day. An uncertainty factor of 10 was applied (for variability in the human population) to give an RfD of 2.75 x10^{-8} mg/kg/day (0.03 ng/kg/d). A Cancer Slope Factor (CSF) of **0.0293 (mg/kg/d)$^{-1}$** was also derived by the US EPA (2024a) for Renal Cell Carcinoma using a critical study in humans (Shearer et al. 2021). Five guidance values, termed RfDs, ranging from **10 - 70 ng/kg/day** were derived for PFOA by the Burgoon et al. (2023) study using five underlying critical studies in animals (Butenhoff et al. 2002, Lau et al. 2006, Loveless et al. 2006, Abbott et al. 2007, DeWitt et al. 2016) as follows. • Butenhoff et al. 2002: A Serum Benchmark concentration (BMC) of 19 µg/mL was identified for increased liver weight in monkeys. An uncertainty factor of 75.6 was applied (3x for animal to human toxicodynamic differences, 3x for human toxicodynamic differences, and 8.4 for human toxicokinetic differences) to give an RfD serum concentration of 0.25 µg/mL corresponding to a RfD of 0.06 µg/kg/day (60 ng/kg/d) using a clearance factor of 0.23 mL/day/kg. • Lau et al. 2006: A No Observed Adverse Effect Level (NOAEL) of 23 µg/mL was identified for dose-dependent growth deficits for gestation days 1–17 in mice. An uncertainty factor of 75.6 was applied (as per above

#	Research Questions	Response	
			description) to give an RfD serum concentration of 0.30 µg/mL corresponding to a RfD of 0.07 µg/kg/day (70 ng/kg/d) using a clearance factor of 0.23 mL/day/kg. • Loveless et al. 2006: A Serum BMC of 4.35 µg/mL was identified for lipid parameters/relative liver weight in mice. An uncertainty factor of 75.6 was applied (as per above) to give an RfD serum concentration of 0.30 µg/mL corresponding to a RfD of 0.07 µg/kg/day (70 ng/kg/d) using a clearance factor of 0.23 mL/day/kg. • Abbott et al. 2007: A NOAEL of 0.3 mg/kg/d (10.4 µg/mL) was identified for neonatal survival in mice. An uncertainty factor of 75.6 was applied (as per above) to give an RfD serum concentration of 0.14 µg/mL corresponding to a RfD of 0.01 µg/kg/day (10 ng/kg/d) using a clearance factor of 0.23 mL/day/kg. • DeWitt et al. 2016: A NOAEL of 0.94 mg/kg/d (assumed to be equivalent to 22 µg/mL based on another study in mice) was identified for immune suppression in mice. An uncertainty factor of 75.6 was applied (as per above) to give an RfD serum concentration of 0.29 µg/mL corresponding to a RfD of 0.07 µg/kg/day (70 ng/kg/d) using a clearance factor of 0.23 mL/day/kg.
5	What are the justifications for choosing the study/ies in the guideline value derivation?	PFOS	• US EPA (2024b) chose the lowest RfDs estimated for a range of effects from human epidemiological studies. They state that "*the available evidence indicates there are effects across immune, developmental, cardiovascular, and hepatic organ systems at the same or approximately the same level of PFOS exposure*" and the "*candidate RfDs within the developmental and cardiovascular outcomes are the same value (i.e. 1 × 10^{-7} mg/kg/day)*" (US EPA 2024b). Further justification is captured in **Appendix A**.
		PFOA	• US EPA (2024a) chose the lowest RfDs estimated for a range of effects from human epidemiological studies. They state that "*the available evidence indicates there are effects across immune, developmental, cardiovascular, and hepatic organ systems at the same or approximately the same level of PFOA exposure*" and "*the candidate RfDs within the immune, developmental, and cardiovascular outcomes are the same value (i.e. 3 × 10^{-8} mg/kg/day)*" (US EPA 2024a). Further justification is captured in **Appendix A**. • Burgoon et al. (2023) presented a range of RfDs from select animal studies chosen by three scientific teams. Importantly, they did not use the human (non-cancer) data or any cancer data (animal or human) as they were not considered to be sufficiently credible as the basis for deriving a PFOA safe dose.
6	Are the guideline values / guidance relevant to the Australian context?	The CSFs derived by USEPA (2024a, 2024b) are not derived consistent with Australian science policy (e.g. enHealth 2012, NEPM 2013), since Australian authorities only use low-dose linear extrapolation and cancer slope factor approaches for carcinogens acting through a mutagenic mode of action. The currently available evidence summarised by the various agencies indicates PFAS are unlikely to cause cancer via a mutagenic mode of action (i.e. there is a threshold below which cancer does not occur). This is also supported by the evaluation of the Butenhoff et al. (2012a) study in **Section 6.2.2**.	

#	Research Questions	Response
		In addition, the critical evaluations of candidate and key studies undertaken in this Addendum (**Sections 5.2** and **6.2**) has concluded that there are various reasons why the epidemiological information for associations of PFAS serum concentrations with various endpoints is not considered suitable in the Australian context for derivation of guidance values for PFAS. Where studies and endpoints selected by US EPA (2024a, b) or Burgoon et al. (2023) were found relevant to the Australian context and of sufficient reliability for adopting / adapting into the Guidelines, the relevant studies have been considered further for candidate guidance / guideline value derivation (see **Sections 5.3** and **6.3**).
7	Is there evidence of any emerging risks (from publications / studies in scope of the expanded review) that have not already been mentioned in the 2024 PFAS Review?	Although adverse health effects *per se* have not been identified in Australian populations from drinking water exposure to these PFAS, based on the various guidance values derived by different jurisdictions, the critical health hazards from exposure to the PFAS evaluated in this review are those identified in the 2024 PFAS Review. The critical health effects considered by Burgoon et al. (2023) have been considered in the 2024 PFAS Review and therefore do not represent evidence of an emerging risk. The US EPA (2024a, 2024b) reviews derived RfDs for both PFOS and PFOA using human epidemiological data and a range of effects. Although the effects considered are consistent with those previously evaluated, some of the critical studies relied upon in the US EPA reviews had not previously been evaluated (Wikström et al. 2020, Dong et al. 2019, Shearer et al. 2021). The emerging epidemiological data and associations being made between PFAS exposure and health risk is evidence of the continuance of the emerging risk that PFAS may present as the toxicological database for these substances continues to grow (irrespective of whether the epidemiological data is considered relevant).

4.2 Summary of PFAS mixture assessment

There is currently a gap in the available knowledge for PFAS to address potential health effects from exposure to PFAS mixtures in water (or any other medium) without the conduct of a complex compound by compound assessment of all PFAS that might be detected in water. To address this knowledge gap, a DWG that covers the gamut of measurable PFAS in water is needed. The following tasks were undertaken to inform this knowledge gap.

- Outline the approaches available for assessing PFAS mixtures in drinking water.
- Summarise some of the approaches used by international regulatory agencies for assessing PFAS mixtures.
- Outline a possible strategy (way forward) for deriving a DWG for the sum of multiple PFAS ($\sum PFAS_n$).

4.2.1 Types of approaches for assessing PFAS mixtures

A framework document that outlines approaches for assessing PFAS mixtures was recently finalised by the US EPA (2024e). Three approaches were described in detail in the US EPA framework document: the Hazard Index (HI) Approach, the Relative Potency Factor (RPF) Approach, and the Mixture benchmark dose (M-BMD) Approach as summarised below in **Table 4-2**. These three data driven approaches can each be used to evaluate the joint toxicity of individual PFAS based on dose addition and have high (toxicological) data requirements. Also summarised in the table below and briefly discussed in the US EPA framework document is the analogue-based read-across methods termed herein as the 'surrogate approach'. The (toxicological) data requirements for a surrogate approach are much fewer than the three data driven approaches described in the US EPA (2024e) framework document. A non-health-based approach using practical considerations, termed herein as a 'Practical (non-health) approach', is also summarised below given it is used by some jurisdictions and does not require toxicological data. Instead, this latter approach relies on what is practically achievable (e.g. laboratory limits of reporting, ability to continually treat, treatment effectiveness, etc.). Each of the approaches have pros and cons associated with them, as summarised in **Table 4-2** below.

Table 4-2 Summary of Potential Approaches to Assess PFAS Mixtures

Approach [1]	Summary Description	Pros	Cons
Hazard Index (HI) Approach	The hazard index is the sum of the hazard quotients (HQs) (i.e. the ratios between exposure and the guidance value) for each component to be evaluated. HI < 1 is considered acceptable. Note: Includes Target-organ-specific Hazard Index (TOSHI) [3].	• Considered likely to be the most health protective approach. • Based on the most sensitive health outcome for each PFAS. • Can derive guidance values *de novo* for PFAS identified as necessary. • Preferred approach with high quality toxicological data. • Transparent and easy to apply.	• Data intensive method. • The majority of PFAS lack sufficient toxicological data [2]. • PFAS dose response curves should have similar shape and slope. • Rapidly evolving toxicological databases. • Lack of consensus on critical effect. • Overestimates risk.
Relative Potency	Provides a mixture toxicity estimate by scaling the	• Common approach used for other chemicals	• Most data intensive method.

Approach (1)	Summary Description	Pros	Cons
Factor (RPF) Approach	potency of component chemicals for a common health effect. Potency for an effect across each component PFAS is scaled to a selected PFAS (typically PFOA or PFOS and referred to as an Index Chemical or IC).	(e.g. dioxins, polycyclic aromatic hydrocarbon or PAHs, polychlorinated biphenyls or PCBs etc.).	• PFAS dose response curves should have similar shape and slope. • Not recommended where the critical effect differs amongst PFAS. • For PFAS, there is a lack of evidence demonstrating that a single receptor mediates toxicity, and PFAS can induce a multitude of toxicities.
Mixture benchmark dose (M-BMD) Approach	This approach uses a dose additivity model to calculate a departure point (e.g. benchmark dose) for the mixture. Compares water concentration with a DWG derived from the M-BMD for the most sensitive effect.	• Provides more accurate predictions of a mixture effect. • No need to identify an IC, RPFs or DWG. • Applicable to PFAS with differing dose response curves.	• Data intensive method. • BMD may be specific to a certain PFAS mixture.
Practical (non-health) Approach	A limit can be set on non-health-based considerations such as reducing exposure, putting forward achievable measures (including laboratory limits of reporting), practicality (e.g. treatment considerations), etc.	• Can be adopted as a precautionary approach. • Toxicological data are not required.	• May not be health protective. • Still need to undertake a comprehensive review of emerging toxicological data. • Not an approved approach in the US EPA Framework (US EPA 2024e).
Surrogate Approach (analogue-based read-across methods)	A common approach for other chemicals is to read across toxicological data from similar compounds in the group (assuming that potency is proportional to carbon chain length).	• Simple approach. • Can adopt data from replete surrogate PFAS with a similar data poor PFAS. • Discussed in the US EPA Framework (US EPA 2024e).	• May not be health protective.

HI = Hazard Index, HQ = Hazard Quotient, RPF = Relative Potency Factor, M-BMD = Mixture benchmark dose, IC = Index Chemical, DWG = Drinking water guidelines, NAMs = New Approach Methodologies.

(1) Publicly available traditional toxicity studies are limited to a small fraction of the available PFAS. Hence, the US EPA (2024e) framework provides for suggestions of integrating validated NAMs such as toxicogenomics (e.g. *in vitro* cell bioactivity) and *in silico* platforms (e.g. structure-activity, read-across) into the HI, RPF, and M-BMD approaches.

(2) Need to consider i) potential exposure, ii) the potency for toxic effect, iii) the duration associated with exposure and toxicity, and iv) qualitative and quantitative uncertainty for each PFAS mixture component.

(3) TOSHI entails calculating component chemical HQs and corresponding mixture HIs for specific target-organ effects/endpoints using only those mixture components with a guidance value for the specified effect.

4.2.2 PFAS mixture approaches used by international organisations

The approaches (and DWG) used by various international agencies to assess PFAS mixtures using a single value are varied as outlined in **Table 4-3** below. The DWGs range from 0.2 to 500 ng/L and include the sum of 3 PFAS to the “totality“ of PFAS. The individual approaches are discussed in detail in **Appendix C**.

The approaches summarised are from a selection of jurisdictions that were encountered when undertaking the initial evidence evaluations for five PFAS including PFOS and PFOA in the 2024 PFAS Review. Approaches as referenced in EC (2022), EU (2020), HC (2018), Mass DEP (2022), Maine DHHS (2021), US EPA (2024e, g), and WHO (2022) were considered as described in **Appendix C**. Attempts were made to identify source documents for the approaches referenced using basic Google® searches. Approaches from two other jurisdictions were identified (from Sweden and Denmark) when reviewing available documents from these select sources (namely WHO 2022) and were briefly summarised. A full literature search was not undertaken.

Table 4-3 PFAS mixture approaches used by select international organisations

Jurisdiction	Approach Used	DWG (ng/L)	PFAS Included	Reference
Health Canada	HI Approach	0.6 (PFOS) and 0.2 (PFOA)	PFOS and PFOA	HC 2023
United States (US)		10 (PFHxS, PFNA, Gen X Chemicals) or 2,000 (PFBS)	PFHxS, PFNA, Gen X Chemicals, PFBS (but does not include PFOS and PFOA).	USEPA 2024e, g
European Union	Practical (non-health) based approach	500	PFAS Total, i.e. the totality of PFAS	EU 2020
		100	Sum of PFAS, i.e. 20 measurable PFAS	
WHO (International)		500	Total PFAS, i.e. 30 measurable PFAS	WHO 2022
		100	PFOA and PFOS each	
European Commission	M-BMD Approach (2)	4.4	∑PFAS4 (PFOA, PFOS, PFNA, and PFHxS)	EC 2022
Massachusetts (US)	Surrogate Approach (3)	20 (CI = PFOA and PFOS)	∑PFAS6 (PFOS, PFOA, PFHxS, PFNA, PFHpA, and PFDA)	Mass DEP 2022, Maine DHHS 2021
Maine (US)				
Other Jurisdictions				
Denmark (Until 2021) (1)	HI Approach	300 (PFOA), 100 (PFOS), & 100 (PFOSA)	PFOS, PFOA and PFOSA	WHO 2022
Sweden (Until 2023) (1)	Surrogate Approach	90 (CI = PFOS)	∑PFAS11 (PFBS, PFHxS, PFOS, 6:2 FTS and PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, and PFDA)	

HI = Hazard Index, RPF = Relative Potency Factor, CI = Index Chemical, DWG = Drinking water guidelines, PFOSA = Perfluorooctanesulfonamide. M-BMD = Mixture benchmark dose. PFOS = Perfluorooctane Sulfonic Acid. PFOA = Perfluorooctanoic Acid. PFHxS = Perfluorohexane Sulfonic Acid. PFNA = Perfluorononanoic Acid. Gen X Chemicals = Hexafluoropropylene Oxide Ammonium Salt and Hexafluoropropylene Oxide Dimer Acid. PFBS = Perfluorobutane Sulfonic Acid. PFHpA = Perfluoroheptanoic Acid. PFDA = Perfluorodecanoic Acid. PFOSA =

Jurisdiction	Approach Used	DWG (ng/L)	PFAS Included	Reference
Perfluorooctanesulfonamide. 6:2 FTS = 6:2 Fluorotelomer Sulfonic Acid. PFBA = Perflorobutanoic Acid. PFPeA = Perfluoropentanoic Acid. PFHxA = Perfluorohexanoic Acid. (1) The Swedish and Denmark approaches have since been updated in line with the RPF approach from the European Commission (EC 2022) and Sum of PFAS approach from the European Union (EU 2020). (2) EC (2022) refers to the approach taken as a RPF approach. Although it is recognised that RPF were estimated for liver effects, the guidance value calculated to derive the applicable DWG used benchmark dose modelling for four PFAS, i.e. a M-BMD Approach as described in the USEPA (2024e) framework. (3) Mass DEP (2022) refer to the approach taken as a RPF Approach. However, RPFs were only derived for liver effects and the PFAS considered are unlikely to be equipotent. Therefore, the approach adopted by Mass DEP is more akin to a Surrogate Approach as summarised in **Table 4-2** with support/justification from estimated RPFs.				

The following is evident from **Table 4-3**.

- Data-driven approaches have been adopted in North America and Europe. For example, the HI Approach was adopted by the US EPA (2024e, g) and HC (2023) whereas an M-BMD Approach was adopted in Europe (EC 2022).
- A Surrogate Approach was adopted in some US States (Mass DEP 2022, Maine DHHS 2021) which was supported by RPFs. The approach is not actually considered to be a RPF approach in this report, even though it is a data-driven approach, given RPFs were only calculated for one health effect that was not the critical effect used to derive guidance values.
- The data driven approaches cover relatively few PFAS (from 2 to 6 PFAS).
- European countries have or are likely to adopt the M-BMD Approach for ∑PFAS 4 outlined by the European Commission (EC 2022) and US States are evaluating the HI Approach outlined in US EPA (2024g).
- Practical (non-health) based approach was adopted by WHO (2022) and the European Union (including many of the EU member countries).
- The practical (non-health) based approach is adopted for many PFAS and can include the sum of measurable PFAS (Sum of PFAS or Total PFAS as defined by WHO 2022) or the “totality” of PFAS as defined by the EU (2020).
- European jurisdictions appear to be adopting a practical (non-health) based approach along with the RPF Approach (∑PFAS 4) as outlined by EC (2022).
- Until recently, Sweden was using a Surrogate Approach with a DWG for PFOS used for the sum of eleven PFAS (∑PFAS11). The Swedish and Denmark approaches have since been updated in line with the RPF approach from the European Commission (EC 2022) and Sum of PFAS approach from the European Union (EU 2020).

4.2.3 PFAS mixture options assessment

There are four options for the derivation/selection of PFAS mixture DWGs or an approach to assess PFAS mixtures. They are the following.

1 Adopt a PFAS mixture DWG derived/established by an overseas jurisdiction.

2 Apply a surrogate approach using existing DWGs.

3 Apply a data-driven approach (e.g. HI approach) for the assessment of a PFAS mixture.

4 Use a combination of surrogate PFAS and HI approaches for the assessment of a PFAS mixture.

Each option is considered separately below. Examples are provided of how to address the gap of assessing PFAS mixtures. It is noted that the options and gaps identified are unlikely to be exhaustive.

4.2.3.1 Option 1: Adopt a PFAS mixture DWG

Adopting an established PFAS mixture DWG from an overseas jurisdiction is not considered appropriate. Existing PFAS mixture DWGs developed using a data-driven approach are mostly based on different critical effects and/or incorporate different assumptions leading to potentially irrelevant and much lower DWGs than adopted in Australia. Effectively, this means the data-driven DWGs considered in **Section 4.2.2** are ruled out for adoption. This only leaves the European DWG for Sum of PFAS of 100 ng/L and Total PFAS of 500 ng/L (WHO 2022 and EU 2020). Both of these values use a practical (non-health) based approach and are below the current Australian DWG for PFOA (560 ng/L), hence they are unlikely to be suitable to be adopted in Australia unless the Australia PFOA DWG is revised (as suggested in this report).

Nevertheless, from a practical perspective, should the DWG for PFOA be lowered below 100 ng/L then adopting a non-health-based guideline value could be part of a suitable screening approach to minimise exposure to other PFAS and/or identify where further assessment may be needed. There are, however, limitations with this approach. For example, for some short-chain PFAS, it is possible that the practical guideline value may be exceeded in certain areas (e.g. potentially where PFHxA or PFBA concentrations are high) and still not necessarily be of a health concern. However, this approach may not be protective for longer chain PFAS without a DWG, such as PFNA and PFDA. If a practical non-health-based guideline value is to be adopted it is suggested that a guidance note be developed that explains the limitations of the approach and to ensure its consistent and appropriate application.

Adopting a DWG from an overseas jurisdiction is not a suitable option in Australia based on the current Australian PFOA DWG but may be applicable if the PFOA DWG is lowered below the European DWG for ‘Sum of PFAS’ of 100 ng/L.

4.2.3.2 Option 2: Surrogate approach to derive a PFAS mixture DWG

Using a surrogate approach is potentially the easiest and quickest manner to derive a DWG for a PFAS mixture as it would rely upon DWGs that are already in use or being derived. Although it is not feasible to select a surrogate DWG for Sum of PFAS given the disparity in current Australian DWGs for PFOA and PFOS, it may make sense to apply a surrogate approach to perfluoro carboxylic acids (PFCAs) and perfluoro sulfonic acids (PFSA). This would simply mean applying the PFOA DWG as a surrogate for PFCAs (and possibly their precursors including fluorotelomers) and the PFOS DWG for PFSAs (and possibly their precursors). Hence, instead of one PFAS group (Sum of PFAS), there would be two (Sum of PFCAs with precursors and Sum of PFSAs with precursors). Such an approach would most likely be conservative and health protective, suitable for a screening assessment of potential exposure risk, and quickly and easily implemented.

As explained, this approach also does not rely upon the preferred data-driven approach to derive/select a PFAS mixture DWG. However, it can be used to capture many more PFAS than data-driven approaches, is a simple approach, and is easily applied/understood. If implemented, it would be beneficial to prepare a technical background document that details the justification of this approach or a brief fact sheet on how to apply the new values. Although not necessary, the preparation of a technical document could be used to

transparently explain the decisions behind the inclusion of the many precursor PFAS (e.g. why they are included in PFCA or PFSA groups) and provide a detailed discussion of available toxicological data to support the conservative nature of such an approach.

4.2.3.3 Option 3: Data-driven approaches to derive a PFAS mixture DWG

Ideally, a data-driven approach would be the basis of a PFAS mixture DWG derived for use in Australia. However, in isolation, such an approach is unlikely to be applicable to more than a handful of PFAS. This is the case in Australia given guidance values and DWGs from the 2024 PFAS Review are likely restricted to five PFAS only, i.e. the five PFAS for which Australian DWGs are either currently available or are being considered. Adopting a RPF approach would only be possible with development of RPFs for developmental effects and potentially other relevant effects and it is not clear whether either of these RPFs could be derived based on available data. Use of a M-BMD approach also requires crucial data that are currently lacking and may only be applicable to certain PFAS mixtures (noting that EC 2022 do not consider PFAS mixtures in application of their ∑PFAS4 DWG).

Deriving a DWG using one of the three data driven approaches described in the US EPA (2024e) framework document is unlikely to provide a PFAS mixture DWG for more than a handful of compounds, will be of limited utility, and require high level of effort to derive a technical background document explaining the new approach.

4.2.3.4 Option 4: Combine approaches for assessment of PFAS mixtures

Despite the shortcomings of data-driven approaches pointed out in **Section 4.2.3.3**, the HI Approach is most amenable for use in Australia. This is because it is easily applied given its widespread use and is easily understood. However, to establish an approach that is applicable to more than five PFAS (such as a Sum of PFAS) then the HI approach would need to be combined with the surrogate approach. To do this, the following would need to occur:

- Additional DWGs (optional) for additional PFAS could be derived (preferable although not entirely necessary). DWGs could be derived for PFBA, PFHxA, PFNA, and 6:2 FTS to cover a wider gamut of PFAS, especially as some of these PFAS are present in Australian waters. There is potentially sufficient toxicological data in the public domain to derive DWGs for these PFAS.
- Assign a surrogate DWG to the other PFAS. As an example (assuming additional DWGs are derived), for PFCAs the PFBA (5 carbons) DWG could be assigned to PFPrA (3 carbons), the DWG for PFHxA (6 carbons) assigned to PFPeA (5 carbons), and the DWG for PFOA (8 carbons) assigned to C7 PFCAs and above (including PFHpA). For PFSAs, the DWG for PFBS (4 carbons) could be assigned to PFPrS (3 carbons) and PFPeS (5 carbons) and the DWG for PFOS+PFHxS assigned to C6 PFSAs and above. The 6:2 FTS DWG (if derived) could be applied to 4:2 FTS, 8:2 FTS and 10:2 FTS (or alternatively the PFOA DWG could be assigned).
- Incorporate a strategy to assign a surrogate DWG to precursor PFAS. This requires knowledge on what type of PFAS the precursor PFAS break down to in the environment (PFCA or PFSA).

This suggested combined approach would allow a HI to be calculated for PFAS mixtures based on the reported concentrations of measurable PFAS. Such a process could easily be automated to calculate a HI, either by reporting laboratories or in data management software (such as ESDAT).

A technical document would be required to derive the additional DWG and justify the HI Approach (including explaining how it should be used).

5.0 Discussion for PFOS

This section provides a discussion of the strengths and limitations of the studies used by the reviews included in this report (i.e. for PFOS this is US EPA 2024b) as candidate or critical studies for derivation of PFOS guidance values for possible adoption/adaption into the Guidelines. Critical evaluation was undertaken for those studies not previously considered / evaluated by FSANZ (2017) or by the 2024 PFAS Review.

5.1 Potential suitability of health-based guidance values for possible adoption/adaption

Candidate guidance values for PFOS from US EPA (2024b) in scope for this expanded evaluation for possible adoption/adaption in Australia have been evaluated using the Assessment Tool provided in **Appendix B**. This tool evaluates each document against administrative and technical criteria that demonstrate transparent and robust guideline development and evidence review processes that meet NHMRC standards for guidelines. The overall potential suitability of the guidance values for adoption/adaption can be gauged at least partially by examining the percentage of 'must-have', 'should-have', and 'may-have' criteria met by each jurisdiction.

The US EPA (2024b) review for PFOS was evaluated using these criteria and met a high proportion of 'must-have' (i.e. 95%), 'should-have' (i.e. 90%) and 'may-have' (i.e. 100%) criteria.

5.2 Critical evaluation of PFOS candidate studies used by US EPA (2024b) to derive guidance values not previously considered by FSANZ (2017) or 2024 PFAS Review

The following studies used by US EPA (2024b) as critical or candidate studies to derive potential guidance values have not been previously considered / cited in the comprehensive review undertaken by FSANZ (2017), the FSANZ (2021) immunological update, or the 2024 PFAS Review with respect to PFOS. The discussion in this section therefore focuses on these relevant studies.

- Three (3) epidemiological studies investigating birth outcomes: Darrow et al. (2013), Sagiv et al. (2018), Wikström et al. (2020).
- Three (3) epidemiological studies investigating cholesterol and liver effect biomarkers: Dong et al. (2019), Gallo et al. (2012), Nian et al. (2019).
- Two (2) epidemiological studies investigating antibody levels: Timmermann et al. (2022), Zhang et al. (2023).
- Two (2) experimental animal studies: full 28-day toxicological study in rats evaluating a large number of endpoints (NTP 2022)[7] and a developmental toxicity study investigating immune system effect markers in mice (Zhong et al. 2016).

Due to there being differing candidate guideline values for PFOS, their overall confidence was assigned as being 'High', 'Moderate', 'Low', or 'Very low' based on expert judgement; this was based on an assessment of underpinning critical study quality, with rationale for the rating provided in the critical evaluation discussions of the respective underpinning study

[7] Note although the NTP (2022) study was included in the 2024 PFAS Review, it was only discussed in relation to PFHxS and PFBS, not PFOS, as it was not one of the studies underpinning internationally derived guidance values for PFOS at the time. It was, nevertheless, considered as a candidate study in the latest US EPA (2024b) review, released after the 2024 PFAS Review, which is why it has now been included in this Addendum report with respect to PFOS.

(see **Sections 5.2.1** to **5.2.10**). This was done to provide the Committee with more information to enable comparison of the different candidate guideline value options against the current Australian guideline value to facilitate an informed decision of whether revision of the existing Australian guideline value is warranted or not.

5.2.1 Darrow et al. (2013) – candidate study in US EPA (2024b)

The authors of Darrow et al. (2013) conducted a population-based survey of PFOA and PFOS and birth outcomes from 2005 through 2010 in a Mid-Ohio Valley community exposed to high levels of PFOA through drinking-water contamination (2005–2006 PFOA serum median = 28 ng/mL *cf.* median PFOA serum level in the U.S. general population in 2003–2004 was 4 ng/mL). Participants who enrolled in the C8 Health Project[8] between 2005 and 2006 (*n* = 69,030) completed a demographic and health questionnaire and provided serum for measurement of PFOA and PFOS. A subset of participants in the C8 Health Project (*n* = 32,254) participated in one or two follow-up interviews between 2008 and 2011. To be included in the analysis, women had to have provided a blood sample at enrolment in the C8 Health Project, completed at least one follow-up interview, and reported at least one live birth between 2005 and 2010. The study evaluated the following endpoints: preterm birth (< 37 weeks gestation), pregnancy induced hypertension (including preeclampsia), low birth weight (< 2,500 g), and full-term (≥ 37 weeks gestation) infant birth weight. The study also evaluated the following covariates: parity, smoking status, maternal age, year of birth, year of conception, education level, body mass index and diabetic status. Binary outcomes were analysed using logistic regression models, and continuous birth weight data were analysed using linear regression. Effect estimates for untransformed PFOA and PFOS concentrations were scaled to an interquartile range increase (i.e. 75th–25th percentile). The primary models included natural log-transformed PFOA or PFOS serum concentrations as continuous exposures. Untransformed continuous concentrations and quintiles of PFOA and PFOS serum concentrations were also modelled. The study also tested for trends across serum concentration quintiles by including an ordinal variable for quintile in the model. The bottom (referent) quintile for PFOA serum concentration would capture approximately 90% of US adult women based on the 2003-2004 US National Health and Nutrition Examination Survey. The bottom quintile for PFOS serum concentration captured approximately 10% of the US general population.

The key findings of the survey were:

- *Serum concentrations of PFOA and PFOS:* Serum levels of PFOA and PFOS were weakly correlated (r = 0.30). The mean serum PFOA was 31.0 ng/mL and the mean serum PFOS was 15.6 ng/mL. The distribution of absolute concentrations was similar between the two chemicals except for the top third of the distribution where PFOA concentrations were more right-skewed (e.g. 95th percentile for PFOA = 114 ng/mL vs. 32 ng/mL for PFOS). Women with normal body mass index, no previous births, or higher education at enrolment had higher PFOA and PFOS serum levels than other women.

[8] From 1950 through 2005, a chemical plant in the Mid-Ohio Valley, West Virginia (USA), emitted PFOA into the surrounding environment. In 2001, a group of residents filed a class action lawsuit alleging health damage from the drinking water supplies drawing on PFOA-contaminated groundwater. Part of the pre-trial settlement of the class action lawsuit included a baseline survey, the C8 Health Project, conducted in 2005-2006, that gathered data from >69,000 persons from six contaminated water districts surrounding the plant.

- *Preterm birth (n = 158 cases):* There was little evidence of an association between PFOA or PFOS serum levels and preterm birth (*p*-trend > 0.4).
- *Pregnancy induced hypertension (n =106 cases):* The effects of PFOS were not statistically significant (*p*-trend > 0.092).
- *Low birth weight (n = 88 cases):* There was little evidence of an association between PFOA or PFOS serum levels and low birth weight (*p*-trend > 0.4).
- *Continuous birth weight in full-term infants (n = 1470 cases):* There was little evidence of an association between PFOA serum level and birth weight in full-term infants. When the data was adjusted for maternal age, educational level, smoking status, parity, body mass index, diabetic status, time between conception and serum measurement and indicator variables for gestational week there was a significant (*p*-trend = 0.045), dose related association between serum PFOS level and reduced birth weight ranging from -25 to -83 g for serum concentrations ranging from 8.6 to ≥ 21.4 ng/mL, but there was no clear dose response. When the data were adjusted to only include the first pregnancy conceived after serum measurement among nonpregnant women the trend was statistically more significant (*p*-trend = 0.006) and the effect level was higher (range - 33 to 105 g for serum concentrations ranging from 8.6 to ≥ 21.4 ng/mL).

Overall, the study found a statistically significant (*p*-trend < 0.05) association (odds ratio > 3) for PFOA serum levels between 8.6 to ≥ 21.4 ng/mL and small (≤ -83 g) reduction in birth weight. The study did not demonstrate an association between PFOA exposure and adversely low birth weight i.e. the changes observed in this parameter are, based on the results of this study, non-adverse.

Importantly the population survey design of the study ranks low on the hierarchy of evidence. Accordingly, the results of the study require confirmation using more powerful study designs and methods before definitive conclusions can be reached. The study does not meet the Bradford-Hill criteria for causation.

US EPA (2024b) used the Darrow et al. (2013) study as a candidate study for derivation of a TRV for PFOS, amongst several other studies. US EPA (2024b) selected a benchmark response level (BMR) of 5% extra risk from the control as per US EPA's *Benchmark Dose Technical Guidance* (US EPA 2012).

US EPA (2024b) estimated a $BMDL_{5RD}$ of 17.4 ng/mL for PFOS from the Darrow et al. (2013) study using a hybrid BMD model and used the updated Verner et al. (2016)[9] Physiologically Based Pharmacokinetic (PBPK) model summarised in **Section 5.2.10** to derive a POD_{HED} of 0.00251 µg/kg/d for PFOS. US EPA (2024b) then applied an uncertainty factor of 10 for human variability to the POD_{HED} to derive a PFOS TRV of 0.000251 µg/kg/d (i.e. 0.3 ng/kg/d).

An effect on birth weight is concordant with effects observed in experimental animal studies in rodent pups. However, it is difficult to reconcile the PFOS serum concentrations at which reductions in pup body weight gain have been observed in experimental studies (e.g. PFOS: maternal and F1 males mean, respectively, of ~18,900 or 45,400 ng/mL, with no effects at ~ 5,280 or 10,500 ng/mL in Luebker et al. 2005) with the human serum PFOS

[9] The model was used to simulate the human equivalent doses (HED) from animal points of departure (PODs) that were obtained from benchmark dose (BMD) modelling of animal toxicological studies; it was also used to simulate selected epidemiological studies to obtain a chronic dose that would result in the internal POD obtained from dose-response modelling (particularly to calculate human equivalent PODs for PODs based on epidemiological observations of maternal serum concentration during pregnancy, cord blood concentration, and serum concentrations in children).

concentrations in the Darrow et al. (2013) study for which statistical associations were found with continuous birth weight in full term infants (i.e. PFOS ≥ 8.6 ng/mL).

In addition, the interquartile range of PFOS serum concentrations in the Darrow et al. (2013) study is very small in terms of absolute values (i.e. 10 ng/mL). The $BMDL_{5RD}$ of 17.4 ng/mL for PFOS derived by US EPA (2024b) is just above the 50th percentile of PFOS maternal serum concentrations measured for the cohort in the Darrow et al. (2013) study (i.e. 13.9 ng/mL). It is difficult to reconcile whether such low serum PFOS concentrations relative to the serum PFOS concentrations observed in experimental animals are to be believed as exerting a true adverse effect.

This indicates there is still marked uncertainty in terms of the appropriateness of using epidemiological data to define the threshold and dose response of effects potentially caused by PFOS (and PFOA) exposure.

Based on the above discussion and uncertainties with respect to using the Darrow et al. (2013) study to define a dose response, although low PFOS doses appear to be associated with decreased birth weight, the data on dose response are not considered sufficiently reliable for use as a key study for derivation of a TRV.

Therefore, the US EPA (2024b) assessment of Darrow et al. (2013) is not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate guidance/guideline value derivation for PFOS in **Section 5.3**.

5.2.2 Dong et al. (2019) – used by US EPA (2024a, b)

Dong et al. (2019) followed on from a previous investigation by Nelson et al. (2010) who examined associations between PFAS and cholesterol in the general US population using NHANES[10] 2003-2004 data. In this updated cross-sectional study, Dong et al. (2019) used the NHANES dataset (for people ages 12-80) to determine whether the associations were consistent or not amongst different rounds of NHANES data and to address the trends in exposure to PFAS in the US population. They analysed for associations with serum PFOA, PFOS, PFDE, PFHxS and PFNA. Where values were below limits of detection (LODs), LODs were divided by 2 for the analysis. The analysis was undertaken with i) total cholesterol (TC), ii) high-density lipoprotein cholesterol (HDLC), and iii) low-density lipoprotein cholesterol (LDLC). Adjustment of common confounders (i.e. age, sex, race, family income index, body mass index, waist circumference, and physical activity in preceding 30 days) was also undertaken. For adults, three additional factors were considered (i.e. diabetes status, smoking status, and number of alcoholic drinks per day in the past 12 months). As previous studies had indicated other factors (such as serum albumin, kidney function, diet) have little effect on the association between serum PFAS and cholesterol, they were not included.

The study authors undertook correlation analyses separately for adolescents and adults. Linear regression was then carried out for each NHANES round dataset; coefficients and

[10] The US National Health and Nutrition Examination Survey (NHANES) is a program of studies designed to assess the health and nutritional status of adults and children in the United States. The survey combines interviews and physical examinations. It is a major program of the National Center for Health Statistics (NCHS), which is part of the Centers for Disease Control and Prevention (CDC). The NHANES program began in the early 1960s and has been conducted as a series of surveys focusing on different population groups or health topics. In 1999, the survey became a continuous program that has a changing focus on a variety of health and nutrition measurements to meet emerging needs. The survey examines a nationally representative sample of about 5,000 people each year, located across the US. The NHANES interview includes demographic, socioeconomic, dietary and health-related questions. The examination component consists of medical, dental and physiological measurements, as well as laboratory tests (including blood PFAS measurements) (https://www.cdc.gov/nchs/nhanes/about_nhanes.htm, accessed 29 July 2024).

95% confidence intervals (CIs) were estimated. Pooled analysis was then also carried out to calculate an overall coefficient. Outliers (>1.5 x above or below interquartile range) were excluded.

Correlation analyses found the most significant association between PFOA and PFOS (R: 0.69). Mean ± standard deviation serum PFOS and PFOA levels and cholesterol levels over the whole dataset (2003-2014) were:

- PFOA: 3.3 ± 2.0 (95% CI 0.77-8.3) ng/mL in adolescents; 3.7 ± 3.4 (95% CI 0.6-10.4) ng/mL in adults.
- PFOS: 12.2 ± 10.4 (95% CI 1.5-38.1) ng/mL in adolescents; 15.6 ± 17.8 (95% CI 1.3-54.7) ng/mL in adults.
- TC: 160 ± 30.9 (95% CI 109-231) mg/dL in adolescents; 196.6 ± 42.5 (95% CI 125-288) mg/dL in adults.
- HDLC: 52.4 ± 12.7 (95% CI 31-81) mg/dL in adolescents; 523 ± 16.1 (95% CI 29-91) mg/dL in adults.
- LDLC: 89.3 ± 26.8 (95% CI 46-153) mg/dL in adolescents; 115.4 ± 35.8 (95% CI 55-192) mg/dL in adults.

Most associations between PFAS serum concentrations and cholesterol for adolescents were insignificant, except for PFOS. Most associations were significant for adults ($p<0.05$), except the PFOS-HDLC association. Regression analysis (only undertaken for adults), presumably adjusted for all confounders but this is unclear, found a positive trend between PFOA and TC (ß = 1.49, 95% CI: 0.2-2.8) as well as PFOS and TC (ß = 0.4, 95% CI: 0.06-0.6). PFOA coefficients were positive for the individual sub-sets but only reached statistical significance in 2003-2004, 2007-2008, 2011-2012 and 2013-2014.

TC levels significantly increased with increasing serum concentration quintiles for PFOS, PFOA, and PFNA, whereas for LDLC this only occurred for PFNA.

Limitations of the study include that it is cross-sectional and therefore cannot be used to attribute causality, only an association. The authors state, similar to other cross-sectional studies, the study cannot answer whether exposure to PFAS elevates cholesterol levels, whether high cholesterol levels simply facilitate PFAS storage in the blood, or whether joint factors simultaneously affect both PFAS and cholesterol. In addition, other potential confounders (e.g. diet, albumin, etc.) may also impact cholesterol and were not adjusted for in the Dong et al. (2019) study. The authors also state that another limitation in their study is that the clinical significance of the elevations in cholesterol were not investigated.

US EPA (2024a, b) used the study for derivation of candidate TRVs for both PFOS and PFOA, along with several other studies. US EPA (2024a, b) selected a BMR of 5% for this study and excluded data for people taking cholesterol medication.

US EPA (2024a, b) estimated a $BMDL_{5RD}$ of 9.34 ng/mL for PFOS and a $BMDL_{5RD}$ of 2.29 ng/mL for PFOA from the Dong et al. (2019) study using a hybrid BMD model and used the updated Verner et al. (2016) PBPK model summarised in **Section 5.2.10** to derive a POD_{HED} of 0.0012 µg/kg/d for PFOS and 0.000275 µg/kg/d for PFOA. US EPA (2024a, b) then applied an uncertainty factor of 10 for human variability to the POD_{HED} to derive a PFOS TRV of 0.00012 µg/kg/d (i.e. 0.1 ng/kg/d) and a PFOA TRV of 0.0000275 µg/kg/d (i.e. 0.03 ng/kg/d).

The dose response data from this study are not considered sufficiently reliable for use as a key study for derivation of a TRV for the reasons discussed in the text above. Therefore, the US EPA (2024a, b) assessment of Dong et al. (2019) is not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate

guidance/guideline value derivation for PFOS or PFOA in **Section 5.3** and **Section 6.3**, respectively.

5.2.3 Gallo et al. (2012) – candidate study in US EPA (2024a, b)

Note the description in this section is largely based on the previous description of this study in the 2024 PFAS Review and focuses on PFOS (as this study has already been evaluated with respect to PFOA).

In a cross-sectional study, Gallo et al. (2012) analysed data for 46,452 adults[11] from the C8 Health Project. They fitted linear regression models for natural log (ln)-transformed values of alanine transaminase (ALT), γ-glutamyltransferase (GGT) and direct bilirubin on PFOA, PFOS, and potential confounders (age, physical activity, body mass index, average household income, educational level, race, alcohol consumption, and cigarette smoking). Logistic regression models were fitted comparing deciles of PFOA or PFOS concentrations in relation to biomarker levels. A multilevel analysis was also undertaken comparing the association of PFOA with liver biomarkers at the individual level within water districts to that at the population level between water districts.

PFOA and PFOS were associated with all potential confounders considered. Ln-transformed values of ALT were significantly associated with ln-PFOA and ln-PFOS in linear regression models [fully adjusted (model 3) coefficient: PFOA, 0.022; 95% CI: 0.018, 0.025; PFOS, 0.020; 95% CI: 0.014, 0.026) with a partial R^2 greater for the association with PFOA (0.002) than for PFOS (<0.001). A steady increase in fitted levels of ALT per decile in PFOA or PFOS serum concentrations was found, with a possible levelling off effect after approximately 30 ng/mL (when ALT was ~22.5 International Units per Litre or IU/L). This positive association was also observed in logistic regression models with a steady increase in odds ratio (OR) estimates across deciles of both PFOA and PFOS concentrations (p = <0.001) and a significant OR for both ln-unit of PFOA (OR = 1.1; 95% CI 1.07, 1.13) and ln-unit of PFOS (OR = 1.13; 95% CI 1.07, 1.18).

No association of PFOS with GGT or direct bilirubin was found.

The authors found significance of associations of ALT outside the 'normal range' used in the study (i.e. cutoffs of 45 IU/L in men and 34 IU/L in women)[12], however only a small proportion of people had ALT values outside the selected 'normal range', making the observed values difficult to interpret in terms of a true adverse effect. Gallo et al. (2012) state that it is not clear if this small increase in ALT levels can lead to clinically diagnosable conditions or if this effect is reversible. Gallo et al. (2012) also state that data from their study cannot be directly used for estimating single-subject damage in relation to PFAS exposure. It is also noted that the reference ranges for ALT can vary depending on the laboratory. For example, Mayo Clinic (2023) cite a standard reference range for ALT of 7 to 55 IU/L. Regardless of the reference range used, the positive associations observed for both PFOA and PFOS with ALT appear to level off within the reference range of ALT (i.e. at ~22.4 IU/L), raising uncertainty with respect to the clinical relevance of the association observed. It therefore becomes arguable whether a cross-sectional study result (recognising it was well conducted and for a relatively large population) for a positive association of serum PFOS or PFOA with a biomarker of a potential effect which seems to level off within the reference

[11] 56,554 adults (≥18 years of age) were considered for the analysis, and a total of 46,452 of those adults (82.1%) were included in the final analysis after exclusion of subjects with missing data on socioeconomic status, alcohol consumption, or cigarette smoking and other potential confounding variables or without PFAS or liver enzyme measurements.

[12] These values are clinically based reference levels used by the International Federation of Clinical Chemistry and Laboratory Medicine and were approximately the 90th percentile of all ALT values in the study.

range for this biomarker should be used as the basis of deriving a health-based guidance value.

The study authors indicate the main limitation of the study is its cross-sectional design, which makes causal inference difficult. However, the consistency of findings with other literature, in particular for the association with ALT, reinforces the hypothesis of a true association (Gallo et al. 2012).

US EPA (2024a, b) used the study for derivation of candidate TRVs for both PFOS and PFOA, along with Nian et al. (2019). US EPA (2024a, b) selected a BMR of 5% for this study.

US EPA (2024a, b) estimated a $BMDL_{5RD}$ of 56.8 ng/mL for PFOS and a $BMDL_{5RD}$ of 17.9 ng/mL for PFOA from the Gallo et al. (2012) study using a hybrid BMD model and used the updated Verner et al. (2016) PBPK model summarised in **Section 5.2.10** to derive a POD_{HED} of 0.00727 µg/kg/d for PFOS and 0.00215 µg/kg/d for PFOA. US EPA (2024a, b) then applied an uncertainty factor of 10 for human variability to the POD_{HED} to derive a PFOS TRV of 0.0007 µg/kg/d (i.e. 0.7 ng/kg/d) and a PFOA TRV of 0.000215 µg/kg/d (i.e. 0.2 ng/kg/d).

The dose response data from this study are not considered sufficiently reliable for use as a key study for derivation of a TRV for the reasons discussed in the text above. Therefore, the US EPA (2024a, b) assessment of Gallo et al. (2012) is not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate guidance/guideline value derivation for PFOS or PFOA in **Section 5.3** and **Section 6.3**, respectively.

5.2.4 Nian et al. (2019) – candidate study in US EPA (2024a, b)

Nian et al. (2019) conducted a cross-sectional study evaluating the association between serum PFAS and liver function biomarkers in the *Isomers of C8 Health Project in China*. The project was set up to investigate associations between PFAS exposure and health outcomes in a high PFAS exposure area in China (Shenyang city). Shenyang city is a heavy industrial city, about 100 km east from Fuxin city which is one of the largest fluoropolymer manufacturing centres in China. Enrolment in the study consisted of a total of 1,605 participants all of whom resided in the city. From July 2015 through October 2016, government employees, including retirees, (n=1,228 adults) were enrolled in the study.

The 500 residents from the community-dwelling locales were stratified into Central, North, East South, and West originating from geographical zones of Shenyang city. Using random sampling, 100 residents from each zone of the community who were 35 years or older and lived in the current residence for more than half a decade were selected. A total of 384 of the randomly stratified participants completed the study (response rate: 77%). Proportions of male and female participants and most serum PFAS concentrations were similar for government workers and community-dwellers and, therefore, were combined for statistical analysis. Study participants completed a self-administered survey and anthropometric measures and donated a fasting blood specimen. Blood was analysed for 18 PFAS and various liver biomarkers (albumin, aspartate aminotransferase or AST, serum alanine aminotransferase or ALT, serum total protein or TP, prealbumin or PA, alkaline phosphatase or ALP, cholinesterase or ChE, total bilirubin or TB, and gamma-glutamyl transferase or GGT). Serum PFAS concentrations lower than the LOD were replaced with the LOD divided by the square root of 2.

Linear regression models were conducted to estimate associations between PFAS as continuous predictors and log transformed serum liver biomarkers as continuous outcomes while also adjusting for the following covariates: age, sex, career (cadres vs. others), income, education, smoking status, alcohol consumption, consumption of giblet, seafood

consumption, regular exercise and body mass index. Sensitivity analysis was conducted by excluding smokers, drinkers and medicine takers[13]. Logistic regression models were also used to estimate associations between PFAS concentrations and abnormal liver function biomarkers, as well as between quartiles of single PFAS as categorical predictors of interest, with binary liver function biomarkers dichotomised according to clinical reference intervals.

The study population (n=1,605) consisted of 17.9% smokers, 38.1% alcohol drinkers and 26.7% medicine takers. Out of the 18 PFAS analysed for in serum, 13 were detected in more than 50% of serum samples. Median (interquartile range) total PFOS and PFOA concentrations were:

- Government workers (n=1,223) = PFOA: 6.37 (4.25-9.61) ng/mL; PFOS: 23.79 (14.5-36.8) ng/mL.
- Community-dwelling people (n=382) = PFOA: 5.28 (3.34-8.61) ng/mL; PFOS: 25.48 (14.79-38.48) ng/mL.
- Combined population (n=1,605) = PFOA: 6.19 (4.08-9.31) ng/mL; PFOS: 24.22 (14.62-37.19) ng/mL.

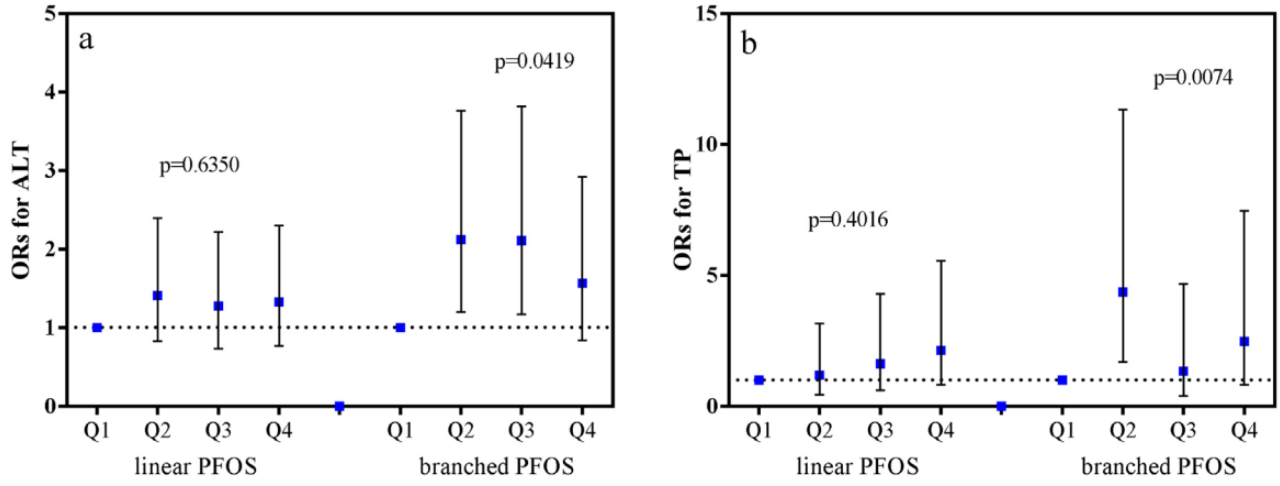
The regression analyses showed positive associations between several serum PFAS concentrations and liver biomarkers. For example, a 1 ln-unit increase in total PFOA was associated with a 7.4% (95% CI: 3.9-11.0%) higher ALT level in serum, 2.9% increased AST (95% CI: 0.7-5.2%), 0.6% increased albumin (0.2-1.0%), 3.7% PA (2.4-4.9%), 2.1% ChE (0.9-3.4%), and 8.6% GGT (4.9-12.3%) ($p<0.05$).

PFOS was significantly associated with higher ALT (4.1%, 95% CI: 0.6-7.7%), albumin (0.6%, 95% CI: 0.2-0.9%), TP (0.6%, 95% CI: 0.2-1.0%), and PA (1.6%, 95% CI: 0.3-2.8%). There were also associations with other PFAS which are not reported in this summary.

In the sensitivity analysis that excluded medicine takers, or smokers or alcohol drinkers whose liver function may be compromised, significant associations for PFOS and PFOA with the relevant markers remained except for PFOS and ALT which became non-significant (3.8%, 95% CI: -0.2, 7.8%).

For PFOS, the analysis for liver biomarkers was also done by quartile (with quartile 1 acting as the referent) with ORs and 95% CI estimated for each biomarker. This analysis was only presented in the paper for PFOS, and not PFOA. Sporadic statistically significant ORs were found between individual PFOS isomers and ALT, TP, ChE, GGT, and TB. However, there was no clear dose response evident for these (Nian et al. 2019, supplementary information, see also **Figure 5-1**).

[13] i.e. anti-hypertensive drugs, antidiabetics, lipid-lowering drugs, uric-acid-lowering drugs, anti-arrhythmic drugs, anti-asthmatic drugs, analgesic-antipyretic, anti-depressant, sedative, hormone drugs, and traditional Chinese medicines.



Adjusted for age, sex, career, income, education, drinking alcohol, smoking, giblet and seafood consumption, exercise and body mass index.

Figure 5-1 ORs (and 95% CIs) for different quartiles of linear and branched PFOS for ALT and TP.

Strengths of the study include its size, the separation of individual isomers of PFOS and PFOA, and adjustment for a number of potential confounders (including medicine use).

The authors indicate that further studies are required to confirm the associations between PFAS serum concentrations and liver biomarkers. The authors discuss the inconsistency in findings between their study and other studies, where some cross-sectional studies have found significant associations with liver biomarkers whereas several prospective studies have reported no associations. Limitations of the study include its cross-sectional design, the fact that liver biomarkers and serum concentrations were only captured in a single point in time (and at the same time) which could result in misclassification of both exposure and outcome, possible reverse causation, binary self-reported data for potential confounders, possible type I error inflation due to the number of combinations assessed (18 PFAS and nine liver biomarkers) and, as with any epidemiological study, not all possible confounders could be controlled for. In addition, multiple PFAS were not evaluated in the same regression model.

US EPA (2024a, b) used the endpoint of increased ALT in the Nian et al. (2019) study for derivation of candidate TRVs for both PFOS and PFOA, along with several other studies. US EPA (2024a, b) selected a BMR of 5% for this study.

US EPA (2024a, b) estimated a $BMDL_{5RD}$ of 15.1 ng/mL for PFOS (females only) and a $BMDL_{5RD}$ of 3.76 ng/mL for PFOA from the Nian et al. (2019) study using a hybrid BMD model and used the updated Verner et al. (2016) PBPK model summarised in **Section 5.2.10** to derive a POD_{HED} of 0.00194 µg/kg/d for PFOS and 0.000451 µg/kg/d for PFOA. US EPA (2024a, b) then applied an uncertainty factor of 10 for human variability to the POD_{HED} to derive a PFOS TRV of 0.0002 µg/kg/d (i.e. 0.2 ng/kg/d) and a PFOA TRV of 0.0000451 µg/kg/d (i.e. 0.05 ng/kg/d).

The dose response data from this study are not considered sufficiently reliable for use as a key study for derivation of a TRV for the reasons discussed in the text above. Therefore, the US EPA (2024a, b) assessment of Nian et al. (2019) is not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate guidance/guideline value derivation for PFOS or PFOA in **Section 5.3** and **Section 6.3**, respectively.

5.2.5 NTP (2022) – candidate study in US EPA (2024b)

NTP (2022) summarises toxicity studies conducted with PFOS, PFBS and PFHxS. Only the studies relevant to PFOS have been summarised, since the other chemicals are not in scope for this addendum.

NTP (2022) evaluated the effects of repeated oral (gavage) exposure of PFOS (>96% purity) for 28 days on Sprague Dawley (SD) rats. Male and female rats (10/sex per dose level) were orally (gavage) dosed at 0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg bw/day for 28 days.

There were no PFOS-associated adverse effects on survival in males, body weights in males and females, clinical pathology findings in both males and females (except for serum thyroid hormone levels), sperm counts in males, sperm motility in males, testicular / epididymal weights in males, or male serum testosterone levels.

One female dosed at 5 mg/kg bw/day died before study termination (other females survived till study termination). Plasma and liver PFOS concentrations are shown in **Table 5-1**.

Significant ($p < 0.05$), dose related increases in liver weight occurred in all PFOS treated animals (up to about 1.6-fold for absolute and up to about 1.7-fold for relative liver weight in males *cf.* control; up to about 1.5-fold for absolute weight and up to about 1.6-fold for relative liver weight in females *cf.* control). These changes were correlated with significantly ($p < 0.05$) increased incidence of hepatocyte vacuolisation (*cf.* control) at 5 mg/kg bw/day (i.e. increased liver weights are regarded as being adverse at 5 mg/kg bw/day). However, there were no biologically relevant changes in serum transaminases, ALP, direct bilirubin, total cholesterol, triglycerides, or total bile acids.

Table 5-1 PFOS Concentrations in the Plasma and Liver of Rats in the 28-day Gavage Study (adapted from Table 18 in NTP 2022)

	Vehicle control	0.312 mg/kg/d	0.625 mg/kg/d	1.25 mg/kg/d	2.5 mg/kg/d	5 mg/kg/d
Molar Dose (mmol/kg/day)	0	0.00062	0.0013	0.0025	0.005	0.01
Male						
n	10	10	10	10	10	10
Plasma concentration (ng/mL)	BD	23,730 ± 1,114	51,560 ± 3,221	94,260 ± 3,144	173,700 ± 9,036	318,200 ± 8,868
Liver concentration (ng/g)	BD	87,170 ± 3,039	160,100 ± 7,209	286,100 ± 7,882	468,200 ± 12,136	867,100 ± 26,802
Liver/plasma ratio	BD	3.76 ± 0.24	3.29 ± 0.35	3.06 ± 0.11	2.75 ± 0.13	2.74 ± 0.08
Female						
n	10	10	10	10	10	9
Plasma concentration (ng/mL)	54 ± 4	30,530 ± 918**	66,970 ± 1,629**	135,100 ± 3,877**	237,500 ± 5,218**	413,556 ± 8,071**

**Significantly different (p<0.01) from the vehicle control group by Shirley's test.
BD = Below detection; group did not have over 20% of its values above the limit of quantification

Blood reticulocyte counts were significantly ($p < 0.05$) reduced (by ≥ 23% *cf.* control) at ≥ 2.5 mg/kg bw/day. Blood segmented neutrophil counts and leukocyte counts were also significantly ($p < 0.05$) reduced *cf.* control at 5 mg/kg bw/day in males only. These changes correlated with a significantly ($p < 0.05$) increased incidence (≥ 40% in males and ≥ 50% in females *cf.* control) of bone marrow hypocellularity at ≥ 2.5 mg/kg bw/day and an increased incidence ($p < 0.05$; ≥ 70% in males and ≥ 80% in females *cf.* control) of splenic extramedullary haematopoiesis. Accordingly, these changes are regarded as being adverse.

Serum total thyroxine (T4) and free T4 were significantly ($p < 0.05$) reduced (by ≥ 62% for total T4 and free T4 in males *cf.* control; by ≥ 50% for total T4 and by ≥ 39% for free T4 in females *cf.* control) at all dose levels. Significantly ($p < 0.05$) reduced (≥ 31% in males *cf.* control and ≥ 19% *cf.* control in females) serum triiodothyronine (T3) occurred at ≥ 0.625 mg/kg bw/day. These effects were not accompanied by any significant ($p < 0.05$) change in serum thyroid stimulating hormone (TSH) concentrations or microscopic anatomic changes in the thyroid glands, nevertheless the magnitude of the changes in serum T3 and T4 levels appear relatively high.

Changes in thyroid hormone concentrations were observed across three PFAS (PFHxSK, PFBS and PFOS) in the NTP (2022) study. The magnitude of the effect was stronger in PFBS and PFOS rats compared to PFHxSK rats. The reason for a lack of a compensatory TSH response in the face of substantially low thyroid hormone concentrations in these PFAS studies is not clear and is not consistent with a classical disruption in the hypothalamic-pituitary-thyroid axis.

It has been shown that PFAS can bind to proteins including albumin and transthyretin, which are transport proteins for thyroid hormones (NTP 2022). NTP (2022) also indicated that several PFOS studies (in rats and monkeys) have shown low free T4 levels as measured by analog radioimmunoassays (RIA) (the method used in the NTP 2022 study), but no change in free T4 levels when measured by equilibrium dialysis followed by RIA (ED-RIA). NTP (2022) considered that these findings are consistent with PFOS competing with free T4 for binding to serum proteins, potentially creating a negative bias in the (competitive-binding) analog RIA method. However, while this explanation may be plausible for primate samples that contain serum/plasma thyroid hormone shepherd proteins such as thyroid binding globulin (the major physiologically important shepherd protein) it is less plausible for rats given that this species lacks thyroid binding globulin in protein and has lower levels of thyroid shepherd proteins in serum and plasma (Lewandowski et al. 2004).

It is nevertheless noted that decreases in total T4 and T3 were found in the rat and monkey studies with PFOS, as well as the NTP (2022) study. NTP (2022) commented that it is plausible that the decreases in total T4 and T3 in rats are related to activation of PPARα and CAR receptors resulting in an increase in thyroxine-UDP glucuronosyltransferase and accelerated degradation of thyroxine by the liver. It is noteworthy that PFHxSK had a lower response in CAR activity with a lower effect observed on thyroid hormones.

Some researchers have concluded that the administration of PFAS (PFDA and PFOS) does not cause a classical hypothyroid state (NTP 2022). Primary hypothyroidism is typically clinically characterised by increased TSH and decreased T4 (in the presence or absence of thyroid histopathology), whereas secondary hypothyroidism is typically the result of a pathological change to the pituitary. It is noted the 28-day NTP (2022) study found no significant changes to TSH levels or histopathological findings in the pituitary or hypothalamus in PFOS dosed rats. It could therefore be argued that the decreased T4 and T3 observed in rats administered PFOS in the NTP (2022) study may not be relevant to humans.

The strongest support for such an argument is the lack of repeatability of the effect in chronic toxicity studies with PFOS. A chronic toxicity study conducted with PFOS in the same breed of rats (i.e. Sprague-Dawley) (Butenhoff et al. 2012a) found no treatment-related effects on the pituitary, nor any thyroid hormone changes. The absence of these findings in other chronic toxicity studies conducted with PFOS provides confidence in the conclusion that the thyroid hormone changes observed in PFOS dosed rats in the 28-day NTP (2022) study are unlikely to be relevant to humans and therefore would not be considered adverse. As no such chronic studies are available for PFHxS and PFBS, the conclusion with respect to potential human relevancy of the thyroid hormone changes in the NTP (2022) study remains more uncertain than for PFOS.

Based on Markov chain analysis, females at ≥ 0.625 mg/kg bw/day had a significantly ($p < 0.05$) higher probability than the vehicle control group of transitioning to extended dioestrus. This is regarded as potentially adverse.

The study lowest observed effect level (LOEL) was 0.312 mg/kg bw/day (the lowest dose tested, corresponding to measured serum PFOS levels of 23,730 ± 1,114 ng/mL in males and 30,530 ± 918 ng/mL in females) due to significant ($p < 0.05$) reductions in serum T4 levels. As indicated above, these changes in rats are considered unlikely to be relevant to humans in light of the lack of repeatability of the finding in chronic toxicity studies with PFOS. Critically, the changes in serum T3 and T4 levels, while of substantial magnitude, were not associated with proportionate or substantial changes in serum TSH or effects on thyroid microscopic anatomy.

US EPA (2024b) selected the effect of extramedullary haematopoiesis in the spleen in male rats from the NTP (2022) study for deriving a candidate guidance value because they regarded the study as being of high confidence, the effect to be histopathologically confirmed, consistent across sexes, accompanied by evidence of bone marrow hypocellularity, and consistent with other studies.

It is noted that the experimental NOAEL for both of these effects (i.e. extramedullary haematopoiesis in the spleen of male rats and bone marrow hypocellularity) was 0.625mg/kg bw/day, corresponding to measured mean serum NOAELs of 51,560 ng/mL in males and 66,970 ng/mL in females. The human relevancy of these effects cannot be discounted based on currently available information.

US EPA (2024b) used the Wambaugh et al. (2013) model to simulate the $C_{last7,avg}$ internal dose metric (this was selected for all non-developmental studies rather than an alternative metric such as C_{max} to provide a consistent internal dose for use across chronic and subchronic studies where steady state may or may not have been reached) for the extramedullary haematopoiesis effect. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk from the control was chosen as per US EPA's *Benchmark Dose Technical Guidance* (US EPA 2012). US EPA (2024b) derived a $BMDL_{10}$ of 9.6 mg/L (i.e. 9,600 ng/mL) in males and 2.3 mg/L (i.e. 2,300 ng/mL) in females from the study for this effect for use as a POD. US EPA (2024b, Appendix) states the selected model (logistic in males, multistage degree 1 in females) showed adequate fit (p>0.1) and had the lowest Akaike Information Criterion (AIC) (for males) or was the lowest BMDL (for females). It is noted for the female data, the model with the lowest AIC (i.e. 48.7 vs. 53 in multistage degree 1 model) was the Weibull model which gave a $BMDL_{10}$ of 5 mg/L (i.e. 5,000 ng/mL), within a factor of 3 of the selected $BMDL_{10}$.

The PODs (2.3 mg/L in females, 9.59 mg/L in males) were converted by US EPA (2024b) to POD_{HED} of 0.291 µg/kg/day (females) and 1.23 µg/kg/day (males) using a clearance value of 0.128 mL/kg/day. US EPA (2024b) applied an uncertainty factor of 300 (3x for interspecies extrapolation of toxicodynamic differences, 10x for human variability, and 10x for use of a subchronic study) to the lowest POD_{HED} value (for females) to derive a guidance value of 0.00097 µg/kg/day (i.e. 1 ng/kg/day).

Whilst the uncertainty factors applied are consistent with what would typically be applied in an Australian context, there are large differences between the modelled $BMDL_{10}$ serum values (2,300 ng/mL in females, 9,600 ng/mL in males) derived by US EPA (2024b) and the experimental measured serum NOAELs for this effect (66,970 ng/mL in females, 51,560 ng/mL in males) in NTP (2022), i.e. differences were approximately 29-fold and 5-fold in females and males, respectively. Therefore use of the measured serum NOAEL from the study as a POD for the critical effects (i.e. extramedullary haematopoiesis and bone marrow hypocellularity) is considered to be associated with a lower degree of uncertainty. US EPA (2024b) did not comment on the reason for this discrepancy.

If the measured male rat serum NOAEL for extramedullary haematopoiesis combined with bone marrow hypocellularity of 51,560 ng/mL is used as a serum POD, this POD is converted to a POD_{HED} value of 6.6 µg/kg/day using the clearance value from US EPA (2024b) of 0.128 mL/kg/day, and subsequently divided by an uncertainty factor of 300 (i.e. 3x for interspecies extrapolation of toxicodynamic differences, 10x for human variability, 10x for use of a subacute study)[14], resulting in a guidance value of 0.022 µg/kg/day (i.e. 22 ng/kg/day). This is essentially the same value as the current Australian guidance value for PFOS (i.e. 20 ng/kg/day) derived by FSANZ (2017), which was based on developmental effects in a different toxicological study. The fact that use of two different sensitive endpoints from two separate experimental toxicological studies result in the same guidance value lends further support for the use of this value.

The NTP (2022) study is a high-quality study and has been conducted appropriately. US EPA (2024b) considered the study to be of high confidence. Thus, the candidate guideline value resulting from adaption of the US EPA (2024b) candidate guidance values (incorporating the use of a serum NOAEL for extramedullary haematopoiesis and bone marrow hypocellularity) is considered to be of high confidence. Therefore, the NTP (2022) study is suitable for adoption/adaption in the Australian context and the study has been included in the candidate guidance/guideline value derivation for PFOS in **Section 5.3**.

5.2.6 Sagiv et al. (2018) – candidate study in US EPA (2024a, b)

Sagiv et al. (2018) conducted a longitudinal cohort study where plasma concentrations of four PFAS (PFOA, PFOS, PFHxS and PFNA) were measured in early pregnancy (median length of gestation, 9 weeks) among 1,645 women in Project Viva, a study of a birth cohort recruited during 1999-2002 in eastern Massachusetts[15]. The authors fitted multivariable models to estimate associations of PFAS with birth weight-for-gestational age z score (foetal growth) and length of gestation, adjusting for several sociodemographic and haemodynamic confounders (i.e. maternal age at enrolment, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding prior to the index pregnancy, pre-pregnancy body mass index, gestational age at blood collection, plasma albumin concentration, plasma creatinine concentration, paternal education, household income and sex of the child). Serum PFAS lower than the LODs (<0.2 ng/mL for PFOS, <0.1 ng/mL for the others) were imputed as the LOD divided by the square root of 2.

PFAS plasma concentrations were moderately correlated with each other, with Spearman correlation coefficients as high as 0.72 for PFOS and PFOA. PFAS plasma concentrations were also moderately correlated with haemodynamic indicators, including positive correlations with plasma albumin, consistent with serum dilution due to blood volume

[14] It is noted that a lower uncertainty factor could possibly apply for use of a subacute study. For example, Guth et al. (2020) indicate an uncertainty factor of 1.5 to 5 may be appropriate for extrapolation from a subacute (28-day) or subchronic (90-day) study.

[15] Blood samples were obtained at the recruitment visit, centrifuged and plasma stored in non-PFAS-containing cryovial tubes, in liquid nitrogen freezers. In 2014, the samples were thawed and analysed for PFAS.

expansion. PFAS were also negatively correlated with eGFR, consistent with an increased flow rate during pregnancy.

The authors state that while patterns were not strictly monotonic, they observed overall decrements in foetal growth across quartiles for PFOS, PFOA and PFNA. It is noted these associations disappeared for PFOS and PFOA when adjustments for albumin and eGFR and creatinine were included. After adjustment for potential confounders (except for albumin), there was only a weak statistically significant association for the continuous interquartile range of PFNA plasma concentrations with birth weight-for-gestational age z score (ß = -0.05, 95% CI: -0.1, -0.01), but there was no clear dose response when individual quartiles were compared to the referent quartile. After adjustment for potential confounders (including albumin, but excluding eGFR and plasma creatinine), there was a similar finding only for PFNA (continuous interquartile range ß = -0.06, 95% CI: -0.11, -0.02) but again with no clear dose response.

For gestational length, after adjustment for confounders (excluding albumin), statistically significant negative associations with PFAS plasma concentrations were observed only for the PFOS highest quartile (34.9-185 ng/mL vs. 0.1-18.8 ng/mL) (ß = -0.31, 95% CI: -0.59, -0.03). After adjustment for confounders (except eGFR and creatinine), significant associations were also only observed for PFOS (ß = -0.37, 95% CI: -0.65, -0.1). OR (adjusted, excluding albumin, eGFR and creatinine) for preterm birth was statistically significantly increased for PFOS in all quartiles compared to the referent quartile (2nd quartile = OR 2.0, 95% CI: 1.1-3.7; 3rd quartile = OR 2.0, 95% CI: 1.1-3.7; 4th quartile = OR 2.4, 95% CI: 1.3-4.4). PFHxS was also associated with preterm birth but only in the third quartile (2.5-3.7 ng/mL) (OR 1.8, 95% CI: 1.1-3.1) (i.e. no clear dose response).

The authors indicate given the low PFNA plasma concentrations in Project Viva compared to other PFAS, the associations of PFNA with birth outcomes should be interpreted with caution. The authors state that a limitation of their study is the use of pregnancy haemodynamic markers measured in early-pregnancy plasma; thus, whether they adequately represent pregnancy haemodynamics is unclear. Strengths of the study included the large sample size with participants recruited before voluntary phase-out of PFOS and PFOA, the use of measured PFAS concentrations, and adjustment for key confounders.

Despite the effect not being statistically significant for PFOA or PFOS after adjustment of confounders in Sagiv et al. (2018), US EPA (2024a, b) used the study for derivation of a candidate TRVs for both PFOS and PFOA, amongst several other studies. US EPA (2024a, b) selected a BMR of 5% for this study.

US EPA (2024a, b) estimated a $BMDL_{5RD}$ of 41 ng/mL for PFOS and a $BMDL_{5RD}$ of 9.1 ng/mL for PFOA from the Sagiv et al. (2018) study using a hybrid BMD model and used the updated Verner et al. (2016) PBPK model summarised in **Section 5.2.10** to derive a POD_{HED} of 0.006 µg/kg/d for PFOS and 0.00121 µg/kg/d for PFOA. US EPA (2024a, b) then applied an uncertainty factor of 10 for human variability to the POD_{HED} to derive a PFOS TRV of 0.0006 µg/kg/d (i.e. 0.6 ng/kg/d) and a PFOA TRV of 0.000121 µg/kg/d (i.e. 0.1 ng/kg/d).

Due to the fact that the effect on birth weight in the Sagiv et al. (2018) study was not statistically significant, and for the same reasons provided for the Wikström et al. (2020) study in **Section 5.2.8**, the dose response data from this study are not considered sufficiently reliable for use as a key study for derivation of a TRV.

Therefore, the US EPA (2024a, b) assessment of Sagiv et al. (2018) is not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate guidance/guideline value derivation for PFOS or PFOA in **Section 5.3** and **Section 6.3**, respectively.

5.2.7 Timmermann et al. (2022) – candidate study in US EPA (2024a, b)

Timmermann et al. (2022), using a cross-sectional study design, examined the diphtheria and tetanus vaccination generated antibody levels in Greenlandic children aged 7 to 12 years during the years 2012 to 2015. The study population blood levels of mercury, polychlorinated biphenyls (PCB) and PFAS were determined.

The 338 children in the studied population were between 7.1 and 12.1 years old (median 9.9 years) at the time of examination and approximately half of them were girls. The majority of children were from Nuuk, Sisimiut and Ilulissat, and the median concentrations of tetanus and diphtheria antibodies were 0.92 and 0.07 IU/mL, respectively. Most (72%) of children had been breastfed at least 6 months, and only 7 children (2%) were never breastfed. Forty-two (12%) had tetanus concentrations below the protective limit and 175 (52%) had diphtheria concentrations below the limit. Among the 175 children with a known vaccination date, 5 (3%) and 72 (41%), respectively, had tetanus and diphtheria concentrations below protective levels.

All seven types of PFAS monitored for (i.e. PFOS, PFOA, PFHxS, PFHpS, PFNA, PFDA and PFUnDA)[16] were detected in more than 90% of the child serum samples. Five children had serum PFUnDA concentrations below the LOD and PCB congeners CB-138 and CB-153 were below the LOD for five children each, and PCB congener CB-180 was below the LOD for 12 children. One child had concentrations of all three PCB congeners below the LOD. The children's concentrations of the contaminants were comparable to those found among the mothers during pregnancy with the exception of PFOS that occurred in lower concentrations among the children. Child concentrations of PFOS were found to be strongly correlated with PFHxS, PFNA and PFUnDA. PFUnDA concentrations were correlated with PFNA levels. PFOS exposures in childhood were only weakly to moderately associated with maternal exposures during pregnancy.

Median measured PFOS and PFOA serum concentrations (interquartile range) in children were 8.68 (6.52-12.23) ng/mL and 2.28 (1.89-2.88) ng/mL, respectively.

Once adjusted for confounding variables [duration of being breastfed (<6 months, 6–12 months, >1 year), area of residence] and when only data from children with a known vaccination booster date were included, the OR of not being protected against diphtheria (i.e. antibody concentration <0.1 IU/mL) for each 1 ng/mL increase in serum concentrations of PFOS and PFOA were 1.14 (95% CI: 1.04-1.26) and 1.41 (0.91-2.19), respectively.

Based on adjusted data for each 1 ng/mL increase in serum concentration of PFOS, the serum anti-tetanus and anti-diphtheria antibody concentrations both decreased by 3% and 9%, respectively. For PFOA, a 1 ng/mL increase in serum concentration was associated with an 8% decrease in serum anti-tetanus antibodies and a -22% decrease in serum anti-diphtheria antibodies. However, in all cases, the confidence intervals were large. No consistent associations were seen between maternal contaminant concentrations and vaccine antibody concentrations in children. Notably the effect level related to PFOS-associated reduction in vaccination-induced humoral immunity is low and is potentially within the range of chance with this type of study design.

Timmermann et al. (2022) used a methodology (cross-sectional survey) that ranks low on the hierarchy of evidence. Accordingly, the results of this exploratory study require replication using study methods with higher reliability. The results also need to be replicated in different geographic populations and over time.

[16] PFUnDA = Perfluoroundecanoic Acid.

The study population in Timmerman et al. (2022) were also exposed to multiple chemicals, a number of which (e.g. PFHxS, PFHpS and PFDA) also had much higher (PFHxS -38/-78% per 1 ng/mL increase in serum concentration for tetanus/diphtheria, respectively; PFHpS -22/-85% per 1 ng/mL increase in serum concentration for tetanus/diphtheria, respectively; PFDA - 29/-59% per 1 ng/mL increase in serum concentration for tetanus/diphtheria, respectively) effect levels. Furthermore, the multivariate linear method of analysis used in Timmermann et al. (2022) assumes that each of the chemicals in the complex mixture to which the study population was exposed behaves discretely (i.e. there was no additive, subtractive effects, no potentiation, no synergistic etc. interactions between chemicals in the mixture). This approach also assumes that none of the chemicals in the mixture act via a common mode of action. Neither of these assumptions has been substantiated.

Timmermann et al. (2022) also assumed linearity of environmental exposures. Significant ($p < 0.05$) deviations from linearity were found for PFOS associations with tetanus antibodies in analyses including only children with a known vaccination date, and for the maternal PFOS association with diphtheria antibodies.

Exposure to multiple other chemicals (particularly to persistent organic pollutants such as the organochlorines and heavy metals; AMAP 2015, Bjerregaard et al. 2001) and substantial (and immunologically-relevant) differences in dietary nutritional quality occur across Greenlandic sub-populations (Bjerregaard and Jeppesen 2009), which were not controlled for or accounted for in the study by Timmermann et al. (2022).

Notably, the current recommendations for human vaccination for diphtheria and tetanus is based on a 5-dose schedule at 2, 4, 6 and 18 months, and 4 years of age (DHAC 2023c). An additional vaccination for tetanus is recommended at age 11 to 13 years. The Danish (Greenlandic) recommendations for diphtheria and tetanus vaccination (DHA 2022) calls for a 4-dose schedule at 3, 5, 12 months and 5 years of age. Based on 2018 to 2019 data, childhood vaccination coverage for diphtheria and tetanus varies from about 70 to 93% across Greenland and there are significant ($p < 0.05$) geographic differences in both level of coverage and number of vaccinations received (Albertsen et al. 2020). While Timmermann et al. (2022) did adjust the results of the analyses for time since last vaccination, the study did not evaluate, or control for, the number of previous vaccinations performed in the study population. Thus a potentially important confounding variable was not controlled for in the study. Furthermore, as noted in Timmermann et al. (2022):

> *"However, delays frequently occur, and children without a known booster date were assumed to have received their most recent vaccination at age 6 years (average booster age among those with a known date), unless it was known that the booster had not been administered, in which case the most recent vaccination was assumed to have been at 12 months of age."*

Timmermann et al. (2022) noted the following study limitations:

- Concentrations of the specific antibodies were fairly low in this study, especially for diphtheria, probably due to the time interval since the most recent vaccination or booster. Tetanus booster vaccinations are not routinely provided in emergency rooms in Greenland. Thus, in the oldest participants, the age-5 booster was probably given seven years prior to study participation, thereby allowing substantial decreases in antibody concentrations over time.
- The date of the most recent vaccine booster was known only for approximately half of the children in the study, and the analyses in which the study used an estimated date of vaccination yielded results that differed from those obtained for the restricted data with exact information on booster time. Using an estimated date of vaccination caused information bias, perhaps in particular due to the long and likely variable time interval since the most recent vaccination.

- Exposure to some of the environmental chemicals were strongly correlated, which makes it difficult to completely separate their effects.

US EPA (2024a, b) used the Timmermann et al. (2022) study as a candidate study for derivation of a health-based guidance value for PFOS and PFOA, amongst several other studies. The agency rationalised that immunosuppression shown by functional assessments of the immune response in experimental animal studies, such as analyses of plaque forming cell and natural killer cell responses, are concordant with decreased antibody responses seen in human populations. US EPA (2024a, b) selected a 0.5 standard deviation (SD) as the benchmark response (BMR) for this study (and other immunological endpoints used in their evaluation), rather than a fixed change in antibody concentration distributions, because i) the health outcome is regarded as developmental, and ii) there is no accepted definition of an adverse level of change or clinical cutoff for reduced antibody concentrations in response to vaccination. It is noted the US EPA (2012) Benchmark Dose technical guidance specifies that a 0.5 SD BMR is generally used for severe effects. It is arguable whether a reduction in antibody concentrations can be regarded as a severe effect; in addition, based on the experimental animal studies, thresholds for effects on immunosuppression have been found.

US EPA (2024a, b) estimated the following $BMDL_{0.5\ SD}$ for PFOS and PFOA.

- For anti-diphtheria antibodies, 5.61 ng/mL for PFOS and 1.49 ng/mL for PFOA.
- For anti-tetanus antibodies, 9.66 ng/mL for PFOS and 2.26 ng/mL for PFOA.

US EPA (2024a, b) then used the updated Verner et al. (2016) PBPK model to derive the following POD_{HED} values.

- For anti-diphtheria antibodies, 0.00103 µg/kg/d for PFOS and 0.00022 µg/kg/d for PFOA.
- For anti-tetanus antibodies, 0.00178 µg/kg/d for PFOS and 0.000334 µg/kg/d for PFOA.

After application of an uncertainty factor of 10 for human variability to the POD_{HED} values, the resulting guidance values were the following.

- For anti-diphtheria antibodies, 0.000103 µg/kg/d (i.e. 0.01 ng/kg/d) for PFOS and 0.000022 µg/kg/d (i.e. 0.002 ng/kg/d) for PFOA.
- For anti-tetanus antibodies, 0.000178 µg/kg/d (i.e. 0.02 ng/kg/d) for PFOS and 0.0000334 µg/kg/d (i.e. 0.03 ng/kg/d) for PFOA.

The agency noted the BMR of 0.5 SD may not be a reasonably good estimate of 5% extra risk and that the BMDL for PFOA was based on a non-significant regression parameter and that no multi-PFAS modelling was conducted.

The comment made in the 2024 PFAS Review with respect to measures of vaccine effectiveness still applies here, where the Australian Immunisation Handbook (DHAC 2023a) indicates that vaccine effectiveness can be assessed in a number of ways including by assessing the following.

- "*How effective the vaccine is at preventing infection.*
- *How effective the vaccine is at preventing hospitalisation for the disease.*
- *The impact of a vaccination program on disease incidence in the population*."

A reduction in antibody concentration, whilst a potential marker of immune response, does not appear to be readily correlated with an adverse response *per se.* In addition, DHAC (2023b) also state that measuring antibody levels by commercial assays is not necessarily a correlate of protection in vaccinated people.

It is also noted that the PFOS and PFOA serum concentrations at which markers of immunosuppression have been shown to be affected in experimental studies in mice (e.g. PFOS mean of ~42,000 ng/mL, with no effects at ~6,000 ng/mL in Zhong et al. 2016; PFOA mean of ~73,100 ng/mL, with no effects at 45,300 ng/mL) are difficult to reconcile with the human serum PFOS and PFOA concentrations in the Timmermann et al. (2022) study for which statistical associations were found with antibody concentrations (i.e. median of 8.68 ng/mL for PFOS; median of 2.28 ng/mL for PFOA). This indicates there is still marked uncertainty in terms of the appropriateness of using epidemiological data to define the threshold and dose response of effects potentially caused by PFOS and PFOA exposure.

Alternative explanations, as discussed above, for the observed effects were also not considered by Timmermann et al. (2022) or US EPA (2024a, b). Furthermore, the study does not meet the Bradford Hill criteria[17] for causation. Given the limitations of the Timmermann et al. (2022) study, it is not regarded as a candidate for generation of health-based guidance value in this report.

Based on the above discussion and uncertainties with respect to using the Timmermann et al. (2022) study to define a dose response, the data are not considered to be reliable for use as a key study for derivation of a health-based guidance value. Therefore, the US EPA (2024a, b) assessment of Timmermann et al. (2022) is not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate guidance/guideline value derivation for PFOS in **Section 5.3** nor for PFOA in **Section 6.3**.

5.2.8 Wikström et al. (2020) – used by US EPA (2024a, b)

Wikström et al. (2020) studied the association between early pregnancy exposure to eight PFAS (PFOS, PFOA, PFHxS, PFNA, PFDA, PFUnDA, PFHpA, PFDoDA) and birth weight in the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study, specifically focusing on differences according to the sex of the child. SELMA is a longitudinal pregnancy cohort study designed to investigate the impacts of early life exposure to environmental factors on growth, development, and chronic diseases in children. In the full cohort, blood serum samples were obtained from 2,355 pregnant women in weeks 3-27 of pregnancy at their first visit at their antenatal care centre between September 2007-March 2010. Median gestation at sampling was 10 weeks. Children born by the participating women for which outcome data, exposure data, and all statistical covariates (i.e. cotinine as marker for smoking, sex, gestational age, maternal weight, maternal age, parity, education levels, pregnancy week of serum sampling, fish intake in the family during pregnancy as a proxy of exposure to PFAS) were available constituted the study group. All twins (n=32) were excluded, leaving n=1,533 infants. All PFAS serum concentration values below the LOD were set to half the LOD for analysis. Infants with birth weight below the 10th percentile for gestational age (GA) and sex were defined as small for gestational age (SGA).

As a large number (>50%) of samples had PFDoDA levels less than the LOD, PFDoDA was excluded from further analysis. In multiple regression models adjusted for sex, GA, maternal weight, parity, and cotinine concentration, increased maternal serum concentration of five out of seven PFAS (PFOS, PFOA, PFNA, PFDA, and PFUnDA) were significantly associated with lower birth weight (BW) and with lower birth weight for sex and gestational age (BW-SDS). In the full sample including both girls and boys, a ln-unit increase in prenatal exposure to PFOS, PFOA, PFNA, and PFDA (all closely corresponding to an increase from

[17] In 1965, Sir Austin Bradford Hill published nine “viewpoints” to help determine if observed epidemiologic associations are causal. These criteria are referred to as the “Bradford Hill Criteria” and include the following: strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy.

the 25th to the 75th percentile) was associated with a decrease in BW in the range of 46–68 g or 0.10–0.15 SDS. Children in the upper quartile of prenatal exposure for PFOS, PFOA, and PFDA were 69–90 g lighter than children born in the lower quartile of prenatal exposure. Prenatal PFAS exposure for PFOS, PFOA, PFNA, and PFDA was also significantly associated with being born SGA when adjusted for potential confounders.

- For PFOS (all children), the upper quartile of PFOS exposure (maternal serum concentration interquartile range was 3.97-7.6 ng/mL) was associated with 80g (95% CI: -144; -16) lower birth weight than the first quartile, and an odds ratio (OR) of 1.56 (95% CI: 1.09; 2.22) for SGA.
- For PFOA (all children), the upper quartile of PFOA exposure (maternal serum concentration interquartile range was 1.11-2.3 ng/mL) was associated with 90g (95% CI: -159; -91) lower birth weight than the first quartile, and an odds ratio (OR) (not significant) of 1.44 (95% CI: 0.86; 2.4) for SGA.

In analyses stratified by sex, the associations between prenatal PFAS exposure and BW were significant only for girls in all cases. Nevertheless, a sex interaction analysis failed to show statistical significance (p=0.06 for PFOS and p=0.07 for PFOA).

The study authors indicate, although the reductions in BW of the size found in the study may have minor impact on an individual infant, they regard the associations observed in the study to be potentially important consequences from a public health perspective due to the potential for increased proportions of infants with low BW or born SGA. Nevertheless, they conclude that more research is warranted to clarify the role of sex and whether the effect persists throughout the entire life course.

It is noted, however, that none of the children in the study were classified as having low birth weight (<2,500g). It is therefore not clear if the association for decreased BW found in this study would also be the same for children who are already close to being classified as low BW (where the effect would become of potential concern).

US EPA (2024a, b) used the Wikström et al. (2020) study as a critical study for derivation of a TRV for both PFOS and PFOA, amongst several other studies. US EPA (2024a, b) selected a BMR of 5% for this study[18].

US EPA (2024a, b) estimated a $BMDL_{5RD}$ of 7.7 ng/mL for PFOS and a $BMDL_{5RD}$ of 2.2 ng/mL for PFOA from the Wikström et al. (2020) study using a hybrid BMD model and used the updated Verner et al. (2016) PBPK model summarised in **Section 5.2.10** to derive a POD_{HED} of 0.00113 µg/kg/d for PFOS and 0.000292 µg/kg/d for PFOA. US EPA (2024a, b) then applied an uncertainty factor of 10 for human variability to the POD_{HED} to derive a PFOS TRV of 0.000113 µg/kg/d (i.e. 0.1 ng/kg/d) and a PFOA TRV of 0.0000292 µg/kg/d (i.e. 0.03 ng/kg/d).

Strengths of the Wikström et al. (2020) study include its longitudinal prospective cohort design, which allowed blood samples to be drawn very early in gestation, which means there is less chance for potential confounding due to pregnancy-related changes in glomerular filtration rate and haemodynamics. The study also had a large sample size. However, as with all epidemiological studies it is not possible to control for all possible confounders.

An effect on birth weight is concordant with effects observed in experimental animal studies in rodent pups. However, it is difficult to reconcile the PFOS and PFOA serum

[18] US EPA (2024b) originally used the exact percentage (8.27%) of live births in the United States in 2018 that fell below the cutoff of 2,500g as the tail probability to represent the probability of extreme ('adverse') response at zero dose (P(0)). However, this percentage of 8.27% was calculated without accounting for the existence of background PFOS and PFOA exposure in the US population. Therefore they used an alternative approach where P(0) was 9.86% if there is no background exposure.

concentrations at which reductions in pup body weight gain have been observed in experimental studies (e.g. PFOS: maternal and F1 males mean, respectively, of ~18,900 or 45,400 ng/mL, with no effects at ~ 5,280 or 10,500 ng/mL in Luebker et al. 2005; PFOA: maternal mean of 40,500 ng/mL, with no effects at 21,900 ng/mL in Lau et al. 2006) with the human serum PFOS and PFOA concentrations in the Wikström et al. (2020) study for which statistical associations were found with decreased BW (i.e. PFOS median of 5.38 ng/mL, PFOA median of 1.61 ng/mL).

In addition, the interquartile range of PFOS and PFOA serum concentrations in the Wikström et al. (2020) study is very small in terms of absolute values (i.e. PFOS: 3.97-7.6, PFOA: 1.11-2.3 ng/mL). The $BMDL_{5RD}$ of 7.7 ng/mL for PFOS and 2.2 ng/mL derived by US EPA (2024a, b) are just above or just below the 75th percentile of PFOS and PFOA maternal serum concentrations, respectively, measured for the cohort in the Wikström et al. (2020) study. As the range of BWs for all children in the study were within the normal range of 3,290-3,998 gram (<2,500g is considered low BW), it is difficult to reconcile whether such low serum PFOS and PFOA concentrations relative to the serum PFOS and PFOA concentrations observed in experimental animals are to be believed as exerting a true adverse effect.

This indicates there is still marked uncertainty in terms of the appropriateness of using epidemiological data to define the threshold and dose response of effects potentially caused by PFOS and PFOA exposure.

Based on the above discussion and uncertainties with respect to using the Wikström et al. (2020) study to define a dose response, although low PFOS and PFOA doses appear to be associated with decreased BW, the data on dose response are not considered sufficiently reliable for use as a key study for derivation of a TRV.

Therefore, the US EPA (2024a, b) assessment of Wikström et al. (2020) is not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate guidance/guideline value derivation for PFOS or PFOA in **Section 5.3** and **Section 6.3**, respectively.

5.2.9 Zhang et al. (2023) – candidate study in US EPA (2024b)

Zhang et al. (2023) undertook a cross-sectional study which included 819 adolescents aged 12-19 years who had detectable rubella and measles antibody levels in serum from the US National Health and Nutrition Examination Survey 2003-2004 and 2009-2010 cycles. The aim of the study was to examine associations of serum concentrations of individual PFAS as well as the total PFAS mixture in relation to rubella, measles, and mumps antibody levels, and to further evaluate if RBC folate modifies associations. However, the authors did not include detection of mumps antibody as a criterion since the seroconversion rate of mumps for measles-mumps-rubella (MMR) vaccination is lower compared with that for measles and rubella.

PFAS (i.e. PFOA, PFOS, PFHxS and PFNA) concentrations below the limit of detection (LOD) were imputed by the LOD value divided by the square root of 2 (imputed for <2% of study participants). In the 2003-2004 cycle, RBC folate concentrations were measured using the Bio-Rad Laboratories “Quantaphase II Folate” radioassay kit, whereas in the 2009-2010 cycle, RBC folate concentrations were calculated from serum folate and whole-blood folate concentrations measured in microbiologic assay. In 2003-2004, serum IgG antibody levels to rubella, measles and mumps viruses were measured with enzyme immune-assay tests developed by the Immunoserology Unit of the California State Department of Health Services (CSDHS), Viral and Rickettsial Disease Laboratory (VRDL). In 2009-2010, the Wampole IgG enzyme-linked immunosorbent assay II test system was used. For both cycles, measles and mumps antibody optical density (OD) index ≥1.1 or rubella antibody

IU/mL ≥10 was considered as detectable of measles, mumps, and rubella antibody in serum, respectively.

The authors stratified the study population into lower (bottom two tertiles) vs. upper folate group with the highest tertile of the survey cycle-specific RBC folate levels as the cut-point (234 ng/mL for 2003-2004, 441.5 ng/mL for 2009-2010). In the associational analyses, PFAS and antibody levels were natural log-transformed to normalise the distributions, reduce the influence of outliers, and improve the interpretations of the associational results. The authors used multivariable linear regressions to examine associations between serum concentration of individual PFAS compounds and antibody levels in the total population, and lower and upper folate groups, respectively. Models were adjusted for age, sex, race, income-poverty ratio, body mass index, serum cotinine concentrations (as a marker of tobacco smoking), survey cycle, and dietary intake of milk and milk products, eggs and meat. The authors used quantile g-computation (QGC) to examine the joint effect of PFAS on natural log-transformed antibody levels.

In the sensitivity analysis, given the different antibody quantification methods in the two cycles, the authors also stratified the analyses by the two cycles to report cycle-specific associations, as well as investigating a redefinition of the lower vs. upper folate group by using the median and lowest tertile cycle-specific RBC folate concentrations as cutoffs.

The authors found inverse associations between serum PFOS (and PFHxS) and rubella antibodies (% change in antibody levels per 2.7-fold increase in PFOS: -11%, 95% CI = -18.08, -3.31%), between PFOA and mumps antibodies (-14.79%, 95% CI: -24.46, -3.89%), and between PFAS mixture and rubella (-9.84%, 95% CI: -15.57, -3.74%) and mumps (- 8.79%, 95% CI: -14.39, -2.82%) antibodies, only among adolescents with red blood cell (RBC) folate concentrations <66th percentile (lower folate group) while not among adolescents with higher RBC folate levels (upper folate group). A per quartile increase in serum concentrations of the total PFAS mixture was associated with a 9.84% (95% CI: -15.57%, -3.74%) decrease in rubella antibody and an 8.79% (95% CI: -14.39%, -2.82%) decrease in mumps antibody concentrations in the lower folate group; no associations were found for the upper folate group. No association was observed for serum concentrations of any individual PFAS compound on measles antibody levels.

The study authors indicated there are several limitations to their study. These included i) the cross-sectional design of the study, which means they could not establish causal relationships between PFAS, folate and antibody concentrations; ii) using seropositivity of rubella and measles antibodies as a proxy for MMR vaccinations since authors did not have vaccination or booster information for the study population; this may not be accurate and the excluded participants could be those who had MMR vaccines but did not produce enough antibodies or whose antibody concentrations depleted over time to below the detection limit. Thirdly, although the authors adjusted for a set of covariates, residual confounding may still be possible. The authors state that although the clinical implications by the estimated changes in rubella and mumps antibody levels associated with PFAS exposure are difficult to interpret at clinical level, additional evidence on PFAS as a risk factor for different types of infections in childhood suggest that these associations are of public health relevance.

US EPA (2024b) used the Zhang et al. (2023) study as a candidate study for derivation of a TRV, amongst several other studies. The agency rationalised that immunosuppression shown by functional assessments of the immune response in experimental animal studies, such as analyses of plaque forming cell and natural killer cell responses, are concordant with decreased antibody responses seen in human populations. US EPA (2024b) selected a 0.5 standard deviation (SD) as the BMR for this study (and other immunological endpoints used in their evaluation), rather than a fixed change in antibody concentration distributions, because i) the health outcome is regarded as developmental, and ii) there is no accepted definition of an adverse level of change or clinical cutoff for reduced antibody concentrations

in response to vaccination. It is noted the US EPA (2012) Benchmark Dose technical guidance specifies that a 0.5 SD BMR is generally used for severe effects. It is arguable whether a reduction in antibody concentrations can be regarded as a severe effect; in addition, based on the experimental animal studies, thresholds for effects on immunosuppression have been found.

US EPA (2024b) estimated a $BMDL_{0.5\ SD}$ of 24.3 ng/mL from the Zhang et al. (2023) study for PFOS and used the updated Verner et al. (2016) PBPK model summarised in **Section 5.2.1** to derive a POD_{HED} of 0.00431 µg/kg/d. US EPA (2024b) then applied an uncertainty factor of 10 for human variability to the POD_{HED} to derive a TRV of 0.000431 µg/kg/d (i.e. 0.4 ng/kg/d).

The comment made in the 2024 PFAS Review with respect to measures of vaccine effectiveness still applies here, where the Australian Immunisation Handbook (DHAC 2023a) indicates that vaccine effectiveness can be assessed in a number of ways including by assessing the following.

- "*How effective the vaccine is at preventing infection.*
- *How effective the vaccine is at preventing hospitalisation for the disease.*
- *The impact of a vaccination program on disease incidence in the population*."

A reduction in antibody concentration, whilst a potential marker of immune response, does not appear to be readily correlated with an adverse response *per se.* In addition, DHAC (2023b) also state that measuring antibody levels by commercial assays is not necessarily a correlate of protection in vaccinated people. People with low levels of vaccine-induced antibodies to rubella, for example, are often protected, whereas some people with measurable antibodies can be reinfected (DHAC 2023b).

It is also noted that the PFOS serum concentrations at which markers of immunosuppression have been shown to be affected in experimental studies in mice (e.g. mean of ~42,000 ng/mL, with no effects at ~6,000 ng/mL in Zhong et al. 2016) are difficult to reconcile with the human serum PFOS concentrations in the Zhang et al. (2023) study for which statistical associations were found with antibody concentrations (i.e. geometric mean of 12.9 ng/mL in lower folate group). In addition, the difference in geometric mean PFOS serum concentrations between the lower and upper folate group (where the former exhibited a statistically significant association with antibody levels, whereas the latter did not) is very slight in terms of absolute values (i.e. 12.9 ng/mL vs. 11.6 ng/mL, respectively). This indicates there is still marked uncertainty in terms of the appropriateness of using epidemiological data to define the threshold and dose response of effects potentially caused by PFOS exposure. It is also noteworthy that there was very little difference between the interquartile ranges of antibody concentrations in adolescents included in the Zhang et al. (2023) study:

- For rubella, the range was 31.47-64.25 IU/mL in the lower folate group and 31.10-64.52 IU/mL in the upper folate group. It is also noted that in laboratory tests a concentration of 10 IU/mL or greater is regarded as positive for rubella antibodies to indicate a current or previous exposure/immunisation to rubella (UoI 2019).
- For mumps, the OD index range was 1.75-3.78 in the lower folate group and 1.72-3.62 in the upper folate group. A laboratory specimen is considered positive for IgG mumps at an OD index ≥ 1.10 (CDC 2010).

Based on the above discussion and uncertainties with respect to using the Zhang et al. (2023) study to define a dose response, although low PFOS doses appear to be associated with immunosuppression, the data are not considered to be reliable for use as a key study for derivation of a TRV. Therefore, the US EPA (2024b) assessment of Zhang et al. (2023) is

not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate guidance/guideline value derivation for PFOS in **Section 5.3**.

5.2.10 Zhong et al. (2016) – candidate study in US EPA (2024b)

Following one week of acclimation and pairing, Zhong et al. (2016) exposed plug-positive female C57BL/6 mice to 0, 0.1, 1, or 5 mg/kg bw/day PFOS (potassium salt, purity >98%) in deionised water with 2% Tween 80 via gavage from gestation day (GD) 1 to 17. Upon delivery (GD19), pregnant dams were singly housed. Only litters of pups delivered during a 5-day window (Monday-Friday) were included in the study. Among these litters, those that contained 6-9 pups were selected for the final study and were kept with their mothers for the first 3 weeks after birth. Male and female F1 pups (n=12/sex per group) were evaluated for a number of endpoints (i.e. body mass, organ weights, various immune parameters, serum levels of testosterone and oestradiol, serum PFOS) at four and eight weeks of age.

At four weeks of age in the 1 mg PFOS/kg bw/day group:

- Plaque forming cell responses (i.e. sheep red blood cell-specific Immunoglobulin M (IgM) production by B-lymphocytes) of splenic cells were significantly decreased in males (15% decrease vs. controls) ($p \leq 0.05$) (effect seemed to recover at eight weeks of age).

At eight weeks of age in the 1 mg PFOS/kg bw/day group:

- Natural killer cell activity was significantly decreased (20.37% vs. 24.98% in controls) in males ($p \leq 0.05$).
- Serum testosterone was significantly decreased in males (62% relative to controls).

At four weeks of age in the 5 mg PFOS/kg bw/day group:

- Spleen and thymus weights in males were reduced ($p < 0.05$) relative to control pups.
- Hepatic indices were increased in males and females by, respectively, 13% and 10%.
- Splenic and thymic cellularity were significantly decreased in males by, respectively, 21% and 17% ($p \leq 0.05$).
- Splenic cellularity was significantly decreased in females by 21% ($p \leq 0.05$).
- CD4+CD8- population among all splenocytes was significantly reduced by 26% in males, and total levels of CD4-CD8+ cells in males were decreased by 20% ($p \leq 0.05$).
- Absolute number of splenic B220+ cells was significantly altered in both males and females ($p \leq 0.05$).
- Average proliferation index of T-lymphocytes was significantly lower in both males and females ($p \leq 0.05$).
- Natural killer cell activity was significantly decreased in males (36.23 vs. 42.54% in controls) ($p \leq 0.05$).
- Plaque forming cell responses (i.e. sheep red blood cell-specific IgM production by B-lymphocytes) of splenic cells were significantly decreased in males (28% decrease vs. controls) and females (24% decrease vs. controls) ($p \leq 0.05$).
- Spontaneous IL-1 formation was decreased in isolated splenocytes from males ($p \leq 0.05$).
- There was also a significant trend toward increased spontaneous IL-4 production by isolated splenocytes from male and female pups.

- Serum testosterone was significantly decreased in males (52% relative to controls).
- Serum oestradiol was significantly increased in males (142% relative to controls).

At eight weeks of age in the 5 mg PFOS/kg bw/day group:

- Thymus weight was significantly decreased in male pups ($p<0.05$).
- Thymic cellularity was significantly decreased in males relative to controls ($p\leq0.05$).
- CD4+CD8- population among all splenocytes was still significantly reduced by 23% in males ($p\leq0.05$).
- Absolute number of splenic B220+ cells was significantly altered in females ($p\leq0.05$), but there did not appear to be a clear dose response for this effect.
- Natural killer cell activity was significantly decreased in males (18.45% vs. 24.98% in controls) and females (15.57 vs. 21.33% in controls) ($p\leq0.05$).
- Significantly increased spontaneous IL-4 production by isolated splenocytes from males.
- Although serum testosterone was decreased (~34% relative to controls) in males, this was not statistically significant.
- Serum oestradiol was not increased in males or females.

There was a dose-related increase in serum PFOS levels in all PFOS-exposed F1 pups. Serum concentrations in the 0, 0.1, 1, and 5 mg/kg/day groups were, respectively, 0.05 ± 0.01, 6.38 ± 0.35, 47.03 ± 3.23, and 118.40 ± 6.27 mg/L in males; 0.04 ± 0.01, 5.16 ± 0.27, 41.81 ± 3.62, and 107.53 ± 4.51 mg/L in females.

Study authors found a significant interaction between sex and PFOS concentration for serum testosterone alteration at both four and eight weeks of age (p-value for interaction = 0.0049 at four weeks and 0.0227 at eight weeks). A positive effect of modification by sex was found for oestradiol alteration at four weeks of age ($p=0.0351$), but not at eight weeks. For other parameters (e.g. natural killer cell function, plaque forming cell levels, spontaneous production of IFNγ, IL-4 and IL-2), the effect modification was not significant at either time point.

The authors indicate their results should be interpreted with care. Although they found sex-specific difference in some functional parameters (i.e. natural killer cell activity and plaque forming cell response) in pups, no significant interaction between sex and PFOS exposure was found. The authors also point out that since sex-specific impacts of PFOS on TH1/TH2 cytokine balance in pups were not confirmed through the collection of additional data, such as phenotypes, antibody isotopes or function tests, and the study design was just a pilot study, future studies will need to be undertaken to verify the conclusions.

US EPA (2024b) selected the Zhong et al. (2016) study for deriving a candidate TRV because the effect on plaque forming cell response to sheep red blood cells was reported by multiple studies and represents effects in the low-dose range for immune effects reported in experimental animal toxicological studies. The population used (i.e. mouse pups) was also considered by US EPA (2024b) to represent a sensitive population and coherent with the epidemiological information on immune parameters in humans. US EPA (2024b) considered the study to be of medium confidence.

The experimental NOAEL dose for the decrease in plaque forming cell response in male pups at four weeks of age from the data provided in the study is 0.1 mg PFOS/kg bw/day, i.e. 6.38 ± 0.35 mg/L (i.e. 6,380 ng/mL) as a serum NOAEL. US EPA (2024b) used the

Wambaugh et al. (2013) model to simulate daily exposure through oral gavage from GD1-GD17 using female CD1 mice parameters (C57BL/6 mice parameters are not available for PFOS). The $C_{avg,pup,gest\_lact}$ internal dose metric was selected for this model by US EPA (2024b) since an average concentration metric is expected to better correlate with the effect that may have resulted from exposure during gestation or lactation. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to one standard deviation from the control mean was chosen as per US EPA's *Benchmark Dose Technical Guidance*[19]. US EPA (2024b) derived a $BMDL_{1\ SD}$ of 1.8 mg/L (i.e. 1,800 ng/mL)[20] from the study for this effect for use as a POD. US EPA (2024b) states the selected model (Hill) showed adequate fit (p>0.1) and presented the most protective BMDL associated with the effect. $BMDL_{1SD}$ from using other models were 6,600 ng/mL (using exponential model), 34,400 ng/mL (using another exponential model) or 38,900 ng/mL (using other models) (US EPA 2024b, Appendix).

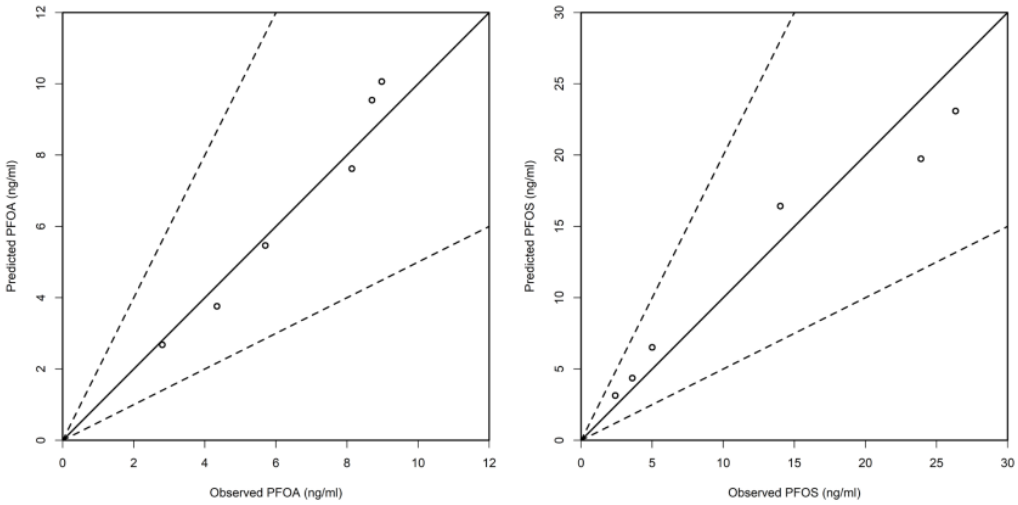
The POD was converted by US EPA (2024b) to a POD_{HED} of 0.288 µg/kg/day using the updated one-compartment human developmental model by Verner (Verner et al. 2016).[21]

In the model, half-life and volume of distribution (V_d) are used to calculate clearance, which is used in the model directly and is also used for calculation of steady-state concentrations in adults. The parameters used in the model were 230 mL/kg for V_d (from Thompson et al. 2010a), 3.4 year half-life (from Li et al. 2018), 0.128 mL/kg/d clearance (calculated from half-life and volume of distribution), a 0.4 cord serum:maternal serum ratio, and a 0.016 milk:serum partition coefficient. US EPA (2024b) states that the use of the Verner et al. (2016) model in humans presents a substantial advancement in approach for endpoints in children compared with the previous US EPA (2016a) assessment for PFOS. The previous assessment did not explicitly model children, but instead applied an uncertainty factor to an RfD based on long-term adult exposure to account for potential for increased susceptibility in children. The approach used by US EPA (2024b) explicitly models PFOS exposure to infants during nursing who are undergoing rapid development, including growth, through childhood, and who do not reach steady state until near adulthood. US EPA (2024b, Appendix) present data validating the use of the model; the data shows that the predicted human serum concentrations line up well when compared to measured human serum concentrations (**Figure 5-2**).

[19] US EPA (2012) Benchmark Dose technical guidance specifies that the preferred approach if there is a minimal level of change in an endpoint that is generally considered to be biologically significant, then the amount of change can be used to define the Benchmark Response (BMR). However, "*in the absence of any other idea of what level of response to consider adverse, a change in the mean equal to one control SD* [standard deviation] *(or lower, e.g., 0.5 SD, for more severe effects) from the control mean should be used."*

[20] The $BMDL_{1\ SD}$ is the lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean response equal to one standard deviation from the control mean.

[21] The model was run starting at birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth at 25 years. The initial concentration in the child is governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the 1-year breastfeeding period. The average serum concentration in the infant through gestation and lactation is determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. A male infant was used for this calculation to match the sex of the animals.



Dashed lines represent a two-fold difference between observed and predicted concentration

Figure 5-2 Comparison of predicted and observed child serum PFOA and PFOS concentrations using the updated Verner et al. (2016) model (reproduced from Appendix F in US EPA 2024b)

Reverse dosimetry for the animal POD used the ratio of standard exposure and internal dose as was applied to PODs from epidemiological data. When a concentration internal dose metric in the pup during lactation and/or gestation was selected, the POD_{HED} is the dose to the mother that results in the same average concentration in the foetus/infant over that period.

The pharmacokinetic modelling code for the model is available freely online, and the model code underwent quality assurance through the established EPA Quality Assurance Project Plan for Physiologically Based Pharmacokinetic (PBPK) models.

Although SLR has not specifically evaluated the model, the documentation, descriptions and validation results provided by US EPA (2024b) confers confidence in PBPK model predictions.

US EPA (2024b) applied an uncertainty factor of 30 (3x for interspecies extrapolation of toxicodynamic differences and 10x for human variability) to the POD_{HED} of 0.288 µg/kg/day to derive a TRV of 0.0096 µg/kg/day (i.e. rounded to 10 ng/kg/day). The uncertainty factors applied are consistent with what FSANZ (2017) applied to other experimental animal studies when deriving the current Australian TRV for PFOS.

It is noted that the range of potential POD_{HED}, when considering the range of serum $BMDL_{1\ SD}$ derived by US EPA (2024b) in benchmark dose modelling for this study, would be 0.288 to 6.2 µg/kg/day (the experimental serum NOAEL was 3.5x-fold higher than the modelled $BMDL_{1\ SD}$; use of this value would result in a POD_{HED} of 1 µg/kg/day). Thus, if any of the other BMD models had been selected for use, the resulting TRV range (using the same uncertainty factor of 30) could be 10 to 210 ng/kg/day (rounded); if the experimental NOAEL from the study was used the resulting TRV would be 34 ng/kg/day (see also **Section 5.3**). It is considered important to highlight and understand the factors that can influence the relative precision of the final TRV value.

The Zhong et al. (2016) study appears to have been conducted appropriately, albeit it was of a pilot study nature; it evaluated a large number of immune system markers, as well as hormone levels and clinical parameters. There was a clear dose response for parameters of the immune system to be affected in male mice. Thus, the candidate guideline value

resulting from adaption of the US EPA (2024b) candidate guidance value is considered to be of medium confidence (see **Section 5.3**). This aligns with the US EPA (2024) findings of medium confidence for this study. Nevertheless, the span of POD_{HED} values derived by US EPA (2024b) may result in very different TRVs depending on which POD is chosen. It is therefore considered appropriate to use the experimental measured serum NOAEL as the POD for adaption of the US EPA (2024b) values for the Australian context.

5.3 Candidate guidance/guideline values for PFOS

As indicated in preceding sections, a number of additional studies (summarised in **Sections 5.2.1** to **5.2.10**) that had not been previously explicitly considered / evaluated in the FSANZ (2017) review of PFAS and the 2024 PFAS Review (for PFOS) were used by US EPA (2024b) as critical or candidate studies for derivation of PFOS guidance values. Of those studies, only the two experimental animal studies (NTP 2022 and Zhong et al. 2016) were considered potentially suitable for adoption/adaption for candidate DWG derivation in the Australian context.

The critical endpoints chosen by US EPA (2024b) from the studies are increased incidence of extramedullary haematopoiesis (NTP 2022) or decreased plaque forming cell responses to sheep red blood cells (Zhong et al. 2016) (see **Table 5-2**). SLR considered the use of the serum NOAEL from the NTP (2022) study to be a less uncertain serum POD than the modelled serum $BMDL_{10}$ derived by US EPA (2024b), due to the large discrepancies between the measured and modelled values, i.e. approximately a 29-fold difference in females and a 5-fold difference in males. The candidate guidance values resulting from adaption of both effects have been presented in **Table 5-2**.

The same toxicokinetic adjustment factor for converting an animal serum concentration to a human dose were used by US EPA (2024b) for both studies. The uncertainty factors used by US EPA (2024b) differed between the two studies in that an additional uncertainty factor of 10 was applied to the POD from the NTP (2022) study to account for the subacute timeframe of exposure (see **Table 6-4**).

With respect to the relative source contribution (RSC) factor, the current factor employed in derivation of the DWGs for PFOS, PFHxS and PFOA in the Guidelines is 0.1 (i.e. 10%) which is also the default factor for the Australian context. It is noted US EPA typically uses an RSC of 0.2 (i.e. 20%) when deriving DWGs but do not provide the rationale for this value with respect to PFAS. It is also noted the final DWG recommended by US EPA (2024c, d) is based on practical considerations rather than a health-based value. Thus, the default factor of 0.1 has been retained in calculating the potential resulting DWGs for PFOS using the guidance values in **Table 5-2**, noting that it yields a lower guideline value than use of an RSC of 0.2.

Also presented in **Table 5-2** is the derivation of the current Australian DWG for PFOS of 70 ng/L. The underpinning study on which the existing Australian PFOS guideline value is based (Luebker et al. 2005) is considered to have high confidence based on study design.

Table 5-2 Potential drinking water guideline values (ng/L) resulting from adaption of PFOS guidance values [1]

Parameter		NHMRC and NRMMC 2011, FSANZ 2017, DOH 2017	NTP 2022 – candidate study in US EPA 2024b	Zhong et al. 2016 – candidate study in US EPA 2024b
Critical study		Luebker et al. 2005b	NTP 2022	Zhong et al. 2016
Study population		Rats	Rats	Mice
Form of PFOA studied		Potassium PFOS	PFOS (>96% pure)	PFOS (potassium salt, purity >98%)
Exposure route		Oral (gavage)	Oral (gavage)	Oral (gavage)
Study timeframe		Two-generation study (male and female rats dosed for 6 weeks prior mating, throughout mating, and, for females, through gestation and lactation, across two generations).	28 days	GD1-17
Critical Effect		Decreased body weight gain and food consumption in F0 generation (parental toxicity); significant decreased pup weight and weight gain during lactation (offspring toxicity).	Extramedullary haematopoiesis and bone marrow hypocellularity	15% decreased plaque forming cell responses (i.e. sheep red blood cell-specific IgM production by B-lymphocytes) of splenic cells in 4-week-old male pups (effect seemed to recover at eight weeks of age).
Serum Point of Departure (mg/L)		- (dose POD = 0.1 mg/kg/d)	Serum NOAEL = 51.56 in males ($BMDL_{10}$ = 2.3 in females) [4]	Serum NOAEL = 6.38 ($BMDL_{1\ SD}$ = 1.8)
Clearance Factor (L/kg-day)		0.0051 (*back-calculated from POD HED*)	0.000128	0.000128
Point of Departure HED (mg/kg/day)		0.00051	0.0066 (0.00029) [4]	0.00082 (0.00023)
Uncertainty factors	UF_A	3	3	3
	UF_H	10	10	10
	$UF_{subchronic}$	1	10	1
	$UF_{database}$	1	1	1

Parameter		NHMRC and NRMMC 2011, FSANZ 2017, DOH 2017	NTP 2022 – candidate study in US EPA 2024b	Zhong et al. 2016 – candidate study in US EPA 2024b
	$UF_{composite}$	30	300	30
Health-based guidance value (ng/kg/day)		20 (rounded up from 17)	22 (1) [4]	27 (7.7) [2]
Relative source contribution (RSC) to drinking water		0.1	0.1	0.1
Resulting adaption to a Health-based DWG [3] (ng/L)		70	77 (3.4) [4]	95 (27) [2]
Confidence in candidate guideline value		High [6]	High [5]	Medium [7]

DWG = Drinking Water Guideline; BMDL = Lower Benchmark Dose; HED = Human Equivalent Dose; GD =Gestation Day. UF_A = Uncertainty factor for extrapolation from animals to humans; UF_H = Uncertainty factor for human variability; UF_{LOAEL} = Uncertainty factor for use of a LOAEL rather than a NOAEL; $UF_{subchronic}$ = Uncertainty factor for extrapolation from a subchronic to a chronic study; $UF_{composite}$ = Composite (i.e. total) uncertainty factor; $UF_{database}$ = Uncertainty factor to account for the limited database of toxicological studies.

(1) As discussed in **Section 5.2** for PFOS, there are various reasons why the epidemiological information for associations of PFAS serum concentrations with various endpoints is not considered suitable in the Australian context for derivation of guidance values for PFAS. For this reason, the epidemiological studies have not been included in this table.

(2) As discussed in **Section 5.2.1**, due to the relatively wide range of potential $BMDL_{1\ SD}$ values derived by US EPA (2024b) using different BMD models, it is considered appropriate to use the experimental measured serum NOAEL as the POD for adaption of the US EPA (2024b) values for the Australian context. The value that would result from using the $BMDL_{1\ SD}$ value from US EPA (2024b) is considered to be of lower confidence and is provided in brackets.

(3) Adaption of guidance value has been undertaken using the default assumptions for derivation of DWGs in Australia using the following equation as outlined in NHMRC (2021):

DWG (ng/L) = [Guidance value (ng/kg bw/day) x 70kg (adult) x 0.1 for adult] ÷ 2 L/day for adult

(4) As discussed in **Section 5.2.5**, the most sensitive effect from the NTP (2022) study is considered to be extramedullary haematopoiesis and bone marrow hypocellularity, as used by US EPA (2024b). Nevertheless, there are large discrepancies between the US EPA (2024b) estimated $BMDL_{10}$ (2.3 mg/L in female rats, 9.6 mg/L in male rats) and the lowest experimental serum NOAEL achieved in the study (66.97 mg/L in female rats, 51.56 mg/L in male rats), i.e. a 29-fold difference in females, and a 5-fold difference in males. Therefore, use of the measured serum NOAEL from the study as a POD for the critical effects is associated with a lower degree of uncertainty. Thus, higher confidence is placed in the health-based guidance value derived using the experimental NOAEL. The value that would result from using the $BMDL_{10}$ value from US EPA (2024b) is provided in brackets.

(5) The NTP (2022) study is a high-quality study and has been conducted appropriately. Thus, the candidate guideline values resulting from adaption of the US EPA (2024b) candidate guidance value (incorporating the use of a serum NOAEL instead of a $BMDL_{10}$ for extramedullary haematopoiesis and bone marrow hypocellularity) is considered to be of high confidence. Less confidence is placed in the candidate guideline value derived using the US EPA modelled $BMDL_{10}$ value.

Parameter	NHMRC and NRMMC 2011, FSANZ 2017, DOH 2017	NTP 2022 – candidate study in US EPA 2024b	Zhong et al. 2016 – candidate study in US EPA 2024b
(6) The Luebker et al. (2005) study appears to have been conducted appropriately, was designed to examine a sensitive effect (i.e. multigeneration study testing relatively large numbers of dose groups and low dose ranges), reported effects as relative to litter, reported serum PFOS concentrations in adults and pups, and examined a large number of endpoints at multiple time points in multiple dose groups. Thus, the confidence in the resulting guideline value is considered to be high. (7) The Zhong et al. (2016) study appears to have been conducted appropriately, albeit it was of a pilot study nature; it evaluated a large number of immune system markers, as well as hormone levels and clinical parameters. There was a clear dose response for parameters of the immune system to be affected in male mice. The candidate guideline value resulting from adaption of the US EPA (2024b) candidate guidance value is considered to be of medium confidence.			

The candidate PFOS DWGs derived by adapting existing US EPA (2024b) guidance values for PFOS range from 3.4 to 95 ng/L depending on the study and endpoint selected, with the existing DWG at 70 ng/L.

However, when excluding the values derived from using US EPA (2024b) BMD modelled serum PODs from the candidate DWGs (see **Table 5-2**), the range is 70 to 95 ng/L. The candidate value of 77 ng/L is derived from a study with high confidence, as is the existing drinking water guideline of 70 ng/L, whereas the value of 95 ng/L is derived from a study with medium confidence.

As discussed previously, the candidate guideline values of 3.4 ng/L (from a high confidence study) and 27 ng/L (from a medium confidence study) are based on the same critical endpoints as the candidate guideline values of 77 ng/L and 95 ng/L, respectively, but the former were derived using serum points of departure modelled by the US EPA whereas the latter using serum points of departure measured in the experimental studies. The difference between modelled and measured values could not be readily reconciled, therefore the use of the measured values from the studies are considered to be associated with less uncertainty.

Concentrations of PFOS in most distributed drinking water in Australia can range up to 6 ng/L in Queensland and Sydney (2024 PFAS Review) but up to 16 ng/L in Australia according to WHO (2022). These concentrations are below the existing Australian DWG and below the candidate guideline value considered to be of highest confidence (77 ng/L) of the four candidate guideline values derived in this report. Due to the uncertainty factors and small RSC incorporated into the derivation of the candidate DWGs and the existing Australian DWG, PFOS is unlikely to present a human health risk from distributed drinking water in most regions of Australia. However, there are many sites of PFAS contamination in Australia, and, if water from these contaminated sites is used as a local source of drinking water (e.g. backyard bore in rural location where distributed water is not available), PFOS may be present at concentrations greater than the candidate DWGs and existing Australian DWG in these cases.

6.0 Discussion for PFOA

This section provides a discussion of the strengths and limitations of the studies used by the reviews subject of this report as candidate or critical studies for derivation of PFOA guidance values for possible adoption/adaption into the Guidelines. Critical evaluation was undertaken for those studies not previously considered / evaluated by FSANZ (2017) or by the 2024 PFAS Review.

6.1 Potential suitability of health-based guidance values for possible adoption/adaption

Candidate guidance values for PFOA from US EPA (2024a) and Burgoon et al. (2023) in scope for this expanded evaluation for possible adoption/adaption in Australia have been evaluated using the Assessment Tool provided in **Appendix B**. This tool evaluates each document against administrative and technical criteria that demonstrate transparent and robust guideline development and evidence review processes that meet NHMRC standards for guidelines. The overall potential suitability of the guidance values for adoption/adaption can be gauged at least partially by examining the percentage of 'must-have', 'should-have', and 'may-have' criteria met by each jurisdiction.

The US EPA (2024a) review for PFOA met a high proportion of 'must-have' (i.e. 95%), 'should-have' (i.e. 90%) and 'may-have' (i.e. 100%) criteria. The Burgoon et al. (2023) paper, as it is a peer-reviewed published article which used information from other international jurisdictions, as to be expected, does not contain as much detail as the US EPA (2024a) review but nevertheless met a high proportion of 'must-have' (i.e. 80%), 'should-have' (i.e. 75%) and 'may-have' (i.e. 50%) criteria.

6.2 Critical evaluation of PFOA candidate studies used by US EPA (2024a) or Burgoon et al. (2023) to derive guidance values not previously considered by FSANZ (2017) or 2024 PFAS Review

The following studies used by US EPA (2024a) or Burgoon et al. (2023) as critical or candidate studies to derive potential guidance values have not been previously considered / cited in the comprehensive review undertaken by FSANZ (2017), the FSANZ (2021) immunological update, or the 2024 PFAS Review[22]. The discussion in this section therefore focuses on these relevant studies.

- Two (2) epidemiological studies investigating birth outcomes: Sagiv et al. (2018), Wikström et al. (2020).
- Three (3) epidemiological studies investigating cholesterol and liver effect biomarkers: Darrow et al. (2016), Dong et al. (2019), Nian et al. (2019).
- One (1) epidemiological study investigating antibody levels: Timmermann et al. (2022).
- Two (2) epidemiological studies investigating patterns of cancer incidence: Shearer et al. (2021), Vieira et al. (2013).

[22] It is noted that in review comments received on a draft version of this Addendum report from FSANZ, FSANZ indicated they did previously consider the DeWitt et al. (2008) and Butenhoff et al. (2012a) studies and did not consider them useful for derivation of a guidance value for PFOA because the studies did not include serum PFOA levels that could be used for calculating a human equivalent external dose.

- Six (6) experimental animal studies: two prenatal developmental toxicity studies in mice (Abbott et al. 2007, Song et al. 2018), two 2-year chronic/carcinogenicity studies in rats (Butenhoff et al. 2012a, NTP 2023) one of which (NTP 2023) included the full developmental life stages, and two 15-day drinking water studies investigating immune system effect markers in mice (Dewitt et al. 2008, 2016).

Due to there being differing candidate guideline values for PFOA, their overall confidence was assigned as being 'High', 'Moderate', 'Low', or 'Very low' based on expert judgement; this was based on an assessment of underpinning critical study quality, with rationale for the rating provided in the critical evaluation discussions of the respective underpinning study (see **Sections 6.2.1** to **6.2.14**). This was done to provide the Committee with more information to enable comparison of the different candidate guideline value options against the current Australian guideline value to facilitate an informed decision of whether revision of the existing Australian guideline value is warranted or not.

6.2.1 Abbott et al. (2007) – used by Burgoon et al. (2023)

The authors of the Abbot et al. (2007) study conducted a prenatal developmental toxicity study in 129S1/SvlmJ wild-type (WT) and PPARα-knockout (KO) mice. Mice (minimum of 4 pregnant dams per dose level; range 4 to 23 per dose level) were orally (gavage) dosed with PFOA (ammonium salt; >98% pure) at 0, 0.1, 0.3, 0.6, 1, 3, 5, 10, or 20 mg/kg body weight (BW)/day over gestational days (GD) 1 to 17. There were no PFOA-associated adverse effects on maternal body weight in surviving dams, embryonic implantation, and total number, or weight of pups at birth.

Significantly ($p < 0.05$) increased percent litter loss (71/70% at 0.6/1 mg/kg bw/d *cf.* 43% in the controls) occurred in WT mice following dosing at 0.6 and 1 mg/kg bw/day. Complete litter loss in WT mice occurred following dosing at ≥ 5 mg/kg bw/day. In WT mice dosed at ≥ 5 mg/kg bw/day, this correlated with a significantly ($p < 0.05$) increased percentage of dams with full litter resorption (≥ 80% *cf.* 5% in the controls). Full litter resorption occurred in all WT dams dosed at 20 mg/kg bw/day.

KO mice were less sensitive to the effects of PFOA on litter resorptions *cf.* WT mice. Significantly ($p < 0.05$) increased percent litter loss (≥ 85% *cf.* 42% in the controls) only occurred following dosing at ≥ 5 mg/kg bw/day. Complete litter loss in KO mice only occurred at 20 mg/kg bw/day. The percentage of KO dams with full litter resorption was significantly ($p < 0.05$) increased (≥ 75% *cf.* 17% in the controls) following dosing at ≥ 5 mg/kg bw/day. However, no dose response was apparent over the 5 to 20 mg/kg bw/day range and 100% full litter resorption did not occur at any tested dose in KO mice. Full litter resorption in both WT and KO mice occurred early in gestation.

Postnatal survival up to postnatal day (PND) 22 was significantly ($p < 0.05$) reduced (≤ 43% *cf.* about 79% in the controls) in WT pups derived from dams dosed at ≥ 0.6 mg/kg bw/day. KO pups derived from dams dosed at up to 3 mg/kg bw/day had ≥ 87% survival to PND 22.

To evaluate whether maternal strain affected the survival, litters of WT, heterozygous (HET) and KO pups were produced using WT dosed at 0 or 1 mg/kg bw/day and KO dams dosed at 0 or 3 mg/kg bw/day of PFOA during gestation. Survival was evaluated up to PND15 (**Figure 6-1**). Postnatal survival was significantly ($p < 0.05$) reduced in both WT and HET pups derived from WT dams dosed at 1 mg/kg bw/day. Notably, survival in HET pups derived from WT dams dosed at 1 mg/kg bw/day was lower (by a factor of about 2-fold) than that of WT pups derived from PFOA dosed WT dams. All HET pups derived from KO dams dosed at 3 mg/kg bw/day died by PND 7. Postnatal survival of KO pups derived from KO dams was not significantly ($p < 0.05$) affected by maternal dosing at 3 mg/kg bw/day. Overall, these data demonstrate that expression of PPARα is required for induction of postnatal lethality by PFOA and that the loss of one functional copy of the gene (i.e. HET

genotype) results in significantly (p <0.05) increased postnatal pup deaths irrespective of the parental genotype.

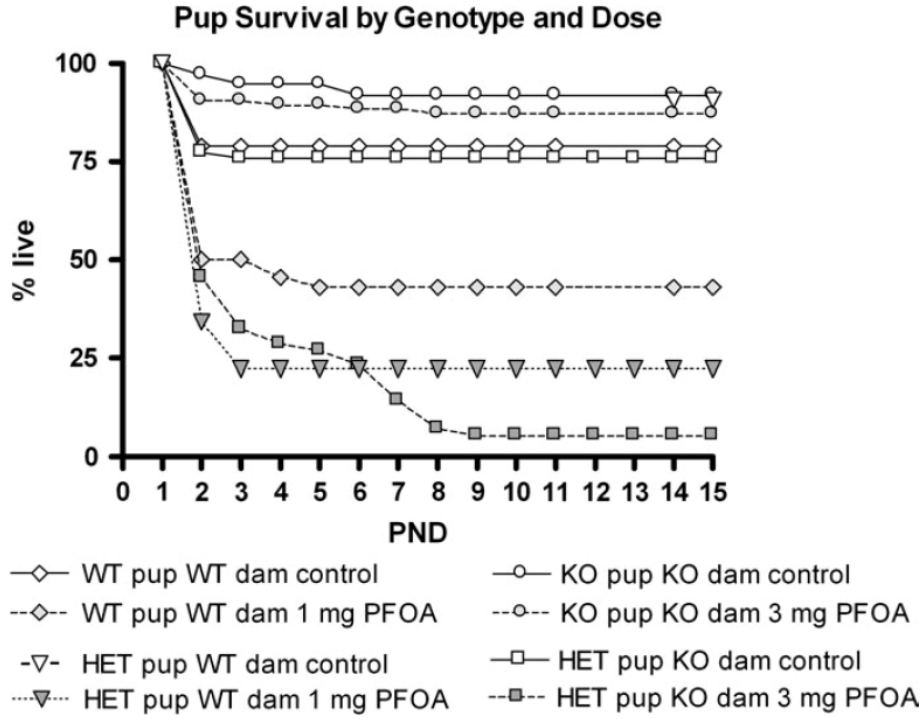


Figure 6-1 The postnatal survival of heterozygous (HET) pups born to either wild type (WT) or knock out (KO) dams

Mean day of bilateral eye opening was significantly (p < 0.05) delayed (14.6 *cf.* 13.8 in the controls) in WT pups derived from dams dosed at 1 mg/kg bw/day. Bilateral eye opening was not significantly (p > 0.05) delayed in KO pups derived from dams dosed at 3 mg/kg bw/day. Significantly (p < 0.05) reduced (> 10% *cf.* control) PND 1 to PND 22 body weight gain and body weight at PND 22 occurred in WT pups of dams dosed at 1 mg/kg bw/day. These effects did not occur in KO pups dosed at up to 3 mg/kg bw/day.

In WT adult females, relative liver weight was significantly (p < 0.05) increased in a dose related manner at ≥ 1 mg/kg bw/day. In KO adult females, relative liver weight was significantly (p < 0.05) increased in a dose related manner at ≥ 3 mg/kg bw/day. While microscopic anatomic pathology of the liver was not performed, there was no evidence of hepatic dysfunction in either WT or KO adults or pups. However, a > 2-fold increase in relative liver weight occurred at 20 mg/kg bw/day in WT adult females and at 10 mg/kg bw/day in adult KO females, implying that increases in liver weight are not solely due to peroxisomal interaction. This magnitude of change is regarded by Allen et al. (2004) and Corton et al. (2020) as adverse, i.e. a > 2-fold increase in relative liver weight. In WT pups, relative liver weight was significantly (p < 0.05), but not adversely (≤ 20%), increased at all dose levels. However, in KO pups, significantly (p < 0.05) increased (non-adverse, ≤ 20%) relative liver weight only occurred at 3 mg/kg bw/day.

There were no significant (p > 0.05) effects of strain or PPARα expression on serum PFOA levels. Likewise, serum PFOA levels in dams and pups were comparable (p > 0.05). Serum concentrations reached a plateau at ≥ 5 mg/kg bw/day in WT mice and at ≥ 3 mg/kg bw/day in KO mice. Lactation resulted in a decrease in serum PFOA concentration. The serum levels of PFOA in non-lactating WT mice was 2.8 to 3.7 times higher *cf.* that in lactating WT

mice (measured at weaning). In non-lactating KO mice, serum PFOA levels were 3–5.4 times higher than in lactating KO mice (measured at weaning).

The developmental NOAEL for WT mice was 0.3 mg/kg bw/day (mean serum PFOA was 10,400 ± 781 ng/mL in adult female mice with no pups at wean, 2,840 ± 387 ng/mL in adult females with pups at wean, and 2,150 ± 324 ng/mL for pups at weaning) due to significantly ($p < 0.05$) increased litter loss and significantly ($p < 0.05$) reduced postnatal survival at the next highest dose of 0.6 mg/kg bw/day (mean serum PFOA was 5,170 ± 913 ng/mL in adult females with pups at wean, and 3,810 ± 562 ng/mL for pups at weaning). Because of altricial development between mice and humans, pup survival provides concordance with the timing of the effect of decreased infant birth weight in humans and is thus regarded as an important human health risk assessment endpoint.

The maternal NOAEL for WT mice has not been determined. The reason for this is that US EPA (2024a) claims that increased WT maternal mortality occurred at ≥ 5 mg/kg bw/day in this study. However, maternal mortality was not clearly reported in the paper. Accordingly, it is difficult to establish whether or not effects on maternal survival occurred.

The developmental NOAEL for KO mice was 3 mg/kg bw/day (mean serum PFOA was 18,400 ± 1,430 ng/mL in adult females with pups at wean, and 10,600 ± 1,010 ng/mL for pups at weaning) due to significantly ($p < 0.05$) increased litter loss at the next highest dose of 5 mg/kg bw/day (mean serum PFOA of dams with no pups at wean was 81,800 ± 3,430 ng/mL, whereas there was no data for pups at weaning due to significant mortality).

The maternal NOAEL for KO mice was 5 mg/kg bw/day (mean serum PFOA of dams with no pups at wean was 81,800 ± 3,430 ng/mL) based on a >2-fold increase in relative liver mass at the next highset dose of 10 mg/kg bw/day (mean serum PFOA of dams with no pups at wean was 78,500 ± 4,340 ng/mL). Given that there was no evidence of hepatic dysfunction at the LOAEL, and the mean serum PFOA concentrations at the NOAEL and LOAEL were similar, the maternal NOAEL is regarded as being conservative.

The reliability of the study for human health risk assessment purposes is considered to be low due to the high background rate of litter loss in the controls, the high level of litter loss at doses greater than 1 mg/kg bw/day, the lack of clear reporting on maternal mortality, the variable statistical power across the different dose groups, the limited descriptions of the study design and the lack of historical control data for the strain of mouse used.

Nevertheless, the study demonstrates that PFOA-induced prenatal and postnatal mortality in mice requires the presence of PPARα. The study also supports a possible role for PPARα in relation to the effects of PFOA on developmental delay manifesting as delayed bilateral eye opening, reduced weight gain and reduced body weight at PND 22.

Critically, the effects of PFOA on offspring survival in mice have been replicated in other studies (e.g. Song et al. 2019, White et al. 2011, Yahia et al. 2010, Wolf et al. 2007 and Lau et al. 2006). The effect of PFOA on delayed eye opening in mice has been replicated in two other studies (Wolf et al. 2007, Lau et al. 2006).

PPARα-mediated non-genotoxic carcinogenic effects in rodents are quantitatively not relevant to humans (Corton et al. 2014, Corton et al. 2018, Foreman et al. 2021, Lai 2004). However, no human relevance mode of action evaluations or adverse outcomes evaluations on PPARα-mediated effects on pre- and post-natal survival, growth and development in rodents have been performed.

An adverse outcomes network (AON) has been proposed for PFAS-associated neonatal mortality and foetal growth restriction/lower birth weight in rodents (**Figure 6-2**; Rogers et al. 2023). The molecular initiating events (MIEs) for adverse outcomes pathway (AOP) 1 are receptor mediated events via PPAR and constitutive androstane receptors (CAR)/pregnane-X receptors (PXR). As noted above, PPARα-mediated carcinogenic effects in rodents are

quantitatively not relevant to humans. However, the possible human relevance of effects in rodents mediated via PPARγ have not yet been completely excluded. Notably, PFOA binds to and activates mouse PPARγ *in vitro* (Yamamoto et al. 2015). CAR-mediated carcinogenic effects in rodents are not relevant to humans (Elcombe et al. 2014, Yamada et al. 2021).

In addition to activation of CAR/PXR and PPAR receptors as molecular initiating events (MIEs), AOP 2 proposes effects on transthyretin resulting in increased T_4 clearance. Critically the binding potency of PFOA for transthyretin is about 16-fold lower than that of T_4 (Weiss et al. 2009). The median human plasma levels of PFOA in the general population 15 years ago was 12 nM, about 79-fold lower than the transthyretin binding median inhibitory concentration (IC_{50}) for PFOA (Fromme et al. 2009, Weiss et al. 2009). Accordingly, putative effects of PFOA acting via transthyretin are likely of limited practical human relevance at current levels of exposure (especially considering serum PFOA levels have also been decreasing in the general population over time, e.g. Toms et al. 2014, 2019). However, the possible human relevance of AOP 2 cannot be absolutely excluded because of the possible role of PFOA's action on PPARγ and because of the limited understanding of the human relevance (or lack of human relevance) of this MIE in humans.

AOP 3 proposes impaired pulmonary surfactant production as a MIE. PFOA inhibits lung surfactant function in human bronchial epithelial cells *in vitro* (Sørli et al. 2020) This MIE is most likely directly relevant to humans.

Based on the evaluation by Rogers et al. (2023), the strengths of the key event relationships in the proposed AON (showing the three AOPs in this network in **Figure 6-2** range from weak to strong.

Overall, the human relevance of the AON proposed by Rogers et al. (2023) (and thus the effects of PFOA on neonatal mortality, foetal growth restriction and post-natal developmental delay demonstrated in Abbott et al. 2007) cannot be absolutely excluded.

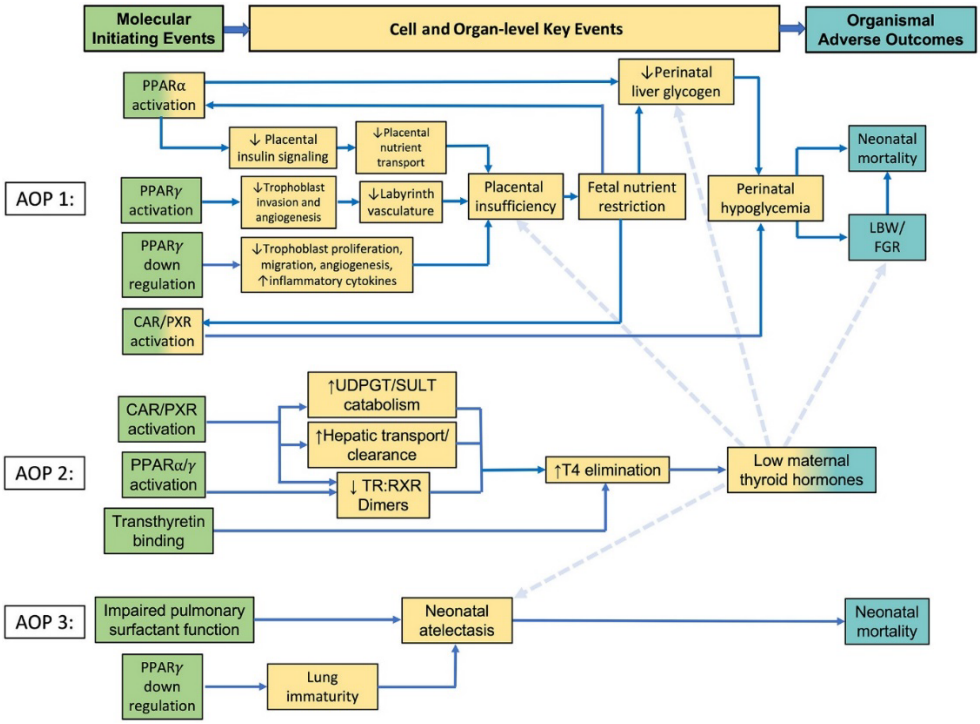


Figure 6-2 The AON for the putative AOPs for foetal growth restriction (FGR)/lower birth weight (LBW), and neonatal mortality in rodents induced by PFAS (Rogers et al. 2023)

Burgoon et al. (2023) derived a candidate health-based TRV of 0.03 µg/kg bw/day based on the developmental NOAEL for WT mice of 0.3 mg/kg bw/day (300 µg/kg bw/day). This NOAEL equated to a measured serum PFOA concentration of 10,400 ng/mL in adult females with no pups at wean. The uncertainty factors applied by Burgoon et al. (2023) were:

- Mouse to human toxicokinetic factor = 1 (factor is not needed since BMD is based on serum concentration).
- Mouse to human toxicodynamic factor = 2.5 [IPCS (2005) default or 3 being US EPA (2014) default].
- Human toxicodynamic factor = 3 [default of IPCS (2005) and EPA (2014)].
- Human toxicokinetic factor = 8.4 [0.79 mL/day/kg arithmetic mean clearance of average group from Zhang et al. (2013, Table 2) ÷ 0.094 mL/day/kg arithmetic 95% lower bound clearance of sensitive group from Zhang et al. (2013, Table 2)].
- Database uncertainty factor = 1.
- Resulting serum concentration = 0.14 µg/mL or 140 ng/mL [10.4 µg/mL ÷ (1 × 3 x 3 × 8.4 x 1) = 0.14].
- TRV = 0.03 µg/kg bw/day or 30 ng/kg bw/day [0.14 µg/mL x 0.23 mL/day/kg [geometric mean clearance from Zhang et al. (2013, Table 2) assuming steady state].

The use of a combined human toxicodynamic and toxicokinetic uncertainty factor of (3 x 8.4=) 25.2 is not in line with what Australian jurisdictions have used in the past to express human variability (i.e. a default factor of 10). Nevertheless, Burgoon et al. (2023) have used a data-driven toxicokinetic uncertainty factor which is considered to be appropriate and in

line with guidance from IPCS (2005) on development of chemical-specific uncertainty factors.

Abbott et al. (2007) was not evaluated by FSANZ (2017). US EPA (2024a) evaluated Abbott et al. (2007). This evaluation concurs with the evaluation in this report except that US EPA (2024a) stated that exposure at ≥ 5 mg/kg bw/day increased WT maternal mortality. Critically, the presence or absence of increased maternal mortality in WT mice is not clearly reported in Abbott et al. (2007), thus contributing to its low reliability for human health risk assessment purposes. Amongst the published studies evaluating the effect of PFOA on mouse pup survival and delayed eye opening, Abbott et al. (2007) was also classified as having low reliability by US EPA (2024a). US EPA based this classification on the high level of litter loss at doses greater than 1 mg/kg bw/day and because other studies (e.g. Song et al. 2018 and Lau et al. 2006) presented data for a larger number of treatment groups spanning broader or lower dose ranges (US EPA 2024a).

The candidate guideline value resulting from adaption of the Burgoon et al. (2023) candidate guidance value is considered to be of low confidence for the reasons cited above (see **Section 6.3**).

6.2.2 Butenhoff et al. (2012a) – candidate study in US EPA (2024a)

The authors of Butenhoff et al. (2012a) conducted a non-GLP, combined chronic toxicity and carcinogenicity study in SD rats. Ammonium perfluoroctanoate (ammonium salt of PFOA, 97.2% purity) was fed in the diet at 0 (*n* = 65/sex), 30 (*n* = 50/sex) or 300 (*n* = 65/sex) ppm (i.e. mg PFOA/kg feed) for 2 years. An interim sacrifice at 1 year involved 15 male and 15 female rats from both the control and high dose groups. The remaining rats in the control and high dose cohorts remained on study for a second year.

Dietary concentrations and homogeneity were analytically confirmed. Based on food consumption, the average test material consumption was 0, 1.3 and 14.2 mg/kg bw/day in males and 0, 1.6 and 16.1 mg/kg bw/day in females. Serum PFOA concentrations were not reported in the paper, but US EPA (2024a, Appendix) reports serum concentrations as 0, 43,263.7 and 167,102.5 mg/L/day (i.e. 0; 43,263,700 and 167,102,500 ng/mL respectively) at 0, 1.3 and 14.2 mg/kg bw/day respectively. It is unclear from US EPA (2024a) whether the serum concentrations reported are measured or modelled data.

There were no consistent treatment-associated adverse effects on survival, observed clinical signs, haematology findings, urinalysis and urine chemistry findings, ophthalmoscopy findings and relative organ weights. Significant ($p < 0.05$) increases in serum ALT levels occurred in males fed at 300 ppm at all measurement timepoints, and for the first 18 months of the study, in males fed at 30 ppm. These increases peaked at the 12-month measurement time point where serum ALT levels in males fed 300 ppm were > 3-fold higher than those in the control group. In males fed 30 ppm, serum ALT levels were about 2.3-fold higher than those in the control group. Increased serum ALT in males at 12 months was accompanied by smaller (up to about 1.7-fold increase compared with controls) but significant increases ($p < 0.05$) in serum AST at ≥ 30 ppm. After 24 months of feeding, significantly ($p < 0.05$) increased (by about 1.5-fold *cf.* control) serum AST only occurred in the high dose male cohort. These changes did not occur in females and were not accompanied by any biologically meaningful changes in serum total bilirubin, serum albumin or serum total protein. Adverse microscopic anatomic pathology correlates in males fed at 300 ppm included significant ($p < 0.05$) evidence of hepatotoxicity manifesting as a 7-fold increased incidence of cystoid degeneration and an approximately 1.3-fold increase in portal mononuclear infiltrates. The incidence of adaptive hepatocellular hypertrophy in the high dose male cohort was 80%. There was no microscopic anatomic pathological evidence of hepatotoxicity in females. A significantly ($p < 0.05$) increased incidence (16% *cf.* 0% in the controls) of adaptive hepatocellular hypertrophy occurred in the high dose female cohort.

A significant ($p < 0.05$) increase (14% *cf.* 0% in concurrent controls) in the incidence of Leydig cell adenomas occurred in the high dose males. The incidence of these tumours (14%) in the high dose male cohort is consistent with the upper range of the historical control incidence (13.7%; WHO 2015). Leydig cell activity and focal hyperplasia in rats are under hormonal control involving the hypothalamus, which releases gonadotrophin releasing hormone, which acts on the adenohypophysis, which in turn releases luteinising hormone (LH), thereby stimulating Leydig cells to produce testosterone. Testosterone, either directly or after conversion to dihydroxytestosterone, or to oestradiol, inhibits LH release. Disruption of this feedback loop is a fundamental key event in several possible modes of action for Leydig cell neoplasia in rats. Of the seven modes of action that have been formulated, many are dependent on LH release (androgen receptor antagonism, 5α-reductase inhibition, testosterone biosynthesis inhibition, aromatase inhibition). Continuous stimulation of increased LH levels is an important key event for Leydig cell tumour induction in rats. PFOA at concentrations ≥ 0.1 µM (i.e. 41 µg/L) inhibited total androgen biosynthesis in rat immature Leydig cells *in vitro* (Zhang et al. 2024). This effect was also observed *ex vivo* following oral (gavage) dosing of 35-day old rats at ≥ 5 mg/kg bw/day for 7 or 14 days (Zhang et al. 2024). This implies an inhibition of testosterone biosynthesis mode of action for the possible PFOA-associated Leydig cell neoplasia in SD rats. Critically, the current weight of evidence indicates that human Leydig cells are quantitatively less sensitive than their rat equivalent in their proliferative response to LH, and hence in their sensitivity to chemically induced Leydig cell tumours (Cook et al. 2008). Given this, the NOAEL for induction of Leydig cell tumours in rodent bioassays provides an adequate margin of safety for protection of human health and the data support a non-linear (i.e. threshold) mode of action (Cook et al. 2008).

The NOAEL for non-neoplastic effects was 30 ppm (equal to 1.3 mg/kg bw/day) in males due to serum chemical and microscopic anatomic pathological evidence of hepatotoxicity at the next highest dietary concentration of 300 ppm (equal to 14.2 mg/kg bw/day in males). The NOAEL for neoplastic effects was the same, i.e. 30 ppm (equal to 1.3 mg/kg bw/day) in males due to an increased incidence of Leydig cell tumours relative to the concurrent control (noting this was equivalent to the upper limit of the historical control incidence) at the next highest dietary concentration of 300 ppm (equal to 14.2 mg/kg bw per day in males). No serum PFOA concentrations were provided in the Butenhoff et al. (2012a) paper; however, according to US EPA (2024a, Appendix), the administered dose group of 1.3 mg/kg bw/day in males had an internal serum PFOA concentration of 43,264,000 ng/mL.

The NOAEL for neoplastic effects is regarded as conservative given that the incidence of Leydig cell tumours was equivalent to the upper limit of the historical control incidence of these neoplasias in SD rats and there is (limited and incomplete) evidence of the inhibition of testosterone biosynthesis leading to increased LH mode of action to which humans are quantitatively less sensitive *cf.* rodents (note US EPA 2024a did not evaluate Zhang et al. 2024). Evaluation of other potential modes of action for PFOA-induced Leydig cell tumours was performed by US EPA (2024a). This evaluation concluded that multiple PFOA-relevant modes of action may result in Leydig cell tumours in rats. This conclusion supports the US EPA (2024a) designation of PFOA as *Likely to Be Carcinogenic to Humans*, as the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor Carcinogenic to Humans. Given that PFOA is not genotoxic, a dose threshold for neoplastic effects is likely to occur. As noted by Cook et al. (2008), the NOAEL for induction of Leydig cell tumours in rodent bioassays provides an adequate margin of safety for protection of human health and the data support a non-linear (threshold) mode of action in these situations. Furthermore, a possible increased incidence of Leydig cell tumours in animals was only observed in one sex in one species (US EPA 2024a).

Despite this, US EPA (2024a), based on their policy considerations and existing US EPA (2005) guidance, derived a cancer slope factor (CSF) of 8.42 $(mg/kg\ bw/day)^{-1}$ for Leydig cell

neoplasia based on low (BMDL) dose extrapolation using the 1st degree multistage model. The US EPA (2024a) CSF was rated by US EPA as having a medium level of reliability.

However, US EPA (2024a) notes that:

> *"Overall, the evidence suggests that PFOA does not induce mutations or operate through a genotoxic mechanism, with the majority of the study data demonstrating a lack of genotoxic effect of PFOA in both in vitro and in vivo assays. A notable exception is aneuploidy and DNA fragmentation of sperm significantly associated with PFOA exposure in humans."*

US EPA (2024a) concerns regarding DNA effects in sperm derive from Governini et al. (2015) where the occurrence of aneuploidy and diploidy in sperm cells, which are normally haploid, was significantly higher in the PFAS-positive samples (PFOA was detected in 75% of the samples) when compared with PFAS-negative samples. Additionally, fragmented chromatin levels were also significantly increased for the PFAS-positive group (average seminal plasma concentration of 7.68 ng/g f.w.) compared with the PFAS-negative group. US EPA (2024a) concluded that this suggests that PFAS (and PFOA exposure in 75% of the samples) is related to errors in cell division leading to aneugenicity. However, sperm DNA fragmentation in humans is not a reliable measure of germ cell genotoxicity since this endpoint is primarily induced by defective maturation and abortive apoptosis occurring within the testis, or by oxidative stress throughout the male reproductive tract (Muratori et al. 2019). During spermatogenesis, chromatin is compacted through histone exchange with transitional proteins and protamines (Agarwal et al. 2020). This is facilitated by the endogenous nuclease topoisomerase II, creating DNA breaks to reduce torsional stress for histone disassembly and chromatin packaging (Agarwal et al. 2020). If these breaks are not repaired, impairment of chromatin packaging may result in defective maturation and the appearance of sperm with increased sperm DNA fragmentation in the ejaculate (Agarwal et al. 2020). Sperm DNA fragmentation can also be induced by abortive apoptosis during spermatogenesis (Agarwal et al. 2020). Apoptosis ensures that no defective germ cells differentiate into spermatozoa, however failure of this process may result in the accumulation of spermatozoa expressing apoptotic markers in the ejaculated semen (Agarwal et al. 2020). Sperm DNA fragmentation can also be induced by oxidative stress (Agarwal et al. 2020). Based on this sperm DNA fragmentation in humans can be caused by multiple modes of action, most of which do not involve direct, chemically induced, DNA damage. Accordingly, sperm DNA fragmentation is not a reliable measure of direct germ cell genotoxicity in humans.

US EPA (2024a) also noted the presence of DNA strand breakage (based on the alkaline comet assay) in human peripheral blood cells at an average blood PFOA concentration of 2.55 ng/mL (Franken et al. 2017). The study subjects (n=600 14–15-year-old children living near areas of industrial activity) in this cross-sectional study (which ranks low on the hierarchy of evidence) had multiple potential chemical exposures including to a range of IARC category 1 carcinogens [including polycyclic aromatic hydrocarbons (PAHs), cadmium, dioxin-like polychlorinated biphenyls (PCBs), arsenic and aromatic amines]. Statistically significant ($p < 0.05$) associations between positive peripheral blood comet assay results in the study population were found for cadmium, chromium, PCBs, PAHs, aromatic amines, nickel, mercury, hexachlorobenzene and di(2-ethyl)hexyl phthalates. Critically, the blood PFOA *was not* correlated ($p= 0.229$) with positive findings for genotoxicity. As noted by US EPA (2024a), increasing serum PFOA levels were associated with higher DNA damage measured by the alkaline comet assay (9.0% [95% CI: 1.5, 17.0%]). However, seven associations, and all with urinary 8-hydroxyguanosine (8-OHdG), remained significant after accounting for multiple comparisons: urinary cadmium (padj < 0.001), urinary chromium (padj < 0.001), blood PCB 156 (padj=0.036), urinary 1-hydroxypyrene (padj < 0.001), urinary t,t-muconic acid (padj=0.006), urinary nickel (padj < 0.001) and hair MeHg (padj=0.041).

Overall, Franken et al. (2017) does not prove a positive cause and effect relationship between PFOA exposure and DNA strand breaks in peripheral blood cells due to:

- The lack of a statistically significant association.
- The presence of multiple chemical exposures of which cadmium, chromium, PCBs, PAHs, aromatic amines and nickel all had statistically significant ($p < 0.05$) associations with DNA damage i.e. other plausible explanations for the observed effects were present in the study.
- The inherent weaknesses in the study design which ranks low on the hierarchy of evidence.

Notably US EPA (2024a) states that the peripheral blood micronucleus assay in mice exposed to PFOA performed in NTP (2023) was positive for genotoxicity. However, NTP (2023) states the following:

> *"Although a positive response was indicated for male rats exposed to PFOA, the response was within the laboratory's historical control range, and therefore the biologic significance of the increase is questionable."*

OECD test guideline 474 (OECD 2016) states the following:

> *"Providing that all acceptability criteria are fulfilled, a test chemical is considered clearly positive if:*
>
> *a) At least one of the treatment groups exhibits a statistically significant increase in the frequency of micronucleated immature erythrocytes compared with the concurrent negative control,*
>
> *b) This increase is dose-related at least at one sampling time when evaluated with an appropriate trend test, and*
>
> *c) Any of these results are outside the distribution of the historical negative control data (e.g. Poisson-based 95% control limits)."*

Based on these criteria, the results of the *in vivo* micronucleus assay in mice in NTP (2023) are negative since the response did not exceed the historical negative control data. Given this, all four *in vivo* micronucleus assays[23] in US EPA (2024a) are negative for genotoxicity. Furthermore *in vivo* (oral gavage) exposure of mice to PFOA at up to 5 mg/kg bw/day for five weeks did not induce DNA strand breaks in either liver or testis (Crebelli et al. 2019).

Given the weaknesses of Governini et al. (2015) and Franken et al. (2017), the misinterpretation of the findings in NTP (2023) and the finding that PFOA is not genotoxic in most regulatory quality genotoxicity studies, the *overwhelming weight of evidence is that PFOA is not genotoxic*. Accordingly, the derivation of health-based guidance values for Leydig cell neoplasia using a non-threshold approach is not justifiable.

Butenhoff et al. (2012a) was not evaluated by Burgoon et al. (2023). US EPA (2024a) estimated a $BMDL_{4RD}$ of 27,089,000 ng/mL from the Butenhoff et al. (2012a) study and used the updated Verner et al. (2016) PBPK model summarised in **Section 5.2.1** to derive a POD_{HED} of 4.75 µg/kg/d. However, due to science policy considerations, US EPA (2024a) did not use the POD_{HED} to derive a TRV, opting for a non-threshold linear model CSF instead. If the threshold-based approach were used instead, as the data suggest is

[23] US EPA (2024a) presents data for *in vivo* micronucleus assays in Table 3-21 of their report. The four micronucleus studies, as cited by US EPA (2024a) in this table, were Crebelli et al. (2019) (negative), Butenhoff et al. (2014) (negative), NTP (2019, updated in 2020) (positive), Murli (1995) (negative). However, as discussed in the text, the result from NTP (2023) is actually considered negative since the response did not exceed historical control data.

appropriate, and a standard uncertainty factor of 30 were applied to the POD estimated by US EPA (2024a), this would result in a TRV of 0.158 µg/kg/day, i.e. 158 ng/kg bw/day. This is considered to be conservative based on the previous discussions in this section. The Butenhoff et al. (2012a) study has been used as a candidate study for possible guidance/guideline for PFOA in **Section 6.3**. Overall, the resulting adapted guideline value is considered to be of medium confidence, as the underpinning study was well-conducted but lacked serum PFOA measurements reported in the study (it is noted US EPA 2024a provided serum data for the study; it is unclear whether this is modelled or measured data).

6.2.3 Darrow et al. (2016) – candidate study in US EPA (2024a)

The authors of Darrow et al. (2016) conducted a population-based survey of PFOA and serum liver biomarkers and liver disease incidence. The C8 Health Project cross-sectional survey was conducted in 2005 and 2006 and included people who were exposed for at least 12 months (at home, work, or school) to water in any of six districts contaminated (to various degrees) by PFOA. The analysis of liver biomarkers was conducted among 30,723 people from the C8 Health Project (including 1,892 people who worked at the chemical plant) with available liver injury biomarker measurements and retrospective serum PFOA estimates. The analysis of liver disease incidence included participants in the C8 Health Project as well as additional workers who were recruited from a previously established occupational cohort (Leonard et al. 2008) of 6,026 people who worked at a chemical plant producing PFOA from 1948–2002; there were 32,254 people from these two cohorts who had completed at least one follow-up survey (administered between 2008–2010 and 2010–2011) and had retrospective serum PFOA estimates (3,713 from the occupational cohort and 28,541 community members who had not worked at the plant). The follow-up surveys covered demographics, residential history, health-related behaviours, and lifetime personal history of various medical diagnoses.

The key results of the study are:

- 11% of the study population was classified as having above normal ALT levels, 14% had above normal GGT, and 1% had above normal direct bilirubin. The incidence of above normal ALT levels in the study is essentially the same as the background incidence (approximately 10%) for this finding in the US population (Oh et al. 2017).
- A significant (p-trend < 0.05) association was found between cumulative serum PFOA (sum of all previous yearly estimated PFOA serum concentrations expressed as year x ng/mL) and 2005/2006 serum PFOA concentrations with serum ALT. There were no associations with serum GGT level. Direct bilirubin was statistically significantly associated with cumulative serum PFOA, but only in the second and third quintile (i.e. there was no clear dose response). The ORs for above normal serum ALT (for cumulative serum PFOA) were 1.14 (95% CI: 1.01-1.29), 1.20 (95% CI: 1.06-1.35) and 1.16 (95% CI: 1.02-1.33) in the third, fourth and fifth quintile, respectively. The association for 2005/2006 serum PFOA with ALT was only significant for the fourth quintile (OR 1.16, 95% CI: 1.03-1.31).
- Moving from the first to the fifth quintile of cumulative PFOA exposure was associated with an estimated 6% increase in ALT level. This corresponds to an increase of 1.6 IU/L for an individual starting at the average ALT level of 26 IU/L, or an increase of 3.3 IU/L for an individual starting at 55 IU/L (the 95th percentile of ALT of the data). The magnitude of these changes, particularly in the absence of any correlate of hepatocellular injury, would not be classified as an abnormal increase in serum ALT.
- The hazard ratios for cumulative PFOA serum concentrations and liver disease (with and without a 10-year lag) ranged from 0.75 (95% CI: 0.54, 1.03) to 1.19 (95% CI:

0.88-1.59). Overall, there was no significant association between serum PFOA levels and liver disease.

Darrow et al. (2016) used a cross-sectional study design that ranks low on the hierarchy of evidence. The study authors have also regarded serum ALT as being an exclusive biomarker of hepatocellular injury. Critically, isoenzyme analysis was not performed. In humans two forms of ALT have been identified, ALT1 and ALT2, encoded by separate genes (Lindblom et al. 2007). In normal human tissue, high expression of ALT1 was found in liver, skeletal muscle, and kidney and low levels in heart muscle and not detectable in pancreas. High ALT2 activity was detected in heart and skeletal muscle. Darrow et al. (2016) did not exclude other sources and causes for the small increases in serum ALT that were observed in the study. Furthermore, Darrow et al. (2016) did not evaluate other biomarkers or correlates of hepatocellular membrane injury (e.g. serum AST). Small increases in serum ALT are not specific indicators of hepatocellular disease, particularly as the proportion of the study population classified as having above ALT levels (11%) is approximately the same as the background incidence (approximately 10%) for this finding in the US population (Oh et al. 2017).

Notably, mildly increased ALT expression can occur in coeliac disease, autoimmune disorders, skeletal muscle injury, renal disease and induction of hepatic metabolic enzymes (Ennulat et al. 2010, Gianini et al., 2005). None of these potentially confounding variables were controlled for or evaluated in Darrow et al. (2016).

The findings in this study regarding serum ALT do not support a cause- and effect-relationship between serum PFOA concentration and adverse hepatocellular injury. Overall, the study is not considered to be a candidate for derivation of health-based guidance values and does not meet the Bradford Hill criteria.

US EPA (2024a) evaluated Darrow et al. (2016) on the assumption that increases in serum ALT associated with PFOA exposure are associated with hepatotoxicity and are of sufficient magnitude to be adverse. This is not supported by the findings in Darrow et al. (2016) as discussed above.

US EPA (2024a) used the endpoint of increased ALT in the Darrow et al. (2016) study for derivation of a candidate TRV for PFOA, along with several other studies. US EPA (2024a) selected a BMR of 5% for this study.

US EPA (2024a) estimated a $BMDL_{5RD}$ of 66 ng/mL for PFOA (for female adults) from the Darrow et al. (2016) study using a hybrid BMD model and used the updated Verner et al. (2016) PBPK model summarised in **Section 5.2.10** to derive a POD_{HED} of 0.00792 µg/kg/d for PFOA. US EPA (2024a) then applied an uncertainty factor of 10 for human variability to the POD_{HED} to derive a PFOA TRV of 0.0008 µg/kg/d (i.e. 0.8 ng/kg/d).

The non-adversity of the findings and the dose response data from this study are not considered sufficiently reliable for use as a key study for derivation of a TRV. Therefore, the US EPA (2024a) assessment of Darrow et al. (2016) is not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate guidance/guideline value derivation for PFOA in **Section 6.3**.

6.2.4 Dewitt et al. (2008) – candidate study in US EPA (2024a)

Dewitt et al. (2008) evaluated the effects of oral exposure to ammonium-PFOA (≥98% pure) on humoral and cell-mediated immune responses in C57BL/6J mice. The experimental design is shown in **Figure 6-3**.

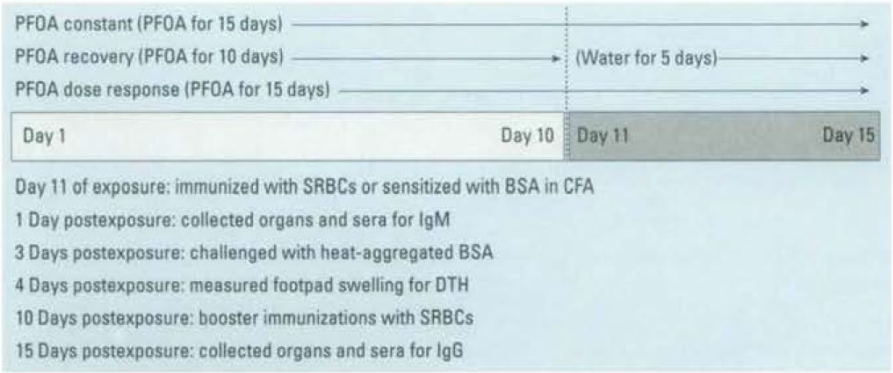


Figure 6-3 Design of dose response and recovery studies in Dewitt et al. (2008)

Continuous exposure and recovery study

In the continuous exposure study arm, female C57/BL6 mice were orally (gavage) dosed with PFOA at 0 or 30 mg/kg bw/day for 15 days. In the recovery arm of the study, mice were orally (gavage) dosed with PFOA at 30 mg/kg bw/day for 10 days and then dosed with water on study days 11 to 15. Sixteen mice per dose were used to measure the IgM and IgG (and lymphoid organ weight) responses to sheep red blood cells (SRBC) and 8 animals per dose were used to measure the delayed hypersensitivity response to bovine serum albumin in Freund's complete adjuvant (BSA-CFU). Cage control animals were included in each endpoint group to ensure that experimental procedures did not alter experimental results and, with the exception of gavage exposure, were treated identically to all other mice within end point groups.

Mean (± standard error) serum PFOA levels measured at one day following the final dose were 25.2 ± 2.0, 32.8 ± 19.5, 84,700 ± 9,814 and 266,500 ± 23,018 ng/mL for the cage control, vehicle control, recovery and constantly exposed animals, respectively. Mean (± standard error) serum PFOA levels measured at 15 days following the final dose were 614.9 ± 66.6, 24.7 ± 2.0, 47,757 ± 2,115 and 67,988 ± 3,823 ng/mL for the cage control, vehicle control, recovery and constantly exposed animals, respectively.

Between study day 8 to 11 mean body weight in PFOA treated animals was reduced by about 8% *cf.* controls. By the end of dosing the mean body weight of the continuously dosed animals was significantly ($p < 0.05$) reduced by about 10.5% *cf.* controls. However, the body weights of PFOA exposed mice in the recovery study arm were not significantly ($p > 0.05$) different *cf.* controls by two days following the cessation of PFOA dosing. By 15 days following the cessation of PFOA dosing, there was no significant difference ($p > 0.05$) in body weight in any of the experimental groups.

Relative liver weights in PFOA treated animals in both the continuous and recovery study arms were significantly ($p < 0.05$) increased (64% *cf.* control) at one day following the cessation of PFOA exposure. Recovery did not occur by 15 days following the end of PFOA dosing. These changes are not regarded as adverse.

One day following the cessation of PFOA dosing, absolute and relative spleen weights and absolute and relative thymus weights were significantly ($p < 0.05$) reduced *cf.* controls in both the continuous exposure and recovery study arms. Full recovery occurred by 15 days following the end of PFOA dosing.

Serum IgM responses to SBRC were significantly ($p < 0.05$) reduced (by about 20%) *cf.* controls in both the continuous exposure and recovery study arms. Exposure to PFOA did not significantly ($p > 0.05$) affect the IgG response to SRBC or delayed type hypersensitivity responses.

Dose response study 1

Female C57/BL6 mice (8 per PFOA concentration per endpoint) were exposed to PFOA in drinking water at concentrations of 0, 25, 50, 100 or 200 mg/L (equivalent to 0, 3.75, 7.5, 15 and 30 mg/kg bw/day) for 15 days.

Mean (± standard error) serum PFOA concentrations at one day following the cessation of dosing were 54.3 ± 4.9, 74,913 ± 2,667, 87,150 ± 3,296, 128,125 ± 6,181 and 162,625 ± 8,434 ng/mL for the 0, 25, 50, 100 and 200 mg/L water PFOA concentration groups, respectively. By 15 days following the cessation of dosing, mean (± standard error) serum PFOA concentrations were 156.4 ± 14.9, 35,325 ± 1,607, 42,771 ± 1,708, 50,025 ± 1,486 and 52,713 ± 3,212 ng/mL for the 0, 25, 50, 100 and 200 mg/L water PFOA concentration groups, respectively.

After 15 days of dosing, mean body weight was significantly ($p < 0.05$) and adversely (reduction *cf.* control of about 15%) reduced following drinking water exposure at 200 mg/L. However, body weight fully recovered in the high dose cohort by 8 days following the cessation of exposure.

Relative liver weights were significantly ($p < 0.05$) increased (by 51 to 71% *cf.* control) in all PFOA exposed mice. Full recovery did not occur by 15 days following the cessation of exposure. These changes are not regarded as being adverse.

At the end of the study, mean spleen weights following exposure at ≥ 100 mg/mL were significantly ($p < 0.05$) reduced (32%/44% at 100/200 mg/L). Thymus weights were significantly ($p < 0.05$) reduced (31%/52% at 100/200 mg/L) at the end of the study. Full recovery occurred by 15 days following the cessation of exposure.

All PFOA exposure levels were associated with significantly ($p < 0.05$) reduced (11 to 29% *cf.* control) serum IgM responses to SRBC. PFOA exposure did not significantly ($p > 0.05$) reduce serum IgG responses to SRBC or delayed type hypersensitivity responses to BSA-CFU.

The LOAEL for dose response study 1 was 25 mg/L (equivalent to 3.75 mg/kg bw/day, the lowest dose tested) due to the reduction of serum IgM responses to SRBC at the next highest concentration of 50 mg/L (equivalent to 7.5 mg/kg bw/day). The measured serum concentration at this LOAEL (one day following cessation of dosing) was 74,913 ± 2,667 ng/mL.

Dose response study 2

Female C57/BL6 mice (8 per PFOA concentration per endpoint) were exposed to PFOA in drinking water at concentrations of 0, 6.25, 12.5, 25 and 50 mg/L (equivalent to 0, 0.94, 1.88, 3.75 and 7.5 mg/kg bw/day) for 15 days.

PFOA exposure at up to 50 mg/L had no significant effect on body weight.

Relative liver weight was significantly ($p < 0.05$) increased (by 35 to 60% *cf.* control) at all PFOA exposure levels. Full recovery did not occur by 15 days following the cessation of exposure. This effect is not regarded as being adverse.

At the end of the study, mean spleen weights were significantly ($p < 0.05$) reduced (16%/18% at 25/50 mg/L) following exposure at ≥ 25 mg/L. Full recovery occurred by 15 days following the cessation of exposure. PFOA exposure had no significant ($p > 0.05$) effect on thymus weight.

PFOA exposure at ≥ 25 mg/L resulted in about a 7% (*cf.* control) reduction ($p < 0.05$) in serum IgM responses to SRBC. No PFOA-associated effects on serum IgG to SRBC and delayed type hypersensitivity responses to BSA-CFU occurred.

The NOAEL for dose response study 2 was 12.5 mg/L (equivalent to 1.88 mg/kg bw/day) due to a reduction in serum IgM responses at the next highest concentration of 25 mg/L (equivalent to 3.75 mg/kg bw/day).

Dewitt et al. (2008) is a well conducted study but did not measure serum PFOA levels in dose response study 2. According to US EPA (2024a, Appendix), the internal serum concentration at the NOAEL dose of 1.88 mg/kg bw/day was 45,300 ng/mL.

Dewitt et al. (2008) was considered a candidate study for derivation of a TRV for PFOA in US EPA (2024a). US EPA (2024a) estimated a $BMDL_{1SD}$ of 18,200 ng/mL from dose response study 1 using a polynomial degree 4 BMD model, and a NOAEL of 45,300 ng/mL from dose response study 2. They then multiplied the results by a human clearance value of 0.00012 L/kg/day to derive POD_{HEDs} of 2.18 and 5.43 µg/kg/d, respectively. They then chose the lower of the two POD_{HEDs} and applied an uncertainty factor of 300 (3 x for interspecies toxicodynamics,10x for human variability, 10x for extrapolation from a subchronic to chronic exposure) to derive a TRV of 0.0073 µg/kg/day (i.e. 7 ng/kg bw/day).

The uncertainty factors applied are consistent with what would typically be applied in an Australian context, however it is considered more appropriate to use the experimental NOAEL from dose response study 2 in Dewitt et al. (2008) as a POD for calculation of a TRV, since this study used tighter dose spacing and found no adverse effects at the dose administered (which is higher than the $BMDL_{1SD}$ modelled from dose response study 1).

Use of the POD_{HED} of 5.43 µg/kg/day and an uncertainty factor of 300 results in a guidance value of 18 ng/kg bw/day (see also **Section 6.3**).

The Dewitt et al. (2016) study appears to have been conducted appropriately and incorporated a recovery phase; it evaluated a number of parameters including immune system markers. There was a clear dose response observed for reduction in IgM response to SRBC in female mice. US EPA (2024a) considered the study to be of medium confidence. Thus, the candidate guideline value resulting from adaption of the US EPA (2024a) candidate guidance value (incorporating the use of a NOAEL instead of a $BMDL_{1SD}$ value) is considered to be of medium confidence (see **Section 6.3**).

6.2.5 Dewitt et al. (2016) – used by Burgoon et al. (2023)

Dewitt et al. (2016) evaluated the role of PPARα in PFOA-mediated inhibition of T-cell-dependent (SRBC) and T-cell-independent (dinitrophenyl-ficoll (DNP)) antibody responses in female PPARα-knockout mice (KO mice) and C57BL/6-Tac wild-type controls (WT mice).

Experiment 1: Effects of PFOA exposure on T-cell-dependent serum IgM responses

Female WT and KO mice (6/strain per concentration level) were exposed to PFOA (ammonium salt, ≥ 98% pure) in drinking water at 0, 50 or 200 mg/L (equivalent to 0, 7.5 or 30 mg/kg bw/day) for 15 days. On study day 11, WT and KO mice were immunised to SRBC. Serum for IgM titre measurement was collected on study day 16 (one day post-exposure; time of peak serum IgM response in WT mice).

WT mouse body weight was significantly ($p < 0.05$) reduced (by ≥ 14% *cf.* control) at ≥ 9 days of PFOA exposure at 200 mg/L. PFOA exposure did not significantly ($p > 0.05$) affect the body weight of KO mice.

PFOA exposure did not significantly ($p > 0.05$) affect relative spleen and thymus weights in KO mice. In WT mice, relative spleen weight was significantly ($p < 0.05$) reduced (by about 30%) following exposure to PFOA at 200 mg/L. In WT mice, relative thymus weight was significantly ($p < 0.05$) reduced (by 55.4%) following exposure at 50 mg/L; however, no dose response was apparent and relative thymus weight in WT mice exposed at 200 mg/L was not significantly different ($p > 0.05$) *cf.* control. Due to the lack of a dose response, the effect of PFOA exposure on relative thymus weight was not regarded as being adverse.

Exposure to PFOA at 200 mg/L significantly ($p < 0.05$) reduced (by 16%/14% in WT/KO mice *cf.* control, respectively) the IgM response to SRBC in both WT and KO mice. Exposure at 50 mg/L did not significantly ($p > 0.05$) affect IgM responses in WT and KO mice. There was no significant ($p > 0.05$) difference in IgM responses between WT and KO mice at any exposure level.

The LOAEL for both WT and KO mice was 200 mg/L (equivalent to 30 mg/kg bw/day), the highest concentration tested. The NOAEL for both WT and KO mice was 50 mg/L (equivalent to 7.5 mg/kg bw/day) due to reduced serum IgM responses at the next highest concentration of 200 mg/L (equivalent to 30 mg/kg bw per day). Serum concentrations of PFOA were not reported in the study.

Experiment 2: Effects of PFOA exposure on T-cell-independent serum IgM responses

Female WT mice (8 per concentration level) were exposed to PFOA in drinking water at 0, 6.25, 12.5, 25, or 50 mg/L (equivalent to 0, 0.94, 1.88, 3.75 or 7.5 mg/kg bw/day) for 15 days. On study day 11, WT mice were immunised to DNP. Serum for IgM titre measurement was collected on study day 17 (two days post-exposure, time of peak serum IgM response in WT mice).

Significant ($p < 0.05$) reduction in relative spleen weight (17% *cf.* control) and thymus (14% *cf.* control) weight occurred in WT mice exposed to PFOA at 50 mg/L.

Exposure to PFOA at ≥ 12.5 mg/L resulted in about a 10 to 11% reduction ($p < 0.05$) in serum IgM responses to DNP. A dose response was not apparent.

The LOAEL was 12.5 mg/L (equivalent to 1.88 mg/kg bw/day). The NOAEL was 6.25 mg/L (equivalent to 0.94 mg/kg bw/day) due to reduction of serum IgM responses at the next highest concentration of 12.5 mg/L (equivalent to 1.88 mg/kg bw/day). Serum concentrations of PFOA were not reported in the study.

Experiment 3: Effect of PFOA on splenic lymphocyte phenotypes

WT mice (4 per concentration level per exposure duration) were exposed to PFOA in drinking water at 0, 25, or 50 mg/L (equivalent to 0, 3.75 or 7.5 mg/kg bw/day) for 10, 13 or 15 days after which their spleens were harvested. Mice exposed for ≥ 13 days were immunised with SRBC on study day 11 and then had their spleens harvested on study days 13 or 15.

PFOA exposure had no significant ($p > 0.05$) effect on splenic lymphocyte subpopulation phenotypes in the 10-day exposure (not immunised) cohort.

In the 13-day (immunised) exposure cohort, a significant ($p < 0.05$) increase (14% *cf.* control) in percentage $CD4^-/CD8^+$ cells occurred at 50 mg/L. This was accompanied by significant ($p < 0.05$) decrease (by 42% *cf.* control) in percentage $CD4^-/CD8^-$ cells (B-cells).

PFOA exposure for 15 days (immunised) had no consistent dose-related effects on splenic lymphocyte subpopulation phenotypes.

In the 13-day (immunised) exposure cohort, the NOAEL was 25 mg/L (equivalent to 3.75 mg/kg bw/day), i.e. the lowest concentration tested, as significant effects on splenic lymphocyte phenotypes were observed at 50 mg/L.

Overall, the study demonstrated that exposure of mice to PFOA resulted in suppression of serum IgM responses to SRBC (T-cell-dependent antigen) and DNF (non-T-cell-dependent antigen) in female mice. The presence or absence of PPARα did not affect either the LOAEL for this effect or the magnitude of the effect. However, the absence of PPARα did protect against PFOA exposure associated reductions in relative splenic and thymic weight. PFOA exposure resulted in an increase in the percentage of splenic $CD4^-/CD8^+$ cells and a decrease in percentage $CD4^-/CD8^-$ (B-cells) cells in WT mice. These results suggest that the

effects of PFOA on humoral immune responses may be mediated by disruption of B-cell/plasma cell function.

Dewitt et al. (2016) was evaluated in Burgoon et al. (2023). Based on a NOAEL of 0.94 mg/kg bw/day (no serum concentrations were available), Burgoon et al. (2023) derived a guidance value of 70 ng/kg bw/day using the following approach:

> Based on Lau et al. (2006), the serum level in mice associated with repeated dosing at 1 mg/kg-day is 23 µg/mL (i.e. 23,000 ng/mL). Therefore, dosing at 0.94 mg/kg/day is estimated to be associated with a serum level of 22 µg/mL (i.e. 22,000 ng/mL). Burgoon et al. (2023) applied the following uncertainty factors to this serum POD.
>
> - Mouse to human toxicokinetic factor = 1 (factor is not needed since POD is based on serum concentration).
> - Mouse to human toxicodynamic factor = 2.5 [IPCS (2005) default or 3 for US EPA (2014) default].
> - Human toxicodynamic factor = 3 [default of IPCS (2005) and EPA (2014)].
> - Human toxicokinetic factor = 8.4 [0.79 mL/day/kg arithmetic mean clearance of average group from Zhang et al. (2013, Table 2) ÷ 0.094 mL/day/kg arithmetic 95% lower bound clearance of sensitive group from Zhang et al. (2013, Table 2)].
> - Database uncertainty factor = 1.
> - Guidance value serum concentration = 0.29 µg/mL (i.e. 290 ng/mL) [22 µg/mL ÷ (1 × 3 x 3 × 8.4 x 1) = 0.29].
> - Guidance value = 0.07 µg/kg bw/day (i.e. 70 ng/kg bw/day) [0.29 µg/mL x 0.23 mL/day/kg [geometric mean clearance from Zhang et al. (2013, Table 2) assuming steady state].

The uncertainty factors used by Burgoon et al. (2023) did not include an uncertainty factor for the short exposure timeframe in the Dewitt et al. (2016) study. Burgoon et al. (2023) also used serum concentrations measured in a different study (Lau et al. 2006) in a different breed of mice (i.e. CD-1 mice) to approximate the serum concentrations that may have been reached in the Dewitt et al. (2016) study. This approximation, in the opinion of the authors of this addendum, renders the resulting health-based guidance value uncertain.

Although the study appears to have been conducted appropriately, the number of animals per dose group were small; this, together with the lack of measured serum PFOA data (and no serum PFOA data provided in another publication) renders the study of very low confidence for guidance value derivation, therefore the study has not been carried forward for candidate guidance value derivation in **Section 6.3**.

6.2.6 Dong et al. (2019) – used by US EPA (2024a, b)

The study by Dong et al. (2019) was already summarised and discussed in **Section 5.2.2** with respect to both PFOS and PFOA.

6.2.7 Nian et al. (2019) – candidate study in US EPA (2024a, b)

The study by Nian et al. (2019) was already summarised and discussed in **Section 5.2.4** with respect to both PFOS and PFOA.

6.2.8 NTP (2023) – candidate study in US EPA (2024a)

NTP (2023) evaluated the non-carcinogenic and carcinogenic effects of repeated dietary exposure of SD rats to PFOA (purity > 98%) over the perinatal (gestational + pre-weaning developmental stages) and post-weaning stages of development.

Study 1. Male and female study

Pregnant F_0 generation females were exposed to PFOA at dietary concentrations of 0 ppm (*n* = 103), 150 ppm (*n* = 36) or 300 ppm (*n* = 36) starting on GD6. On the day the last litter reached PND18 of age, F_1 rats were selected for the 2-year study with weaning occurring on PND 21 – 23. F_1 males (60/dietary concentration) were exposed to PFOA in the diet at 0 ppm, 150 ppm or 300 ppm for 2-years starting on PND 21-23. F_1 females (60/dietary concentration) were exposed to PFOA in the diet at 0, 0/150, 150/300, 0/300 or 300/1000 ppm (prenatal/postnatal exposure levels). F_1 females were exposed to higher dietary concentrations because of the shorter half-life seen in this gender (2 to 12 hours) compared with males (2 to 15 days), i.e. female rats have a lower systemic exposure *cf.* males due to a faster PFOA elimination rate than males, so a higher feed exposure concentration was provided to female rats postweaning.

The results of this study can be summarised as follows.

F_0 generation: There were no PFOA-associated adverse effects on maternal body weight or body weight gain during pregnancy and lactation. Feed consumption during gestation was marginally (3% to 4%) lower in the 150 and 300 ppm groups compared to the control groups. Feed consumption was also marginally (but significantly, $p < 0.05$) lower (up to 4%) in the 300 ppm group from lactation days (LDs) 1 to 10 and over the LD 1 to 14 period. PFOA consumption was 10.9 and 21.7 mg/kg bw/day at 150 and 300 ppm (respectively) during gestation. PFOA consumption was 23.3 and 45.2 mg/kg/day for the 150 and 300 ppm groups over LD 1–14, respectively.

F_1 generation litters: There were no PFOA-associated effects on total number and number of live litters. Male and female pup body weights on PND 1 ($p < 0.05$) were 5% lower at 300 ppm *cf.* control and 5% to 8% lower ($p < 0.05$) on PND 7-21.

F_1 generation 16 week interim sacrifice: In the 16 week interim F_1 generation evaluation cohort, there were no PFOA-associated adverse effects on mortality or feed consumption in females after ≥ study week 5 (reduced consumption, most likely due to palatability issues, occurred over study weeks 1 to 4). There were also no PFOA associated adverse effects on clinical chemistry parameters in females.

However, due to overt toxicity (manifesting as low body weight, > 10% reduction in food consumption *cf.* control ($p < 0.05$) and overt hepatotoxicity) in males, the male portion of the 2-year study was terminated at 21 weeks (24 weeks of age). A significantly ($p < 0.05$) increased incidence of minimal thyroid gland follicular cell hypertrophy, renal tubule mineralisation and glandular stomach submucosal inflammation also occurred in males.

Plasma and liver PFOA concentrations are shown in **Table 6-1** for males and **Table 6-2** for females. Plasma PFOA concentrations in males were consistent between groups with and without exposure during the perinatal period and were within 10% of each other between the 0/150 and 150/150 ppm groups and between the 0/300 and 300/300 ppm groups. Plasma concentrations in females showed a similar pattern to the males (e.g. minor differences between perinatal exposures and liver patterns); however, PFOA concentrations were much lower *cf.* males even though female exposure (mg/kg/day) was 2 to 3 times higher. Plasma concentrations were approximately 12-fold lower in the 0/300 ppm female group compared to the 0/300 ppm male group.

Table 6-1 Summary of Plasma and Liver Concentration Data for Male Rats at the 16-week Interim of the Two-year Feed Study with PFOA (adapted from NTP 2023) (1)

	Perinatal Exposure	Postweaning Exposure		
		0 ppm	150 ppm	300 ppm
n		10	10	10
Plasma concentration (ng/mL)	0 ppm	BD	193,000 ± 11,325	242,500 ± 12,731
	150 ppm	-	175,390 ± 14,956	-
	300 ppm	-	-	223,400 ± 8,422
Liver concentration (ng/g)	0 ppm	BD	157,400 ± 5,418	171,000 ± 7,578
	150 ppm	-	144,300 ± 5,752	-
	300 ppm	-	-	193,800 ± 9,704
Liver/Plasma Ratio	0 ppm	BD	0.84 ± 0.05	0.73 ± 0.06
	150 ppm	-	0.86 ± 0.06	-
	300 ppm	-	-	0.88 ± 0.05

Pairwise comparisons across perinatal exposures (0/150 vs. 150/150 ppm and 0/300 vs. 300/300 ppm) did not show any statistically significant differences.

BD = Below detection; group did not have over 20% of its values above the limit of quantification.

(1) Data presented as mean ± standard error on the mean. Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests (unless otherwise noted).

Table 6-2 Summary of Plasma and Liver Concentration Data for Female Rats at the 16-week Interim of the Two-year Feed Study with PFOA (adapted from NTP 2023) (1)

	Perinatal Exposure	Postweaning Exposure		
		0 ppm	300 ppm	1,000 ppm
n		10	10	10
Plasma concentration (ng/mL)	0 ppm	BD	20,420 ± 1,212	72,250 ± 4,351
	150 ppm	-	20,800 ± 1,043	-
	300 ppm	-	-	70,160 ± 6,895
Liver concentration (ng/g)	0 ppm	BD	16,420 ± 787	69,040 ± 3,942
	150 ppm	-	16,660 ± 750	-
	300 ppm	-	-	67,840 ± 5,681
Liver/Plasma Ratio	0 ppm	BD	0.82 ± 0.03	0.96 ± 0.04
	150 ppm	-	0.81 ± 0.03	-
	300 ppm	-	-	0.99 ± 0.05

Pairwise comparisons across perinatal exposures (0/300 vs. 150/300 ppm and 0/1,000 vs. 300/1,000 ppm) did not show any statistically significant differences.

BD = Below detection; group did not have over 20% of its values above the limit of quantification.

(1) Data presented as mean ± standard error on the mean. Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests (unless otherwise noted).

Group mean body weights of females in the 0/300 and 150/300 ppm groups were within 10% of the 0/0 ppm control group (no PFOA-associated adverse effects). Mean body weights for the 0/1,000 and 300/1,000 ppm female groups were approximately 10–15% less (i.e. adversely reduced) than the 0/0 ppm control group throughout most of the postweaning period. For the females evaluated at 16 weeks, mean body weights for the 0/1,000 and 300/1,000 ppm groups were 12% less (i.e. adversely reduced) than that of the 0/0 ppm control group.

The group mean absolute and relative liver weights of the 0/1000 and 300/1000 ppm female groups were significantly ($p < 0.05$) increased (up to 32%) *cf.* control. Histological correlates included significantly ($p < 0.05$) increased incidences of hepatocyte hypertrophy (adaptive), cytoplasmic alteration (considered adverse), and pigmentation. Because of the presence of cytoplasmic alteration, the increase in liver weights is regarded as being adverse.

Acyl-CoA oxidase enzyme activity, a marker of PPARα activity, was significantly ($p < 0.05$) increased (1.4-fold at 0/300 and 150/300 ppm and 5.5- and 6.5-fold at 300/1,000 and 0/1,000 ppm *cf.* control). No changes were observed in liver aromatase activity in females.

In females, serum ALT activity was significantly ($p < 0.05$) increased (by up to 1.4-fold *cf.* control) in the 0/1,000 and 300/1,000 ppm groups. Serum sorbitol dehydrogenase (SDH) was also significantly ($p < 0.05$) increased (by ≥ 2.5-fold *cf.* control) at ≥ 150 ppm. Serum ALP was also significantly ($p < 0.05$) increased (by up to 2.3-fold *cf.* control) at ≥150 ppm. These changes were accompanied by significantly ($p < 0.05$) increased (up to 5-fold *cf.* control) serum bile acids at ≥ 150 ppm.

An increased incidence (40% *cf.* 0% in the control) of minimal thyroid gland follicular cell hypertrophy occurred in females exposed at 300 ppm. An increased ($p < 0.05$) incidence (70% *cf.* 20% in the control) of minimal renal tubular mineralisation and an increased ($p < 0.05$) incidence (40% *cf.* 0% in the control) of minimal renal papillary urothelial hyperplasia occurred in females exposed at 1000 ppm.

F_1 generation 2-year exposure cohort (females only): There were no PFOA-associated adverse effects on survival or clinical observations. Exposure-related decreases in mean body weights occurred at 0/1000 and 300/1000 ppm (reduced by 19% and 27% *cf.* control, respectively). PFOA consumption after weaning was 18.2, 18.4, 63.4 and 63.5 mg/kg bw/day in the 0/300, 150/300, 0/1000 and 300/1000 ppm groups, respectively.

The incidences of ulcer, epithelium hyperplasia, and chronic active inflammation of the submucosa in the 0/1000 and 300/1000 ppm groups were significantly ($p < 0.05$) increased *cf.* control. Both exposed groups had a single case of a squamous cell papilloma in the forestomach.

Hepatoxicity manifesting as significant ($p < 0.05$), dose-related increases in the incidence of hepatocyte cytoplasmic alteration, hepatocyte single cell death and hepatocyte pigmentation occurred at all exposure levels. An increased ($p < 0.05$) incidence of hepatic necrosis and bile duct hyperplasia occurred at 1000 ppm.

The incidences of hyperplasia of the renal papillary epithelium were significantly ($p < 0.05$) increased in the 0/300, 0/1000, and 300/1000 ppm groups. The incidences of papilla necrosis were significantly ($p < 0.05$) increased in the 0/1000 and 300/1000 ppm groups. The incidence of renal tubule mineral was significantly ($p < 0.05$) increased in the 0/1000 ppm group. In general, the incidences of these kidney lesions increased with increasing exposure concentration. There were some statistical differences observed in groups with and without perinatal exposure. The incidence of hyperplasia of the renal papillary epithelium in the 150/300 ppm group was significantly decreased compared to the 0/300 ppm group. It is not clear if this was related to perinatal exposure as this only occurred with the 150 ppm

perinatal exposure and not the 300 ppm perinatal exposure. In addition, there was a decrease of renal tubule mineral in the 300/1000 ppm group compared to the 0/1000 ppm group. Similarly, it is unclear if this is related to perinatal exposure.

The incidences of follicular cell hypertrophy in the 0/1000 and 300/1000 ppm groups were significantly ($p < 0.05$) greater than those in the 0/0 ppm control group. No differences between groups with perinatal and without perinatal exposures were observed.

A significantly ($p < 0.05$) increased incidence (overall rate of 16% and litters rate of 23% versus up to 3% in the concurrent control and up to 2% in the historical control) of uterine adenocarcinoma occurred following exposure to 1000 ppm. No differences between groups with perinatal and without perinatal exposures were observed.

Study 2. Two-year study in males with 16-week interim evaluation

In this second study, the pregnant females were exposed to a single feed concentration of 300 ppm PFOA because this exposure was well tolerated. Following weaning at PND 21 males (50/dietary concentration level) were exposed to PFOA via the diet at 0, 20, 40 or 80 ppm for two years. The findings of the study can be summarised as follows.

F_0 generation: PFOA dietary exposure at 300 ppm had no adverse effects on pregnancy status, survival, number of dams that littered, body weight, body weight gain, feed consumption and live litter size.

PFOA consumption was 21.8 mg/kg/day during gestation and 48.3 mg/kg/day over PND 1 to 14. Maternal plasma concentrations of the 300 ppm group were 75.1 µM (i.e. 31,097 ng/mL) on GD 18 and 74.2 µM (30,724 ng/mL) on PND 4.

Concentrations of PFOA from foetuses pooled by litter on GD 18 were 23 µM (i.e. 9,524 ng/mL), indicating some maternal transfer with maternal plasma concentrations at 75 µM. On PND 4, concentrations from whole male and female pups were comparable at 11 µM (4,555 ng/mL) and 10 µM (4,141 ng/mL), respectively, indicating some lactational transfer. Concentrations of PFOA were below detection in the control group.

F_1 generation: Maternal PFOA dietary exposure at 300 ppm did not adversely affect survival and body weight between PND 4 to 21.

16-week interim evaluation: There were no PFOA-associated adverse effects on survival or clinical observations. PFOA consumption for the first 13 weeks postweaning averaged 1.9 mg/kg bw/day for the 0/20 and 300/20 ppm groups, 4.0 mg/kg bw/day for the 0/40 and 300/40 ppm groups, and 7.9 and 8.0 mg/kg bw/day for the 0/80 and 300/80 ppm groups, respectively. In general, chemical consumption increased in proportion with dietary concentration. Plasma and liver PFOA concentrations are shown in **Table 6-3**. Mean body weight was significantly ($p < 0.05$) and adversely reduced (by ≥ 14% *cf.* control) at ≥ 40 ppm.

Liver weights were significantly ($p < 0.05$) increased (relative weight increased by up to 66% *cf.* control) in all PFOA exposed animals except for the 300/0 ppm cohort. This was associated with microscopic anatomic pathology evidence of hepatotoxicity (present in all PFOA-exposed animals). No differences were observed between groups with and without perinatal exposures.

Hepatic acyl-CoA oxidase enzyme activity was significantly ($p < 0.05$) increased (up to about 10-fold *cf.* control) in all postweaning exposed groups *cf.* control. No biologically meaningful differences were observed between groups with and without perinatal exposures. Hepatic aromatase activity was significantly ($p < 0.05$) increased (by about 2-fold *cf.* control). No biologically meaningful differences were observed between groups with and without perinatal exposures.

Absolute and relative spleen weights were significantly ($p < 0.05$, except for relative weight in the 80 ppm cohorts where the weight was still reduced *cf.* control) decreased (by up to

29% *cf.* control and without microscopic anatomic pathology correlates) in all PFOA-exposed animals.

Serum globulin concentrations were significantly ($p < 0.05$) decreased (by up to 44% *cf.* controls) in all PFOA exposed animals. This was correlated with a significant ($p < 0.05$) decrease in serum total protein and increased serum albumin/globulin ratios.

While significant ($p < 0.05$) increases in serum hepatic enzymes (ALT, ALP, SDH) and bile salts occurred, the magnitude of the changes (< 2-fold *cf.* control) was not biologically significant. Significant ($p < 0.05$) increases (changes were not observed in the controls) in the incidence of hepatocyte cytoplasmic alterations (100% incidence at all exposure levels), hypertrophy (90 to 100% incidence) and single cell death (50 to 90% incidence) occurred at all levels of PFOA exposure. An increased incidence of hepatic necrosis and pigmentation occurred at ≥ 40 ppm.

Table 6-3 Summary of Plasma and Liver Concentration Data for Male Rats at the 16-week Interim of the Two-year Feed Study with PFOA (adapted from NTP 2023) (1)

	Perinatal Exposure	Postweaning Exposure			
		0 ppm	20 ppm	40 ppm	80 ppm
n		10	10	10	10
Plasma concentration (ng/mL)	0 ppm	BD	81,400 ± 2,715	130,780 ± 7,560	159,600 ± 8,303
	300 ppm	36 ± 12**	78,030 ± 2,976**	117,060 ± 4,189**	144,100 ± 5,480**
Liver concentration (ng/g)	0 ppm	BD	83,550 ± 4,658	108,280 ± 5,412	147,400 ± 10,629
	300 ppm	BD	85,960± 3,635	109,210 ± 3,039	133,310 ± 4,625
Liver/Plasma Ratio	0 ppm	BD	1.02 ± 0.03	0.84 ± 0.04	0.92 ± 0.03
	300 ppm	BD	1.11 ± 0.04	0.94 ± 0.03	0.94 ± 0.05

Statistical significance for a treatment group indicates a significant pairwise test compared to the respective control group (0/0 or 300/0 ppm). Statistical significance for the 0/0 ppm or 300/0 ppm control group indicates a significant trend test.

Pairwise comparisons across perinatal exposures (0/20 vs. 300/20, 0/40 vs. 300/40, and 0/80 vs. 300/80 ppm) did not show any significantly significant differences.

** Statistically significant at p ≤ 0.01.

BD = Below detection; group did not have over 20% of its values above the limit of quantification.

(1) Data presented as mean ± standard error on the mean. Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests (unless otherwise noted).

F_1 generation 2-year exposure cohort: There were no PFOA-associated adverse effects on clinical observations, feed consumption or survival. Adversely reduced (13% *cf.* control) body weight occurred in the 300/80 ppm exposure cohort. After weaning, PFOA consumption for rats in the 0/20, 0/40, and 0/80 ppm groups and the 300/20, 300/40, and 300/80 ppm groups averaged 1.1, 2.2, and 4.6 mg/kg bw/day and 1.0, 2.1, and 4.6 mg/kg bw/day, respectively.

Significantly ($p < 0.05$) increased incidences of hepatocyte cytoplasmic alterations (up to 92% incidence with no occurrence in the controls) occurred in all animals exposed to PFOA following weaning. Significantly ($p < 0.05$) increased incidences of hepatocyte hypertrophy (up to 86%), hepatocyte single cell death (up to 58%), hepatic necrosis (up to 42%), and pigmentation (up to 60%) occurred in all PFOA exposed animals. A significant ($p < 0.05$) increase (up to 28% in PFOA exposed animals *cf.* 30% in controls) in focal hepatic

inflammation and hepatic cystic degeneration (up to 22% in PFOA exposed animals *cf.* 0% in controls) occurred in the 80 ppm cohorts. The incidences of pancreatic acinus hyperplasia were also significantly ($p < 0.05$) increased in all postweaning-only exposure groups and in the 300/40 and 300/80 ppm groups of the perinatal and postweaning study. This lesion is considered to be a potentially preneoplastic lesion.

A significantly ($p < 0.05$) increased incidence of hepatocellular adenomas (up to 22%) and carcinomas (up to 12%) occurred at all PFOA exposure levels. Significant ($p < 0.05$) increases in the incidence of pancreatic acinar adenoma (up to 64%) and adenocarcinoma (up to 6%) occurred at all PFOA exposure levels.

Overall conclusions

A NOAEL was not achieved in the study. The overall study LOAEL was 20 ppm (equal to 1.0 mg/kg bw per day), the lowest dietary concentration tested, due to an increased incidence of hepatic and pancreatic neoplasia at this dietary concentration. This was combined with microscopic anatomic pathology evidence of hepatotoxicity at 20 ppm. Based on the weight of evidence evaluation presented in the summary of Butenhoff et al. (2012a; **Section 6.2.2**), PFOA is likely not genotoxic. Accordingly, a threshold mode of action and dose response likely applies to the hepatic and pancreatic neoplasia observed in NTP (2023).

Hepatic neoplasia, based on data in other studies, is at least in part due to actions at PPARα receptors and thus may not be relevant to humans. However, US EPA (2024a) have noted that multiple other modes of action may occur.

With respect to the acinar hyperplasia, adenoma and adenocarcinoma observed in PFOA-treated rats, it is noted the induction of pancreatic acinar cell proliferation in rats has been observed with other PPARα agonists and has also been noted previously in another 2-year PFOA cancer bioassay with CD male rats in which pancreatic acinar tumours were also observed (Biegel et al. 2001). Other PPARα agonists that have been shown to cause both hepatocellular neoplasia and pancreatic acinar cell neoplasia in rats include butylbenzyl phthalate, cinnamyl anthranilate, clofibrate, di(2-ethylhexyl) phthalate, gemfibrozil, 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123), methylclofenapate, nafenopin, and pirinixic acid (WY-14643) (Klaunig et al. 2003). A proposed mode of action for the pancreatic lesions involves the following (Caverly Rae et al. 2014, Klaunig et al. 2012):

1. PPARα is activated in the liver which triggers
2. a decreased bile acid flow and/or altered bile acid composition which leads to
3. cholestasis.
4. Decreased bile acid synthesis leads to increased cholecystokinin (CCK) release from the intestinal mucosa. This acts to stimulate
5. acinar cell proliferation, leading to
6. development of acinar cell tumours.

CCK has been shown to be a growth factor for rat pancreatic acinar cells, which have numerous CCK1 receptors. Increased CCK therefore causes hyperplasia of the acinar cells, increasing the likelihood of neoplastic change. This mode of action is unlikely to be relevant to humans, as human pancreatic cells do not have functioning CCK1 receptors and do not respond to CCK *in vitro* (Caverly Rae et al. 2014).

Klaunig et al. (2012) presented mode of action human relevancy evaluations for both the liver and pancreatic tumours which had also been observed in previous chronic rat studies with PFOA. For the liver, the authors found "*the extensive experimental evidence of PPAR alpha activators including PFOA five step key events for the PFOA MOA [mode of action]*

including the activation of the PPARα receptor which induces the gene expression of hepatic cell proliferation genes, leading to hepatocyte proliferation and specifically the selective clonal expansion of preneoplastic hepatic cells leading to hepatic neoplasia. These key events are supported by the experimental information and available mechanistic data. They also exhibit dose-response, temporal concordance, consistency, specificity and biological plausibility. In reviewing the application of the MOA of PFOA for liver tumors observed in the rat to humans, it appears that the induction of liver cancer to human by PFOA is not relevant."

Based on the above, there is high confidence that the hepatic neoplastic lesions are unlikely to be relevant to humans.

Upon reviewing the proposed mode of action for the pancreatic tumours, Klaunig et al. (2012) stated "*a proposed Mode of Action….. notes the activation of PPARα in the liver which in turn reduces bile acid flow and alters bile acid composition which produces cholestasis. This in turn increases CCK levels resulting in pancreatic cell proliferation and eventually tumor formation. While there is relatively good temporal concordance, consistency, and specificity to the key events additional experimental support for PFOA functioning through this proposed Mode of Action is needed. However, based on the available information, the proposed MOA for the PACT* [pancreatic acinar tumours] *in the rat by PFOA to human health does not appear to be relevant to humans based on the lack of concordance of a number of the proposed key events in the pancreatic Mode of Action in rats to humans."*

Whilst an update to the mode of action human relevancy assessment undertaken by Klaunig et al. (2012) could not be readily found by SLR in the literature consulted briefly for this Addendum report, the following information lends additional support to the likely lack of relevancy of the pancreatic tumours to humans:

- The histological type of tumour seen in the rodent is distinctly different from tumours of the exocrine pancreas most commonly observed in humans (Caverly Rae et al. 2014). While rodent tumours typically display acinar differentiation, the majority of human pancreatic neoplasms are of the ductular type; true ductular neoplasms of the pancreas are rare in rats (Caverly Rae et al. 2014).
- While rodent tumours typically have no noticeable effects on the rodent's morbidity or mortality, the majority of human pancreatic neoplasms are associated with a 5-year survival rate of 6% (Caverly Rae et al. 2014).
- Mutation causing activation of the proto-oncogene KRAS occurs in >90% of human pancreatic ductular adenocarcinomas, and this mutation is found in even the earliest precancerous pancreatic lesions in humans, whereas PFOA has not been shown to have mutagenic or clastogenic activity (Caverly Rae et al. 2014).
- The Washington State Advisory Committee on Firefighter Presumption (WASAC 2024) recently reviewed the potential association between PFAS exposure and pancreatic cancer incidence in firefighters. The review found that, whilst the International Agency for Research on Cancer (IARC) has classified PFOA as 'carcinogenic to humans' (Group 1), there is insufficient evidence to suggest that PFAS is specifically associated with pancreatic cancer in both the general public nor in firefighters (who have been observed to have elevated PFAS levels in blood compared to non-firefighters). Indeed, epidemiological studies of pancreatic cancer in firefighters have not demonstrated that firefighters are at an increased risk of developing pancreatic cancer compared to non-firefighters. The committee did not find sufficient evidence for an association between PFAS exposure and pancreatic cancer (WASAC 2024).

However, while there is a reasonable basis to assume that the PPARα mode of action for pancreatic neoplasia is likely not relevant to humans, there is some evidence that there are other modes of action that can occur simultaneously with the PPARα mode of action, i.e. pancreatic neoplasia in rodents may be the result of mixed modes of action. There is *in vitro* evidence of possible involvement of an oxidative mode of action (Abudayyak et al. 2021). However, these findings have not been replicated or demonstrated *in vivo*.

Based on the above information, it is concluded that the neoplastic pancreatic effects observed with PFOA in the NTP (2023) study are also unlikely to be relevant to humans based on currently available information.

US EPA (2024a) selected the hepatocellular necrosis effect in male rats from the NTP (2023) study for deriving a candidate guidance value for non-neoplastic effects because they regarded the study as being of high confidence and the effect was accompanied by cytoplasmic alteration in the liver. Data from females were not considered for POD derivation as they appear to be less sensitive, potentially due to toxicokinetic differences between the sexes in rats.

A 90-day oral study by Goldenthal et al. (1978) in rhesus monkeys did not find evidence of hepatocellular necrosis at the doses administered in the study (n=4 animals/group, doses were 3, 10, 30 or 100 mg PFOA/kg bw/day), albeit all monkeys at the top dose and 3 out of 4 monkeys at the 30 mg/kg bw/day dose died before the scheduled end of the study. In a 6-month repeat dose oral study of PFOA with cynomolgus monkeys (n=6/group) (Butenhoff et al. 2002), there were no alterations in serum markers for liver damage at doses of 3 or 10 mg/kg bw/day. Effects on liver enzymes in the highest dose group (30 mg/kg bw/day, decreased to 20 mg/kg bw/day from day 22 of the study) were inconsistent, with one monkey killed in moribund condition on day 29. Elevated liver enzymes were found in this monkey as well as in another monkey where dosing was halted on day 66, but not in the other four monkeys in the group. The monkey that was killed on day 29 exhibited liver degeneration and necrosis, but the monkeys killed at the end of the study did not. These findings in monkeys indicate that the effect of hepatic necrosis and potential human relevancy is uncertain.

ATSDR (2021) noted there is ‘limited’ epidemiological data for potential associations between serum PFAS and liver disease, with considerable variability across studies. Associations with small shifts in liver enzyme levels in communities exposed to PFOA due to environmental contamination were noted but were not accompanied with adverse outcomes such as enlarged liver, fatty liver or cirrhosis (C8 Science Panel 2012). In a Phase 1 trial conducted with ammonium perfluorooctanoate (AFPO, APFO is metabolised to PFOA) for potential use as a therapeutic agent in patients with cancer, patients (n=49) were dosed weekly for up to six weeks at 50-1200 mg APFO (Convertino et al. 2018). This resulted in circulating levels of PFOA of more than four orders of magnitude higher than those measured in epidemiological studies (i.e. 9-1530 µM, 3,730-633,527 ng/mL). There were no treatment-related changes in serum levels of liver enzymes (Convertino et al. 2018). Thus, the human relevancy of the hepatic necrosis effect observed in rodents in the NTP (2023) study is uncertain.

Although there is uncertainty with respect to the dose at which non-neoplastic hepatic effects may occur in humans and it is recognised by SLR that rats are likely more sensitive to this effect than humans, SLR considers there is insufficient information to rule out human relevancy of this effect based on currently available information.

US EPA (2024a) used the Wambaugh et al. (2013) model to simulate the $C_{avg\_pup\_total}$ internal dose metric (this adds the area under the curve in gestation/lactation to the area under the curve from diet and then divides by 2 years) for the hepatic necrosis effect. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk compared to the

control was chosen as per US EPA's *Benchmark Dose Technical Guidance* (US EPA 2012). US EPA (2024a) derived a $BMDL_{10}$ of 26.9 mg/L (i.e. 26,900 ng/mL) from the study for this effect for use as POD. US EPA (2024a, Appendix) states the selected model (Multistage Degree 1 model) showed adequate fit (p>0.1) and had the lowest AIC of the models remaining after consideration of ratios between PODs.

The POD was converted by US EPA (2024a) to a POD_{HED} of 3.23 µg/kg/day using a clearance value of 0.128 mL/kg/day. US EPA (2024a) applied an uncertainty factor of 30 (3x for interspecies extrapolation of toxicodynamic differences, 10x for human variability) to the POD_{HED} value to derive a guidance value of 0.108 µg/kg/day (i.e. 100 ng/kg/day).

The uncertainty factors applied are consistent with what would typically be applied in an Australian context.

US EPA (2024a) also conducted BMD modelling for the neoplastic effects in the study, where $BMDL_{10RD}$ values ranged from 15.2 to 93 mg/L, with the lowest $BMDL_{10RD}$ identified for the pancreatic acinar cell adenomas or adenocarcinomas. The best-fitting model for the most sensitive neoplastic effect was the Multistage Degree 3 model based on adequate p-values (p > 0.1), BMDLs showing <3-fold difference among adequately fitting models and having the lowest AIC. US EPA (2024a) derived a CSF using a linear non-threshold approach; as discussed in **Section 6.2.2**, such an approach is not considered appropriate for PFOA.

As indicated above, SLR considers the neoplastic pancreatic effects observed with PFOA in the NTP (2023) study are unlikely to be relevant to humans, and there is uncertainty regarding the potential human relevance of the non-neoplastic hepatic necrosis effect based on currently available information. Nevertheless, $BMDL_{10RD}$ values derived by US EPA (2024a) for both the non-neoplastic (hepatic necrosis) and neoplastic (acinar pancreatic tumours) effects considered by US EPA (2024a) have been adapted from US EPA (2024a) to derive candidate guideline values for PFOA in **Section 6.3**.

The NTP (2023) study is a high-quality study and has been conducted appropriately. Thus, there is relatively high confidence in the potential candidate guideline values resulting from adaption of the US EPA (2024a) candidate guidance values (see **Section 6.3**), whilst noting the uncertainty associated with the human relevancy of the effects based on currently available information.

6.2.9 Sagiv et al. (2018) – candidate study in US EPA (2024a, b)

The study by Sagiv et al. (2018) was already summarised and discussed in **Section 5.2.6** with respect to both PFOS and PFOA.

6.2.10 Shearer et al. (2021) – used by US EPA (2024a)

Shearer et al. (2021) conducted a prospective nested case-control study where they used prediagnostic serum PFAS (PFOA, PFOS, EtFOSAA, MeFOSAA, PFHxS, PFNA) concentrations in 324 renal cell carcinoma (RCC) cases (diagnosed an average of 8.8 years after phlebotomy) and 324 individually matched controls within the Prostate, Lung, Colorectal and Ovarian Screening Trial (PLCO)[24]. Multivariate conditional logistic regression was used to estimate odds ratios (ORs) and 95% CIs to investigate the association between serum PFAS concentrations and RCC risk. Individual PFAS were modelled continuously (log_2-transformed) and categorically, with adjustment for kidney function (i.e. glomerular

[24] PLCO is a randomised screening trial that recruited approximately 150,000 adults ages 55-74 years from study centres in 10 US cities between 1993 and 2001; participants in the screening arm provided non-fasting blood samples.

filtration rate, eGFR) and a number of other potential confounders (body mass index, smoking status, history of hypertension, prior freeze-thaw cycles, and calendar year of blood draw). Category cut-points were assigned based on quartiles of serum concentrations of each PFAS among controls.

The study authors found a positive association with RCC risk for PFOA (doubling in serum concentration, $OR_{continuous}$ = 1.71, 95% CI = 1.23-2.37, p=0.002). The OR was also significant for those in the highest quartile of serum PFOA concentrations (>7.3-27.2 ng/mL) vs. the lowest (<4 ng/mL) (OR = 2.63, 95% CI = 1.33-5.2, p_{trend} = 0.007). The association with PFOA remained after adjustment for PFOS and PFHxS concentrations ($OR_{continous}$ = 1.68, 95% CI = 1.07-2.63, p=0.02). Although PFOS and PFHxS were also found to be associated with RCC, the association disappeared after adjustment for other PFAS.

It is noted a higher proportion of cases had diminished kidney function (eGFR < 60 mL/min/1.73m^2) compared with controls (9% vs. 5.6%), but this difference was not statistically significant (p=0.25).

The study by Shearer et al. (2021) is a well-conducted study in the general population. Strengths of the study include direct assessment of serum PFAS concentrations and prospective follow-up; the authors also adjusted for a range of potential confounders, including kidney function (reduced kidney function is a known cause of RCC) and other PFAS. Nevertheless, there are still a number of limitations as pointed out by the study authors. These include that exposures were based on serum measurements collected at a single point in time and that limited data were available on race; it is also noted no data were available for socioeconomic status. In addition, as with any epidemiological study, it was not possible to control for other potential chemical exposures that are known to be associated with RCC, e.g. cadmium.

It is also difficult to reconcile the human PFOA serum concentrations at which associations with RCC were observed in the Shearer et al. (2021) study (i.e. >7.3-27.2 ng/mL) with the serum concentrations in rats from the Butenhoff et al. (2012a) study at which no adverse effects on the kidneys were found (e.g. 43,264,000 ng/mL as per US EPA 2024a, Appendix). Furthermore, the absolute differences in serum PFOA concentrations between controls and cases in the Shearer et al. (2021) study is quite minimal as can be seen in **Figure 6-4**. Such a steep dose response is not supported by the experimental animal toxicological information.

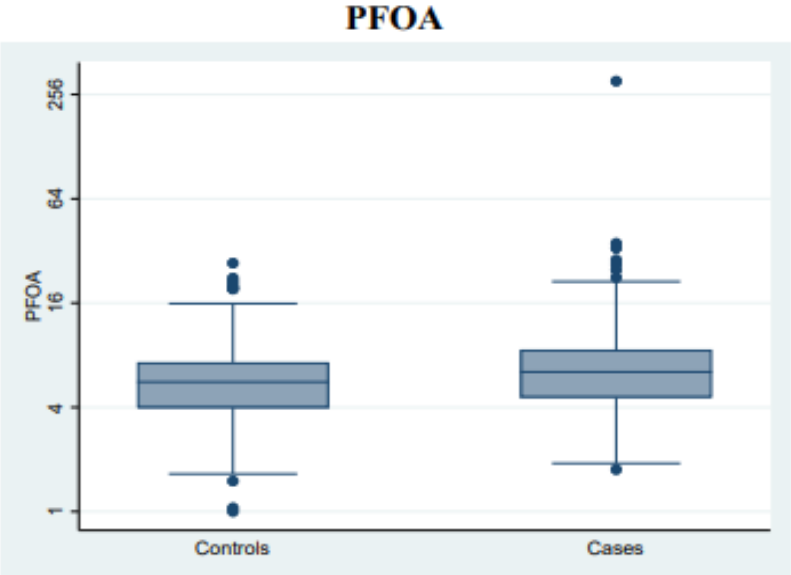


Figure 6-4 Box plot for serum PFOA concentrations (ng/mL) among RCC cases and controls from Shearer et al. (2021) study

US EPA (2024a) used the association between RCC and PFOA serum concentrations from the Shearer et al. (2021) study for dose-response modelling. A cancer slope factor (CSF) of 0.0293 $(ng/kg/day)^{-1}$ was calculated by US EPA (2024a) using non-threshold linear regression. This is a policy decision that US EPA makes for all substances that are regarded as probable carcinogens. However, experimental animal data (Butenhoff et al. 2012a, summarised in **Section 6.2.2**) and data showing lack of genotoxicity at non-cytotoxic concentrations (ATSDR 2021) support a threshold mode of action for carcinogenesis. Thus, calculation of a CSF for PFOA is not in line with Australian science policy.

Although the data in Shearer et al. (2021) suggest there may be an association between PFOA exposure and RCC, a threshold for the potential associations cannot be readily discerned from the data in the study. This, together with Australian science policy considerations with respect to assessment of non-genotoxic carcinogens, indicate that the study by Shearer et al. (2021) is not considered to provide appropriate information for use as a key study for derivation of a TRV.

Therefore, the US EPA (2024a) assessment of Shearer et al. (2021) is not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate guidance/guideline value derivation for PFOA in **Section 6.3**.

6.2.11 Song et al. (2018) – candidate study in US EPA (2024a)

Song et al. (2018) investigated the effects of PFOA on reproductive function and imprinted genes of male mice offspring. Pregnant Kunming mice (n=10/group) were administered 0 (saline control), 1, 2.5, or 5 mg/kg bw PFOA (>98% purity) daily by gavage from GD1-17. On PND21, offspring were separated from dams by sex. Male offspring were sacrificed after blood sampling at age 21 or 70 days. Blood samples were analysed for testosterone. Relative testicular weight was obtained, and testis were examined histologically. Gene expression studies (for target genes Dlk-1-Dio3 imprinting gene cluster in testis) were also carried out.

Results of the study can be summarised as follows:

- Number of surviving offspring mice at weaning (PND7) was statistically significantly reduced in the highest dose group (5 mg/kg bw/day).
- Testicular weight relative to body weight (evaluated as ‘testicular index’) was not significantly different from the control group at PND21 and PND70.
- Serum testosterone significantly decreased with increasing dose on PND21 ($p<0.01$), whereas at PND70 testosterone levels were significantly higher than controls at 1 mg/kg bw/day ($p<0.01$) but significantly lower than controls at 2.5 and 5 mg/kg bw/day ($p<0.01$).
- In terms of histopathology, at PND21 and PND70 inter-cellular substance areas were significantly increased and number of Leydig cells significantly decreased at ≥ 2.5 mg/kg bw/day. Vacuolisation of Sertoli cells and decrease or disappearance of spermatozoa was seen at 5 mg/kg bw/day on PND21. Vacuolisation was not seen at PND70. This indicates some recovery with the growth and development of offspring.

- Significant decreased expression of Dlk1-Dio3 imprinted gene cluster (for three of the four investigated) in the testis was observed on PND21 at ≥ 2.5 mg/kg bw/day. However no significant difference in gene expression was observed at PND70.

The methodology of the paper does not report on the analysis of PFOA in mouse serum, however in the discussion of the paper a concentration of PFOA in mice serum is mentioned (i.e. 170 ng/mL), but it is unclear from the information provided in the paper at what time point this serum measurement was taken and in which exposure group. Thus, the serum PFOA data as reported in this study are considered to be unreliable.

US EPA (2024a) selected the endpoint of pup survival for derivation of a candidate TRV. The agency provides dose-response modelling data for the effect (US EPA 2024a, Appendix), where the internal modelled serum concentrations ($C_{avg,pup,gest,lact}$) are 15,400, 25,300, and 29,600 ng/mL for the 1, 2.5 and 5 mg/kg bw/day dose groups, respectively. The $BMDL_{0.5\ SD}$ (as pup mortality is a severe effect) modelled using various models were all relatively similar, with the best fitting $BMDL_{0.5\ SD}$ for $C_{avg,pup,gest,lact}$ being 12,300 ng/mL[25].

The POD was converted by US EPA (2024a) to a POD_{HED} of 0.64 µg/kg/day using the updated one-compartment human developmental model by Verner (Verner et al. 2016).

In the model, half-life and V_d are used to calculate clearance, which is used in the model directly and is also used for calculation of steady-state concentrations. The parameters used in the model were 170 mL/kg for V_d (from Thompson et al. 2010a), 2.7 year half-life (from Li et al. 2017), 0.120 mL/kg/d clearance (calculated from half-life and volume of distribution), a 0.83 cord serum:maternal serum ratio, and a 0.049 milk:serum partition coefficient. US EPA (2024a) states that the use of the Verner et al. (2016) model in humans presents a substantial advancement in approach for endpoints in children compared with the previous US EPA (2016a) assessment for PFOA. US EPA (2024a, Appendix) present data validating the use of the model; like for PFOS, the data show that the predicted human serum PFOA concentrations line up well when compared to measured human serum concentrations.

It is noted however that multiplying the POD of 12,300 ng/mL by the clearance value of 0.12 mL/kg/d given by US EPA (2024a) gives 1.5 µg/kg/day, i.e. different from the result given as the POD_{HED} for this study of 0.64 µg/kg/day in Table 4-8 of US EPA (2024a). The difference for this result is not clear from SLR's reading of the agency documentation.

US EPA (2024a) applied an uncertainty factor of 30 (3x for interspecies extrapolation of toxicodynamic differences and 10x for human variability) to the POD_{HED} of 0.64 µg/kg/day to derive a TRV of 0.021 µg/kg/day (i.e. rounded to 20 ng/kg/day). The uncertainty factors applied are consistent with what FSANZ (2017) applied to other experimental animal studies when deriving the current Australian TRV for PFOA.

The Song et al. (2018) study focused on specific endpoints of interest in mice, therefore it did not follow standardised protocols for developmental toxicity experiments screening for a larger suite of endpoints. The reported serum PFOA concentration in the paper is also considered unreliable. Although no statistical difference was reported between litter sizes at PND0 (i.e. at birth), statistical analysis of the various endpoints did not include the litter in the model to guard against an inflated Type I error rate. Thus, relatively low confidence is assigned to the candidate guidance/guideline value derived using the Song et al. (2018) study by adaption of the US EPA (2024a) BMD analysis (which relied on the modelled serum concentrations provided by US EPA). Candidate guidance/guideline values have been estimated using both the US EPA (2024a) cited POD_{HED} as well as the POD_{HED} that would

[25] When $C_{avg,pup,gest}$ was used, the best fitting $BMDL_{0.5\ SD}$ was 8,600 ng/mL. When $C_{avg,pup,lact}$ was used, the best fitting $BMDL_{0.5\ SD}$ was 15,200 ng/mL. When $C_{max,pup,gest}$ was used, the best fitting $BMDL_{0.5\ SD}$ was 13,400 ng/mL, and when $C_{max,pup,lact}$ was used, the best fitting $BMDL_{0.5\ SD}$ was 20,300 ng/mL.

result by converting the POD using the human clearance value given in US EPA (2024a) (**Section 6.3**).

6.2.12 Timmermann et al. (2022) – candidate study in US EPA (2024a, b)

The study by Timmermann et al. (2022) was already summarised and discussed in **Section 5.2.7** with respect to both PFOS and PFOA.

6.2.13 Vieira et al. (2013) – used by US EPA (2024a)

Vieira et al. (2013), a study forming part of the C8 Science Panel[26], used geographic methods to investigate the relationship between exposure to PFOA and patterns of cancer risk in the mid-Ohio River Valley using data from the Ohio (OH) and West Virginia (WV) cancer registries. The final dataset included 7,869 geocoded OH cases and 17,238 WV cases of 18 cancer categories (i.e. bladder, brain, female breast, cervix, colon/rectum, kidney, leukaemia, liver, lung, melanoma of the skin, multiple myeloma, non-Hodgkin lymphoma, ovary, pancreas, prostate, testis, thyroid and uterus).

Median PFOA serum concentrations for each district had previously been estimated by pairing geographical location with measured PFOA levels in drinking water. Study authors applied logistic regression to individual-level data using registry-based cancer controls to calculate adjusted odds ratios (AORs) and confidence intervals (CIs) for each cancer category, with the other cancer categories excluding kidney, pancreatic, testicular, and liver cancers (which had been linked to PFOA exposure in animal and human studies previously) serving as controls. Models were adjusted for age, sex, diagnosis year, smoking status, and insurance provider. Geocoding for OH cases (at time of diagnosis) allowed study authors to assign case addresses to contaminated water district areas or to the unexposed group, whereas due to data restrictions WV cases were assigned to county or unexposed areas. For OH data, serum concentrations (both at time of diagnosis and 10 years prior) were modelled (using data of PFOA exposure in water and air) by assuming that cases lived at the address of diagnosis for 10 years.

The study found statistically significant (where CIs did not cross one) AORs for the following:

- Kidney cancer:
    - 2.0 (1.3-3.1) for residents living in Tuppers Plains water district. However, the AOR for the highest exposed district, Little Hocking, was not statistically significant (1.7, 95% CI: 0.9-3.3).
    - When examining the OH serum level results, AOR of 2.0 (1.3-3.2) in the high exposure group (30.8-109 ng/mL) (n=22 cases), with a non-significant AOR of 2.0 (1.0-3.9) in the very high exposure group (110-655 ng/mL) (n=9 cases).
- Lung cancer:

[26] As part of a settlement from a large class action lawsuit against DuPont, the C8 Science Panel was established to investigate potential health effects resulting from PFOA exposure, and a 1-year cross-sectional survey (2005-2006) was conducted among >69,000 residents with ≥ 1-year residency in public water districts contaminated by PFOA (i.e. the latter referred to as the C8 Health Project, which was previously described). Measured mean PFOA levels in public drinking water supplies at the time of the survey ranged from 0.03 µg/L in Mason, WV, to 3.49 µg/L in Little Hocking, OH, and in private drinking water, PFOA measured at levels of ≤ 22.1 µg/L. The median serum PFOA level in this cross-sectional population was 28.2 ng/mL, with a range of 0.2-22,412 ng/mL (Vieira et al. 2013).

  - 1.3 (1.1-1.5) for residents living in Mason water district, but not any other water district (which all had higher exposures).
  - When examining the OH serum level results, no elevated AORs were found for any exposure level category.
- Testicular cancer:
  - 5.1 (1.6-15.6) for residents living in Little Hocking (n=8 cases).
  - When examining the OH serum level results, no elevated AORs were found for any exposure level category.
  - Study authors indicate an inverse association between testicular cancer and lower exposure groups was observed and all of the estimates were imprecise due to small numbers of cases.
- Brain cancer:
  - When examining the OH serum level results, AOR of 1.8 (1.1-3.2) in the medium exposure group (12.9-30.7 ng/mL) (n=16 cases), but no dose-response as there were no cases found in the very high exposure group and no elevated AOR in the high exposure group.

The authors indicated AORs for lower exposure categories generally did not support a dose-response relationship for any cancers. Results were very similar for associations with the cumulative exposure measure and for exposure estimates that did not account for latency, which were highly correlated with exposure estimates that assumed a 10-year latency (Spearman's rank correlation r=0.997, p<0.001).

The authors state that a limitation of their study is the use of other types of cancer as controls (referents), where referent cancers were assumed not to be associated with exposure to PFOA. The water district and very high exposure group analyses were limited by small numbers of individual cancer cases. In addition, only limited covariates could be adjusted for due to availability of data. Authors were unable to adjust for other risk factors of potential interest.

Another limitation is that exposure estimates in the study were not individual exposure estimates but rather dependent on residency location at time of cancer diagnosis, along with mean/average values of exposure as measured over the course of one year. This could lead to exposure misclassification, but every effort was made by the study authors to reduce this risk.

The study authors concluded that the geographic analyses of cancer registry data in their study provide some evidence that higher PFOA serum levels may be associated with certain cancers. The association in the highest PFOA exposure group was largest but very imprecise for testicular cancer, and smaller but more precise for kidney cancer. They indicate the data contributes to evidence for the conclusion of the C8 Science Panel of a probable link between PFOA exposure and testicular and kidney cancers.

US EPA (2024a) used the association between kidney cancer and PFOA serum concentrations from the Vieira et al. (2013) study for dose-response modelling. A cancer slope factor (CSF) of 0.00401 $(ng/kg/day)^{-1}$ was calculated by US EPA (2024a) (excluding the highest exposure group, as this gave a better fit for the data) using non-threshold linear regression. This is a policy decision that US EPA makes for all substances that are regarded as probable carcinogens. However, experimental animal data (Butenhoff et al. 2012a, summarised in **Section 6.2.2**) and data showing lack of genotoxicity at non-cytotoxic concentrations (ATSDR 2021) support a threshold mode of action for carcinogenesis. Thus, calculation of a CSF for PFOA is not in line with Australian science policy.

Although the data in Vieira et al. (2013) suggest there may be an association between PFOA exposure and kidney cancer (and perhaps testicular cancer), the analysis is not supported by a clear dose-response and a threshold for the potential associations cannot be readily discerned. This, together with Australian science policy considerations with respect to assessment of non-genotoxic carcinogens, indicate that the study by Vieira et al. (2013) is not considered to provide sufficient information for use as a key study for derivation of a TRV.

Therefore, the US EPA (2024a) assessment of Vieira et al. (2013) is not suitable for adoption/adaption in the Australian context and the study has not been included in the candidate guidance/guideline value derivation for PFOA in **Section 6.3**.

6.2.14 Wikström et al. (2020) – used by US EPA (2024a, b)

The study by Wikström et al. (2020) was already summarised and discussed in **Section 5.2.8** with respect to both PFOS and PFOA.

6.3 Candidate guidance/guideline values for PFOA

As indicated in preceding sections, a number of additional studies (summarised in **Sections 6.2.1** to **6.2.14**) that had not been previously explicitly considered / evaluated in the FSANZ (2017) review of PFOA or the 2024 PFAS Review were used by US EPA (2024a) or Burgoon et al. (2023) as critical or candidate studies for derivation of PFOA guidance values. Of those studies, five experimental animal studies (Abbott et al. 2007, Butenhoff et al. 2012a, Dewitt et al. 2008, NTP 2023, Song et al. 2018) were considered potentially suitable for adoption/adaption for candidate DWG derivation in the Australian context.

The critical endpoints chosen by US EPA (2024a) or Burgoon et al. (2023) from the studies differ depending on the study (see **Table 6-4**). Both reviews met a high proportion of technical / administrative criteria for potential adoption/adaption into the Guidelines (**Section 6.1**). However, it is noted that, due to various considerations, the confidence in the resulting adapted candidate guideline values ranges from low to high.

The candidate / critical studies from the two reviews have different endpoints for derivation of guidance values, at times have used slightly different toxicokinetic adjustment factors for converting an animal serum concentration to a human dose, and the choices of uncertainty factors also differ between the two reviews (see **Table 6-4**).

With respect to the relative source contribution (RSC) factor, the current factor employed in derivation of the DWGs for PFOS, PFHxS and PFOA in the Guidelines is 0.1 (i.e. 10%) which is also the default factor for the Australian context. It is noted US EPA typically uses an RSC of 0.2 (i.e. 20%) when deriving DWGs but do not provide the rationale for this value with respect to PFAS (Burgoon et al. 2023 did not derive DWG from the guidance values). It is also noted the final DWG recommended by US EPA (2024c, d) is based on practical considerations rather than a health-based value. Thus, the default factor of 0.1 has been retained in calculating the potential resulting DWGs for PFOA using the guidance values in **Table 6-4**, noting that it yields a lower guideline value than use of an RSC of 0.2.

Also presented in **Table 6-4** is the derivation of the current Australian DWG for PFOA of 560 ng/L. The underpinning study on which the existing Australian PFOA guideline value is based (Lau et al. 2006) was evaluated in the 2024 PFAS Review to have high confidence.

Table 6-4 Potential drinking water guideline values (ng/L) resulting from adaption of PFOA guidance values from different jurisdictions [(1)]

Parameter	NHMRC and NRMMC 2011, FSANZ 2017b, DOH 2017	Abbott et al. 2007 – used by Burgoon et al. 2023	Butenhoff et al. 2012a – candidate study in US EPA 2024a	Dewitt et al. 2008 – candidate study in US EPA 2024a	NTP 2023 – candidate study in US EPA 2024a	Song et al. 2018 – candidate study in US EPA 2024a
Critical study	Lau et al. 2006	Abbott et al. 2007	Butenhoff et al. 2012a	Dewitt et al. 2008	NTP 2023	Song et al. 2018
Study population	Mice	Mice	Rats	Female mice	Rats	Pregnant mice
Form of PFOA studied	PFOA Ammonium salt (98.9% linear / 1.1% branched)	PFOA (ammonium salt; >98% pure)	Ammonium PFOA (ammonium salt of PFOA, 97.2% purity)	Ammonium-PFOA (≥98% pure)	PFOA (> 98% purity)	PFOA (>98% purity)
Exposure route	Oral (gavage)	Oral (gavage)	Oral (diet)	Oral (drinking water)	Oral (diet)	Oral (gavage)
Study timeframe	Throughout pregnancy (GD1-17)	Throughout pregnancy (GD1-17)	2 years	15 days, 15 days recovery	Perinatal (gestational + pre-weaning developmental stages) and post-weaning stages of development	Throughout pregnancy (GD1-17)
Critical Effect	↓ pre-weaning growth rate in pups	↓ pup survival	Microscopic anatomic pathological evidence of hepatotoxicity & Leydig cell tumours	Reduction in IgM response to SRBC (7% *cf.* controls at LOAEL)	Non-neoplastic: Hepatocellular necrosis (Neoplastic: Pancreatic acinar adenomas & adenocarcinomas) [(11)]	↓ pup survival

Parameter		NHMRC and NRMMC 2011, FSANZ 2017b, DOH 2017	Abbott et al. 2007 – used by Burgoon et al. 2023	Butenhoff et al. 2012a – candidate study in US EPA 2024a	Dewitt et al. 2008 – candidate study in US EPA 2024a	NTP 2023 – candidate study in US EPA 2024a	Song et al. 2018 – candidate study in US EPA 2024a
Serum Point of Departure (mg/L)		NOAEL = 35.1	NOAEL = 10.4	$BMDL_{4RD}$ = of 27,089 (Area under the curve)	NOAEL = 45.3 (7)	$BMDL_{10RD}$ = 26.9 ($BMDL_{10RD}$ = 15.2)	$BMDL_{0.5\ SD}$ = 12.3
Clearance Factor (L/kg-day)		0.00014 (*back-calculated from POD HED*)	0.00023	Not stated	0.00012	0.000128	a) 0.000052 (*back-calculated from US EPA POD_{HED})* (2) b) 0.00012 (clearance as given by US EPA)(2)
Point of Departure HED (mg/kg/day)		0.0049	0.0024	0.00475	0.0054	0.0034 (0.0019)	a) 0.00064 (2) b) 0.0015 (2)
Uncertainty factors	UF_A	3	3	*3* (6)	3	3	3
	UF_H	10	25.2 (4)	*10* (6)	10	10	10
	$UF_{Subchronic}$	1	1	1	10	1	1
	$UF_{database}$	1	1	1	1	1	1
	$UF_{composite}$	30	75.6	*30* (6)	300	30	30
Health-based guidance value (ng/kg/day)		160	32	158	18	115 (65) (11)	a) 21 (2) b) 49 (2)
Relative source contribution (RSC) to drinking water		0.1	0.1	0.1	0.1	0.1	0.1
Resulting adaption to a Health-based DWG (3) (ng/L)		560	111	554	63	402 (227)	a) 75 (2) b) 172 (2)

Parameter	NHMRC and NRMMC 2011, FSANZ 2017b, DOH 2017	Abbott et al. 2007 – used by Burgoon et al. 2023	Butenhoff et al. 2012a – candidate study in US EPA 2024a	Dewitt et al. 2008 – candidate study in US EPA 2024a	NTP 2023 – candidate study in US EPA 2024a	Song et al. 2018 – candidate study in US EPA 2024a
Confidence in candidate guideline value	High (7)	Low (5)	Medium (9)	Medium (10)	High (12)	Low (8)

DWG = Drinking Water Guideline; BMDL = Lower Benchmark Dose; HED = Human Equivalent Dose; GD =Gestation Day. $BMDL_{4RD}$ = Lower Benchmark Dose for a 4% response level. SRBC = Sheep Red Blood Cells. IgM = Immunoglobulin M. UF_A = Uncertainty factor for extrapolation from animals to humans; UF_H = Uncertainty factor for human variability; $UF_{Subchronic}$ = Uncertainty factor for use of subchronic study instead of a chronic study; $UF_{composite}$ = Composite (i.e. total) uncertainty factor; $UF_{database}$ = Uncertainty factor to account for the limited database of toxicological studies. ↓ = Decreased. ↑ = Increased. APFO = ammonium perfluorooctanoate.

(1) As discussed in **Section 6.2** for PFOA, there are various reasons why the epidemiological information for associations of PFAS serum concentrations with various endpoints is not considered suitable in the Australian context for derivation of guidance values for PFAS. For this reason, the epidemiological studies have not been included in this table.

(2) As discussed in **Section 6.2.9**, multiplying the POD of 12,300 ng/mL by the clearance value of 0.12 mL/kg/d given by US EPA (2024a) gives 1.5 µg/kg/day, i.e. this differs from the result given as the POD_{HED} for this study of 0.64 µg/kg/day in Table 4-8 of US EPA (2024a). The difference for this result is not clear from SLR's reading of the agency documentation. For this reason, both POD_{HED} values are shown in this table.

(3) Adaption of guidance value has been undertaken using the default assumptions for derivation of DWGs in Australia using the following equation as outlined in NHMRC (2021):

DWG (ng/L) = [Guidance value (ng/kg bw/day) x 70kg (adult) x 0.1 for adult] ÷ 2 L/day for adult

(4) Burgoon et al. (2023) used a default human toxicodynamic uncertainty factor of 3, and chemical-specific human toxicokinetic uncertainty factor of 8.4 [0.79 ml/day/kg arithmetic mean clearance of average group from Zhang et al. (2013, Table 2) ÷ 0.094 ml/day/kg arithmetic 95% lower bound clearance of sensitive group from Zhang et al. (2013, Table 2)]. The use of a combined human toxicodynamic and toxicokinetic uncertainty factor of (3 x 8.4=) 25.2 is not in line with what Australian jurisdictions have used in the past to express human variability (i.e. a default factor of 10). Nevertheless, Burgoon et al. (2023) have used a data-driven toxicokinetic uncertainty factor which is considered to be appropriate and in line with guidance from IPCS (2005) on development of chemical-specific uncertainty factors.

(5) As discussed in **Section 6.2.1**, the reliability of the Abbott et al. (2007) study for human health risk assessment purposes is considered to be low due to the high background rate of litter loss in the controls, the high level of litter loss at doses greater than 1 mg/kg bw/day, the lack of clear reporting on maternal mortality, the variable statistical power across the different dose groups, the limited descriptions of the study design and the lack of historical control data for the strain of mouse used.

(6) US EPA (2024a) only derived a non-threshold guidance value (in the form of a cancer slope factor) using the Butenhoff et al. (2012a) study for PFOA. As discussed in **Section 6.2.2,** data do not support a non-threshold mode of action for the Leydig cell tumours observed in male rats in the study, therefore the POD_{HED} derived by the US EPA (2024a) was adapted to the Australian context with the use of a standard uncertainty factor of 30 (as used in the current Australian guidance value for PFOA).

(7) As discussed in **Section 6.2.4**, the POD_{HED} derived by US EPA (2024a) from the NOAEL in dose response study 2 from Dewitt et al. (2008) was considered to be more appropriate to use for derivation of a candidate guidance value, since this study used tighter dose spacing and found no adverse effects at the dose administered (which is higher than the $BMDL_{1SD}$ modelled from dose response study 1). Although no serum concentrations were provided for dose response study 2 in Dewitt et al. (2008), US EPA (2024a, Appendix) provided serum concentrations at each administered dose.

Parameter	NHMRC and NRMMC 2011, FSANZ 2017b, DOH 2017	Abbott et al. 2007 – used by Burgoon et al. 2023	Butenhoff et al. 2012a – candidate study in US EPA 2024a	Dewitt et al. 2008 – candidate study in US EPA 2024a	NTP 2023 – candidate study in US EPA 2024a	Song et al. 2018 – candidate study in US EPA 2024a
(8) Considered to be of low confidence as the Song et al. (2018) study focused on specific endpoints of interest in mice, therefore it did not follow standardised protocols for developmental toxicity experiments screening for a larger suite of endpoints. The reported serum PFOA concentration in the paper is also considered unreliable. Although no statistical difference was reported between litter sizes at PND0, statistical analysis of the various endpoints did not include the litter in the model to guard against an inflated Type I error rate (**Section 6.2.9**). (9) Overall the resulting adapted guideline value is considered to be of medium confidence, as the underpinning study was well-conducted but lacked serum PFOA measurements reported in the study (it is noted US EPA 2024a provided serum data for the study; it is unclear whether this is modelled or measured data) (see also **Section 6.2.2**). (10) The Dewitt et al. (2016) study appears to have been conducted appropriately and incorporated a recovery phase; it evaluated a number of parameters including immune system markers. There was a clear dose response observed for reduction in IgM response to SRBC in female mice. The candidate guideline value resulting from adaption of the US EPA (2024a) candidate guidance value (incorporating the use of a NOAEL instead of a $BMDL_{1SD}$ value) is considered to be of medium confidence for these reasons (**Section 6.2.4**). (11) US EPA (2024a) used the NTP (2023) study to derive a candidate guidance value based on non-neoplastic effects (i.e. liver cell necrosis), however the agency also present BMD modelling for the neoplastic effects. The $BMDL_{10RD}$ for neoplastic effects has also been presented in brackets in this table, although it is recognised that the acinar pancreatic neoplastic lesions are unlikely to be relevant to humans based on currently available information (see **Section 6.2.8**). Although there is uncertainty with respect to the dose at which non-neoplastic hepatic effects may occur in humans and it is recognised by SLR that rats are likely more sensitive to this effect that humans, SLR considers there is insufficient information to rule out human relevancy of this effect based on currently available information. (12) The NTP (2023) study is a high-quality study and has been conducted appropriately. Thus, the candidate guideline value resulting from adaption of the US EPA (2024a) candidate guidance value (and POD for non-neoplastic effects) is considered to be of high confidence.						

The candidate PFOA DWGs derived by adapting existing guidance values for this PFAS range from 63 to 554 ng/L depending on the endpoint selected and uncertainty factors used, with the existing DWG at 560 ng/L.

The candidate values are derived from studies ranging from low to high confidence; the values of 227 ng/L and 402 ng/L are derived from a study with high confidence, as is the existing guideline value of 560 ng/L, whereas other values are derived from studies with low or medium confidence. Nevertheless, it is recognised that the candidate guideline value of 227 ng/L is based on the development of acinar pancreatic neoplastic lesions in rats, which are unlikely to be relevant to humans based on currently available information. The value of 402 ng/L is based on non-neoplastic hepatic necrosis in rats. Although there is also uncertainty with respect to the dose at which non-neoplastic hepatic necrosis may occur in humans and it is recognised by SLR that rats are likely more sensitive to this effect than humans, SLR considers there is insufficient information to rule out human relevancy of this effect based on currently available information.

In Australian distributed drinking waters, PFOA concentrations generally may range up to 10 ng/L in various locations (2024 PFAS Review). This maximum concentration is below the candidate DWGs of 63 to 554 ng/L and well below the existing Australian guideline value of 560 ng/L. Due to the uncertainty factors and small RSC incorporated into the derivation of the candidate DWGs and the existing Australian DWG, PFOA is unlikely to present a human health risk from distributed drinking water in uncontaminated regions of Australia. However, there are many sites of PFAS contamination in Australia, and, if water from these contaminated sites is used as a local source of drinking water (e.g. backyard bore in rural location where distributed water is not available), PFOA may be present at concentrations greater than the candidate DWGs and the existing Australian DWG in these cases.

7.0 Conclusions

The targeted expanded evaluation conducted in this report for PFOS and PFOA focused on three recent reviews (US EPA 2024a, b; Burgoon et al. 2023) and critically evaluated numerous studies that had been used as key or candidate studies for derivation of guidance values that were considered for potential adoption/adaption into the Guidelines. The studies evaluated in this addendum were those studies not previously evaluated / considered by FSANZ (2017, 2021) or the 2024 PFAS Review with respect to the PFAS subject of this addendum.

A summary of the conclusions and DWG options from potential adoption/adaption of suitable information for PFOS and PFOA is provided in **Table 7-1**. Bolded guideline values in the table below are considered to be most relevant to the Australian context in terms of confidence in the underlying study, selection of uncertainty factors and endpoints.

Table 7-1 Conclusions and DWG options from potential adoption/adaption of suitable information for PFOS and PFOA from US EPA (2024a, b) and Burgoon et al. (2023)

PFAS	Candidate DWGs (ng/L) (1)	Conclusion
PFOS	• **3.4 or 77** (2) ng/L using 28-day high confidence toxicology study in rats (NTP 2022) (candidate study in US EPA 2024b), or • **27 or 95** (2) ng/L using medium confidence developmental	Any of these values would be considered to be potentially suitable and conservative as they all incorporate an uncertainty factor ranging from 30 to 300 in TRV development, endpoints which are the equivalent of a dose resulting in no adverse effects, as well as a relative source contribution of 10% of the TRV to drinking water.

PFAS	Candidate DWGs (ng/L) [1]	Conclusion
	toxicology study in mice (Zhong et al. 2016) (candidate study in US EPA 2024b). SLR considered the use of the serum NOAELs from the NTP (2022) and Zhong et al. (2016) studies to be a more appropriate serum POD than the modelled serum BMDs derived by US EPA (2024b), due to the large discrepancies between the measured and modelled values. It is suggested the information does not warrant revision of the existing Australian guideline value for PFOS (70 ng/L).	The candidate guideline values of 3.4 ng/L (from a high confidence study) and 27 ng/L (from a medium confidence study) are based on the same critical endpoints as the candidate guideline values of 77 ng/L and 95 ng/L, respectively, but the former were derived using serum points of departure modelled by the US EPA whereas the latter have been derived using serum points of departure measured in the experimental studies. The difference between modelled and measured values could not be readily reconciled, therefore the use of the measured values from the studies are considered to be associated with less uncertainty. As the candidate guideline values using measured serum PODs (77 or 95 ng/L) are higher than the existing Australian guideline value, it is suggested the updated information does not warrant revision of the existing Australian guideline value for PFOS (70 ng/L). Concentrations of PFOS in most distributed drinking water in Australia can range up to 6 ng/L in Queensland and Sydney (2024 PFAS Review) but up to 16 ng/L in Australia according to WHO (2022). These concentrations are below the existing Australian DWG. Due to the uncertainty factors and small RSC incorporated into the derivation of the candidate DWGs and the existing Australian DWG, PFOS is unlikely to present a human health risk from distributed drinking water in uncontaminated regions of Australia. However, there are many sites of PFAS contamination in Australia, and, if water from these contaminated sites is used as a local source of drinking water (e.g. backyard bore in rural location where distributed water is not available), PFOS may be present at concentrations greater than the candidate DWGs and existing Australian DWG in these cases.
PFOA	• **63** [2] ng/L using 15-day medium confidence toxicology study in mice (Dewitt et al. 2008) (candidate study in US EPA 2024a), or • 75 or 172 ng/L using low confidence developmental toxicology study in mice (Song et al. 2018) (candidate study in US EPA 2024b), or • 111 ng/L using low confidence developmental toxicology study in mice (Abbott et al. 2007) (used by Burgoon et al. 2023), or • **227** or **402** [2] ng/L using high confidence 2-year rat study	Any of these values would be appropriately conservative as they all incorporate uncertainty factors ranging from 30 to 300 in TRV development, an endpoint which is the equivalent of a dose resulting in no adverse effects, as well as a relative source contribution of 10% of the TRV to drinking water. It is therefore suggested the updated information is of high enough quality to warrant revision of the existing Australian guideline value for PFOA (560 ng/L). In Australian distributed drinking waters, PFOA concentrations generally may range up to 10 ng/L in various locations (2024 PFAS Review). This maximum concentration is below the candidate range of DWGs of 63 to 554 ng/L and well below the existing Australian guideline value of 560 ng/L. Due to the uncertainty factors and small RSC incorporated into the derivation of the candidate DWGs and the existing Australian DWG, PFOA is unlikely to present a human

PFAS	Candidate DWGs (ng/L) (1)	Conclusion
	(NTP 2023) (candidate study in US EPA 2024a), or • **554** (2) ng/L using medium confidence 2-year rat study (Butenhoff et al. 2012a) (candidate study in US EPA 2024a). The values of 227 ng/L and 402 ng/L are derived from a study with high confidence. Nevertheless, it is recognised that the candidate guideline value of 227 ng/L is based on the development of acinar pancreatic neoplastic lesions in rats, which are unlikely to be relevant to humans based on currently available information. The value of 402 ng/L is based on non-neoplastic hepatic necrosis in rats. Although there is also uncertainty with respect to the dose at which non-neoplastic hepatic necrosis may occur in humans and it is recognised by SLR that rats are likely more sensitive to this effect than humans, SLR considers there is insufficient information to rule out human relevancy of this effect based on currently available information. It is suggested the information is of high enough quality to warrant revision of the existing Australian guideline value for PFOA (560 ng/L).	health risk from distributed drinking water in uncontaminated regions of Australia. However, there are many sites of PFAS contamination in Australia, and, if water from these contaminated sites is used as a local source of drinking water (e.g. backyard bore in rural location where distributed water is not available), PFOA may be present at concentrations greater than the candidate DWGs and the existing Australian DWG in these cases.

DWG = Drinking Water Guideline. TRV = Toxicity Reference Value. UF = Uncertainty Factor. RSC = Relative Source Contribution.

(1) Values that are **bolded** are considered to be most relevant to the Australian context in terms of selection of uncertainty factors and endpoints, and represent those of medium and high confidence (see detailed discussions in **Section 5.0** to **6.0** for further information).

(2) These are values that would result from a change to the selected uncertainty factors and/or endpoint type by a particular jurisdiction; the suggested changes are considered to be in line with the Australian context such as to provide consistency with the approach taken to uncertainty considerations by FSANZ (2017).

A review of different approaches used currently or in the past by international jurisdictions to evaluate / assess PFAS mixtures in drinking water revealed the approaches can be grouped into the following five categories; i) hazard index (HI) approaches, ii) RPF approaches, iii) M-BMD approaches, iv) practical (non-health) based approaches, and v) surrogate approaches. Each has its own pros and cons, and some are more data-intensive than others. Based on the review of these approaches, a PFAS mixture options assessment was presented which outlines four possible options for developing a PFAS mixture DWG in Australia, noting the options provided are not necessarily exhaustive.

As a potential way forward, the HI approach is most amenable for use in Australia. However, to establish an approach that is applicable to more than just a select number of PFAS for

which there are DWGs, the HI approach is suggested to be combined with the surrogate approach as it is still health-based, does not require marked amounts of data, and can be readily explained and applied. A technical document would be required to derive the additional DWG and justify the approach (including explaining how it should be used). Alternatively, a simple surrogate approach could be easily applied to a large number of measurable PFAS, noting this approach is not data-driven and would be highly conservative.

8.0 Review Team

Name	Position	Responsibilities
Ms Tarah Hagen, MSc, DABT, FACTRA	Technical Director – Toxicologist & Risk Assessor, SLR	Principal report author and technical oversight
Mr Giorgio De Nola, MSc, RACTRA	Principal Consultant – Toxicologist & Risk Assessor, SLR	Data extraction, internal peer review
Dr Rhian Cope, PhD, DABT, DABVT, FACTRA	Technical Director – Toxicologist & Risk Assessor, SLR	Assistance with study evaluations

9.0 Declared Interests

Team Member	Declaration of Interest
Ms Tarah Hagen	As part day-to-day consulting activities at SLR Consulting and ToxConsult Pty Ltd, Ms Hagen has: • Provided the report "Assessment of International and National Agency Processes for Deriving HBGVs and DWGs" to NHMRC. This has been used to inform the methodological framework for the 2024 PFAS Review and this review as described in the Research Protocol of the 2024 PFAS Review. • Been involved in preparation and/or review of draft and final Technical and Evaluation Reports for previous consultancies with NH&MRC (evidence evaluations for 11 inorganic chemicals, full reviews for 4 inorganic chemicals, 2024 PFAS Review). • Conducted numerous health risk assessments for clients where PFAS were the chemicals of potential concern requiring assessment. • Was co-author of the review *Drew, R. and Hagen, T. (2016) Immunomodulation by PFASs: A brief literature review. ToxConsult document ToxCR300816.* (As quoted in FSANZ 2017). This paper is mentioned in the 2024 PFAS Review in the context of the previous review by FSANZ (2017).
Mr Giorgio De Nola	As part day-to-day consulting activities at SLR Consulting Mr De Nola has: • Been involved in preparation and/or review of draft and final Technical and Evaluation Reports for previous consultancies with NH&MRC (evidence evaluations for 11 inorganic chemicals, full reviews for 4 inorganic chemicals, 2024 PFAS Review). • Been involved in numerous health risk assessments as part of contaminated land audits as well as for other clients where PFAS were chemicals of potential concern requiring assessment.
Dr Rhian Cope	No interest to declare.

10.0 Acknowledgements

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Appendix A Data Extraction Tables – Health-based Guidance/Guidelines

Addendum to PFAS Evidence Evaluation for Australian Drinking Water Guidelines Chemical Fact Sheets

Addendum / Work Expansion for 2024 NHMRC PFAS Review of Australian Health-based Guideline Values

National Health and Medical Research Council

SLR Project No.: 640.031365.00001

17 October 2024

SLR

A.1 PFOS Existing Health-based Guidance

A.1.1 US EPA (2024b, c, d)

Agency Report Reference: US EPA (2024c). 40 CFR Parts 141 and 142: PFAS National Primary Drinking Water Regulation, Final Rule. Federal Register, Vol. 89, No. 82. Friday April 26, 2024.
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Supporting Documentation: US EPA (2024b). FINAL Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts, United States Environmental Protection Agency. April 2024. EPA Document No. 815R24007. https://www.epa.gov/system/files/documents/2024-04/main_final-toxicity-assessment-for-pfos_2024-04-09-refs-formatted_508c.pdf.

General Information	Date of data extraction	09 July 2024
	Authors	U.S. Environmental Protection Agency, Office of Water (4304T). Office of Science and Technology. Health and Ecological Criteria Division, Washington, DC 20460.
	Publication date	April 2024
	Literature search timeframe	For the literature searches, the search strings focused on the chemical name (PFOS and its related salts) with no limitations on lines of evidence (i.e. human/epidemiological, animal, *in vitro*, *in silico*) or health outcomes. The EPA conducted a literature search in 2019 (covering January 2013 through April 11, 2019), which was subsequently updated by a search covering April 2019 through September 3, 2020 prior to SAB review of the draft assessment (2020 literature search), a third search covering September 2020 through February 3, 2022 prior to release of the draft assessment for public comment (2022 literature search), and a final supplemental search covering February 4, 2022 through February 6, 2023.
	Publication type	Agency Guidance Value Document
	Peer reviewed?	The agency sought peer review from the U.S. Environmental Protection Agency (EPA) Science Advisory Board (SAB) PFAS Review Panel on key scientific issues, including the systematic review approach for evaluating health effects studies, the derivation of oral toxicity values, the relative source contribution (RSC), and the cancer classification for PFOS.
	Country of origin	US
	Source of funding	Not stated.
	Possible conflicts of interest	Not stated.
Health considerations	Guideline value type (e.g. oral TRV, drinking water guideline)	• Short-term and Chronic oral reference dose (RfD). • Maximum Contaminant Level (MCL) in drinking water. • Cancer slope factor (CSM).

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	Exposure timeframe	Lifetime. The overall reference dose (RfD) for PFOS is applicable to both short-term and chronic risk assessment scenarios.
	Critical human health endpoint	The co-critical effects are as follows: *1) Decreased infant birth weight* (Wikström et al. 2020). *2) Increased serum total cholesterol in adults* (Dong et al. 2019). *3) Combined hepatocellular adenomas and carcinomas* (Butenhoff et al. 2012b, Thomford 2002b).
	Justification provided by agency for critical endpoint	The critical studies that serve as the basis of the RfD are all medium or high confidence epidemiological studies. The critical studies are supported by multiple other medium or high confidence studies in both humans and animal models and have health outcome databases for which EPA determined evidence indicates that oral PFOS exposure is associated with adverse effects. Additionally, the selected critical effects can lead to clinical outcomes in a sensitive lifestage (children) and therefore, the overall RfD is expected to be protective of all other noncancer health effects in humans. The available evidence indicates there are effects across immune, developmental, cardiovascular, and hepatic organ systems at the same or approximately the same level of PFOS exposure. In fact, candidate RfDs within the developmental and cardiovascular outcomes are the same value (i.e. 1×10^{-7} mg/kg/day). Therefore, EPA has selected an overall RfD for PFOS of 1×10^{-7} mg/kg/day. The developmental and cardiovascular RfDs based on endpoints of decreased birth weight and increased total cholesterol, respectively, serve as co-critical effects for this RfD. Notably, the RfD is protective of effects that may occur in sensitive populations (i.e. infants and children), as well as immune and hepatic effects that may result from PFOS exposure. Further justifications are as follows: *1) Decreased infant birth weight (developmental effects):* Three high confidence epidemiological studies were considered for candidate RfD derivation for the endpoint of decreased birth weight (Wikström et al. 2020; Sagiv et al. 2018; Darrow et al. 2013). These candidate studies assessed maternal PFOS serum concentrations before birth (Darrow et al. 2013) or primarily in the first trimester (Wikström et al. 2020; Sagiv et al. 2018) minimising concerns for bias due to pregnancy-related haemodynamic effects. All three studies were high confidence prospective cohort studies with many strengths

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		including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias. Between these three studies, PFOS exposure concentrations observed in Wikström et al. (2020) are more comparable to current exposure levels in the United States and therefore may be more relevant to the general population than the candidate RfD derived from Sagiv et al. (2018) or Darrow et al. (2013). Additionally, the BMDL derived from Wikström et al. (2020) was based on a statistically significant regression parameter. For these reasons, the RfD for decreased birth weight from Wikström et al. (2020) was selected as the basis for the organ-specific RfD for developmental effects. The resulting health outcome-specific RfD is 1×10^{-7} mg/kg/day. Note that all three candidate RfDs based on epidemiological studies for the developmental outcome were within one order of magnitude of the selected health outcome-specific RfD. 2) *Increased serum total cholesterol in adults (cardiovascular outcomes):* Two medium confidence epidemiological studies were selected for candidate RfD derivation for the endpoint of increased total cholesterol (Dong et al. 2019; Steenland et al. 2009). These candidate studies offer a variety of PFOS exposure measures across various populations. Dong et al. (2019) investigated the NHANES population (2003–2014), while Steenland et al. (2009) investigated effects in a high-exposure community (the C8 Health Project study population). Both of these studies excluded individuals prescribed cholesterol medication which minimises concerns of confounding due to medical intervention. The candidate RfD for increased total cholesterol (TC) from Dong et al. (2019) was ultimately selected for the health outcome-specific RfD for cardiovascular effects as there is marginally increased confidence in the modelling from this study. Steenland et al. (2009) presented analyses using both PFOS and TC as categorical and continuous variables. The results using the natural log transformed TC and the natural log transformed PFOS were stated to fit the data slightly better than the ones using untransformed PFOS. However, the dramatically different changes in regression slopes between the two analyses by Steenland et al. (2009) resulting in different PODs raise concerns about the appropriateness of using the data for RfD derivation. Therefore, the resulting health outcome-specific RfD based on results from Dong et al. (2019) is 1×10^{-7} mg/kg/day. Note that the candidate RfDs for the cardiovascular outcome were the same. 3) *Combined hepatocellular adenomas and carcinomas:* This endpoint was selected because: 1) there is concordance

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		between the observed hepatocellular tumours in rats with the liver cancer observed in human epidemiological studies; 2) the derived candidate cancer slope factor (CSF) is representative of both malignant and benign tumours; 3) the endpoint is supported by the observation of hepatocellular adenomas in male rats; 4) there was a statistically significant increase in tumour incidence in the highest dose group; and 5) a statistically significant trend of increased incidence with increasing PFOS concentrations across dose groups.
	Critical study(ies) underpinning point of departure	The co-critical studies are as follows: 1) *Decreased infant birth weight (High confidence): Wikström S., Lin P. I., Lindh C. H., Shu H. and Bornehag C. G. (2020). Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatr Res 87(6): 1093-1099.* 2) *Increased serum total cholesterol in adults (medium confidence): Dong Z., Wang H., Yu Y. Y., Li Y. B., Naidu R. and Liu Y. (2019). Using 2003-2014 U.S. NHANES data to determine the associations between per- and polyfluoroalkyl substances and cholesterol: Trend and implications. Ecotoxicol Environ Saf 173: 461-468..* 3) *Combined hepatocellular adenomas and carcinomas (High confidence): Butenhoff, J.L.; Chang, S.C.; Olsen, G.W.; Thomford, P.J. (2012b). Chronic dietary toxicity and carcinogenicity study with potassium perfluorooctanesulfonate in Sprague Dawley rats. Toxicology 293: 1-15., / Thomford, P.J. (2002b). 104-week dietary chronic toxicity and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in rats (pp. 002148-002363). (Study No. 6329-183). Madison, WI: Covance Laboratories.*
	Species for critical study(ies)	1) *Decreased infant birth weight:* PFOS serum concentrations in first and second trimesters. 2) *Increased serum total cholesterol:* Males and females to age 20- 80 years. 3) *Combined hepatocellular adenomas and carcinomas:* Female rats.
	Point of departure type (e.g. NOAEL, LOAEL, $BMDL_{10}$, etc.)	• Lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in response ($BMDL_{5RD}$). • POD human equivalent doses (POD_{HEDs}). • Reference Dose (RfD).

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		• Benchmark dose level corresponding to the 95% lower confidence limit of a 10% change ($BMDL_{10}$).
	Point of departure value (include units)	*1) Decreased infant birth weight:* 7.7 ng/mL ($BMDL_{5RD}$) equivalent to 1.13 x 10^{-6} mg/kg/day (POD_{HED}). *2) Increased serum total cholesterol in adults:* 9.34 ng/mL ($BMDL_{5RD}$) equivalent to 1.20 x 10^{-6} mg/kg/day (POD_{HED}). *3) Combined hepatocellular adenomas and carcinomas:* 19.8 mg/L (AUC normalized per day (AUCavg)) ($BMDL_{10}$ Multistage Degree 1 Model) converted to 2.53 x10^{-3}mg/kg/day (POD_{HED}).
	Uncertainty factor(s) & rationale	EPA applied a composite uncertainty factor (UF_C) of 10 to the POD_{HEDs} from selected epidemiological studies (UF_C = 10: Composite $UF_C = UF_A \times UF_H \times UF_S \times UF_L \times UF_D$): • UF_A = 1: A UF_A of 1 is applied to effects observed in epidemiological studies as the study population is humans. • UF_H = 10: A UF_H of 10 is applied when information is not available relative to variability in the human population. • UF_S = 1: A UF_S of 1 is applied when effects are observed in adult human populations that are assumed to have been exposed to a contaminant over the course of many years. A UF_S of 1 is applied for developmental effects because the developmental period is recognised as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure. • UF_L = 1: A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL. • UF_D = 1: A UF_D of 1 is applied when the database for a contaminant contains a multitude of studies of adequate quality that encompass a comprehensive array of endpoints in various lifestages and populations and allow for a complete characterisation of the contaminant's toxicity.
	Guideline value (include units)	*1) Decreased infant birth weight:* RfD = 1.13 x 10^{-7} mg/kg/day rounded down to 1 x10^{-7} mg/kg/day (0.1 ng/kg/d). *2) Increased serum total cholesterol in adults:* RfD = 1.20 x 10^{-7} mg/kg/day rounded down to 1 x 10^{-7} mg/kg/day (0.1 ng/kg/d). *3) Combined hepatocellular adenomas and carcinomas:* CSF = 39.5 $(mg/kg/d)^{-1}$, NB: CSF = $BMDL_{10} \div POD_{HED}$. MCL: 4 ng/L, i.e. a practical quantification limit (PQL) (USEPA 2024c). Other relevant information:

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		• Equations used: RfD = POD_{HED} ÷ UF_C., CSF = $BMDL_{10}$ ÷ POD_{HED}. • CSF = Increase in Cancer Risk per 1 ng/(kg*d) increase in dose.
	Mode of action for critical health endpoint	1) *Decreased infant birth weight:* The available mechanistic studies suggest that the developing liver, developing heart, and placenta may be affected by PFOS at the molecular level (i.e. differential methylation of genes, gene expression changes, mitochondrial dysregulation), which may be related to developmental health effects described in Sections 3.4.4.1 and 3.4.4.2 of US EPA (2024b). Some effects tend to vary by sex or by developmental timepoint of outcome evaluation (e.g. early gastrulation, late gestation, lactation). Oxidative stress in parallel with epigenetic alterations in the placenta were consistently reported. 2) *Increased serum total cholesterol in adults:* The mechanisms underlying the positive associations between PFOS and serum TC, LDL, and blood pressure in humans have yet to be determined. Data from the C8 Health Project demonstrated that serum PFOS was positively associated with expression of genes involved in cholesterol mobilisation and transport (NCEH1 and PPARα) in samples from women, while there were no associations in men. The results for PFOS-induced changes in serum lipid levels contrast between rodents (generally decreased) and humans (generally increased). PFOS exposure led to upregulation of genes that encode fatty acid binding proteins in zebrafish, which play a role in lipid binding, particularly in the heart. Evidence is ultimately limited in regard to clear demonstration of mechanisms of alterations to serum lipid homeostasis caused by PFOS exposure. 3) *Combined hepatocellular adenomas and carcinomas:* The available mechanistic data continue to suggest that multiple MOAs may underlie the hepatocellular tumours observed after PFOS exposure. Specifically, the available studies provide varying levels of support for the role of several plausible MOAs: PPARα activation, CAR activation, HNF4α suppression, cytotoxicity, genotoxicity, oxidative stress, and immunosuppression: • *PPARα Activation*: There is considerable debate over the relevance of PFAS-induced hepatic tumours to human health. Exposure to some PFAS have been shown to activate PPARα, which is characterised by downstream cellular or tissue alterations in peroxisome proliferation, cell cycle control (e.g. apoptosis and cell proliferation), and lipid metabolism. Notably, human expression of

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		PPARα mRNA and protein is only a fraction of what is expressed in rodent models, though there are functional variant forms of PPARα that are expressed in human liver to a greater extent than rodent models. Therefore, for PPARα activators that act solely or primarily through PPARα-dependent mechanisms (e.g. Wyeth-14,643, di-2-ethyl hexyl phthalate), the hepatic tumorigenesis observed in rodents may be expected to be reduced in frequency or severity or not observed in humans. The published *in vivo* and *in vitro* literature suggests that PFOS is a relatively weak PPARα agonist compared with other known PPARα agonists such as PFOA. While *in vitro* PPARα activation assay results indicate overall effective activation of PPARα by PFOS, the magnitude of that activation has been found to be relatively lower than chemicals that induce toxicity primarily through PPARα activation (e.g. di-2-ethyl hexyl phthalate). There is *in vivo* rodent assay evidence of PFOS-induced PPARα-associated transcriptional and enzymatic responses (e.g. upregulation of Acox1 and acyl-CoA activity) as well. However, consistent with the *in vitro* activation assays, these *in vivo* responses were relatively weaker than PFOA and/or other PPARα activators and were often reported to be accompanied by transcriptional responses associated with other nuclear receptor signalling pathways (e.g. CAR and PPARγ), consistent with multiple modes of action. • CAR Activation: there is both *in vivo* and *in vitro* evidence that PFOS can activate CAR and initiate altered gene expression and associative events. Some studies, such as NTP (2022), report greater activation of CAR with PFOS treatment compared with PPARα, depending on the sex and/or model of interest. As with PPARα-mediated tumorigenesis, there are claims that CAR-mediated tumorigenesis is not relevant to humans because CAR activators such as phenobarbital have been shown to induce cell proliferation and subsequent tumorigenesis in rodents but do not induce cell proliferation in human cell lines. However, as outlined above, several studies have reported increased cell proliferation or markers of cell proliferation due to PFOS treatment in human cell lines. Further study is needed to understand the mechanistic underpinnings of PFOS-induced hepatic cell proliferation and whether it is related to CAR activation.

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		• HNF4α suppression: HNF4α is known as a master regulator of hepatic differentiation and plays a role in tumour suppression as well as general liver maintenance and function. Interestingly, PFOS exposure appears to downregulate HNF4α and its target genes. Studies utilising primary human hepatocytes, HepG2 cells, and *in vivo* mouse models have reported decreased HNF4α protein expression as well as corresponding changes in downstream HNF4α target genes with PFOS treatment. • Cytotoxicity: There is suggestive evidence that PFOS may act through a cytotoxic MOA. The available data indicate a corresponding dose response for cytotoxicity and the formation of liver tumours. • Genotoxicity: The available *in vivo* evidence suggests that exposure to PFOS at levels resulting in cytotoxicity (e.g. hepatotoxicity, bone marrow toxicity) can lead to secondary genotoxicity in target tissues. At this time, there are no generally accepted mechanistic explanations for PFOS directly interacting with genetic material. Additionally, while there is some *in vivo* evidence of PFOS-induced mutagenicity as primarily evidenced by micronuclei formation in rats, mice, and zebrafish, there are several uncertainties that limit the interpretation of these results. There is currently no robust evidence to support a mutagenic MOA for PFOS, though overall, genotoxicity cannot be ruled out as a potential MOA or key event in PFOS tumour formation. • Oxidative stress: PFOS appears to induce oxidative stress, another initiating event of carcinogens, particularly in hepatic tissues. Several studies in rats and mice showed evidence of increased oxidative stress and reduced capacity for defence against oxidants and oxidative damage in hepatic tissues. Results provide some support for disruption of the oxidative stress response in hepatic tissues leading to accumulation of reactive oxygen species (ROS) and subsequent oxidative damage. • Immunosuppression: It is difficult to discount immunosuppression as a potential MOA for PFOS, given the limited database for rats and stronger databases indicating immunosuppression in mice and humans.
	Genotoxic carcinogen?	• The available *in vivo* evidence suggests that exposure to PFOS at levels resulting in cytotoxicity (e.g. hepatotoxicity, bone marrow toxicity) can lead to secondary genotoxicity in target tissues. At this time, there are no generally accepted

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		mechanistic explanations for PFOS directly interacting with genetic material. Additionally, while there is some *in vivo* evidence of PFOS-induced mutagenicity as primarily evidenced by micronuclei formation in rats, mice, and zebrafish, there are several uncertainties that limit the interpretation of these results. There is currently no robust evidence to support a mutagenic MOA for PFOS, though overall, genotoxicity cannot be ruled out as a potential MOA or key event in PFOS tumour formation. • There is also limited evidence supporting additional potential MOAs of genotoxicity, immunosuppression, and oxidative stress. • The positive multi-site, multi-sex chronic cancer bioassay is supported by mechanistic data indicating that PFOS is associated with events generally known to be associated with tumour formation such as inducing nuclear receptor activation, cytotoxicity, genotoxicity, oxidative stress, and immunosuppression. Notes on carcinogenicity: • EPA reviewed the weight of the evidence and determined that PFOS is Likely to Be Carcinogenic to Humans, as "the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor Carcinogenic to Humans."
	Identified sensitive sub-populations	The selected critical effects can lead to clinical outcomes in a sensitive lifestage (children). The RfD is protective of effects that may occur in sensitive populations (i.e. embryo and foetus, infants, and young children), as well as hepatic effects in adults that may result from PFOS exposure. There is uncertainty about whether there are susceptible populations, such as certain racial/ethnic groups, that might be more sensitive to the health effects of PFOS exposure because of either greater biological sensitivity or higher exposure to PFOS and/or other environmental chemicals.
	Any non-health-based considerations?	Yes. The MCL is based on a PQL.
Risk Summary	Any risks to human health from drinking water identified in agency document?	Ingestion of drinking water is a potentially significant source of exposure to PFOS. Serum PFOS concentrations are known to be elevated among individuals living in communities with drinking water contaminated from environmental discharges. Under the Third Unregulated Contaminant Monitoring Rule (UCMR 3), 36,972 samples from 4,920 public water systems

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Supporting Documentation: US EPA (2024b). FINAL Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts, United States Environmental Protection Agency. April 2024. EPA Document No. 815R24007. https://www.epa.gov/system/files/documents/2024-04/main_final-toxicity-assessment-for-pfos_2024-04-09-refs-formatted_508c.pdf.

		(PWSs) were analysed. PFOS was found in 292 samples at 95 systems above the UCMR 3 minimum reporting level (40 ng/L). More than one-third of states that conducted nontargeted monitoring detected PFOA and/or PFOS at more than 25% of systems. Among the detections, PFOS concentrations ranged from 0.24 to 650 ng/L with a range of median concentrations from 1.21 to 12.1 ng/L. Monitoring data for PFOA and PFOS from states that conducted targeted monitoring efforts, including 15 states, demonstrate results consistent with the nontargeted state monitoring. Glassmeyer et al. (2017, as cited in US EPA 2024b) sampled source and treated drinking water from 29 drinking water treatment plants for a suite of emerging chemical and microbial contaminants, including 11 PFAS. PFOS was reported in source water at 88% of systems, with a median concentration of 2.28 ng/L and maximum concentration of 48.30 ng/L. Similarly, in treated drinking water, PFOS was detected in 80% of systems, with a median concentration of 1.62 ng/L and maximum concentration of 36.90 ng/L.
	Any emerging risks identified?	Mixture analysis is an emerging research area, and there is no scientific consensus yet on the best approach for estimating independent effects of PFOS within complex PFAS mixtures. Additionally, multipollutant analyses from studies included in this assessment did not provide direct evidence that associations between exposure to PFOS and health effects are confounded by or are fully attributable to confounding by co-occurring PFAS. A detailed discussion of statical approaches for accounting for co-occurring PFAS and results from studies performing multipollutant analysis is provided in Section 5.1.1 of the US EPA (2024b) review.
Any other relevant information that should be captured?		When assessing the associations between PFOS and a health effect of interest (e.g. decreased birth weight), there is concern for potential confounding by other PFAS when there is a strong correlation between the occurrence of PFOS and another PFAS and when the magnitude of the association between the co-exposure and the health effect is large. Note: Confounding only considered for birth weight changes. Overall, there is no evidence that the consistently observed associations between exposures to PFOS and the four priority noncancer health outcomes are confounded or are fully attributable to confounding by co-occurring PFAS.
Assessed in Appendix B?		Yes

A.2 PFOA Existing Health-based Guidance

A.2.1 US EPA (2024a, c, d)

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General Information	Date of data extraction	4 July 2024
	Authors	U.S. Environmental Protection Agency, Office of Water (4304T). Health and Ecological Criteria Division, Washington, DC 20460.
	Publication date	April 2024
	Literature search timeframe	US EPA assembled a database of epidemiological, animal toxicological, mechanistic, and toxicokinetic studies based on three main data streams: 1) literature published from 2013 through February 6, 2023 identified via literature searches conducted in 2019, 2020, 2022 and 2023 of a variety of publicly available scientific literature databases, 2) literature identified via other sources (e.g. searches of the grey literature, studies shared with US EPA by the SAB, studies submitted through public comment), and 3) literature identified in US EPA's 2016 Health Effects Support Document for Perfluorooctanoic Acid (PFOA).
	Publication type	Agency Guidance Value Document
	Peer reviewed?	Yes, the final toxicity assessment was peer reviewed by the US EPA SAB PFAS Review Panel in November 2021 and underwent public comment in March 2023. It incorporated expert scientific recommendations received from SAB in 2022 as well as feedback from the public comment period. The final assessment builds upon the literature review presented in the 2016 Health Effects Support Document for Perfluorooctanoic Acid (PFOA) and is an update of the SAB review draft *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* and the subsequent 2022 *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water.*
	Country of origin	USA
	Source of funding	Not stated.
	Possible conflicts of interest	Not stated.
	Guideline value type (e.g. oral	• Short-term and Chronic oral reference dose (RfD).

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Health considerations	TRV, drinking water guideline)	• Maximum Contaminant Level (MCL) in drinking water. • Cancer slope factor (CSM) and Interim CSF (CSF_{Serum}).
	Exposure timeframe	Lifetime. The overall RfD for PFOA is applicable to both short-term and chronic risk assessment scenarios.
	Critical human health endpoint	Co-critical effects are as follows: *1) Decreased serum anti-tetanus & anti-diphtheria antibodies in children: (*Budtz-Jørgensen and Grandjean 2018 and Timmerman et al. 2021). *2) Decreased infant birth weight (*Wikström et al. 2020). *3) Increased total cholesterol in adults (*Dong et al. 2019). *4) Renal cell carcinoma (*Shearer et al. 2021).
	Justification provided by agency for critical endpoint	"*These co-critical effects were selected based on the procedures outlined in the protocol and consistent with EPA peer-reviewed human health risk assessment methodology*." The selected critical effects can lead to clinical outcomes in a sensitive lifestage (children) and therefore, the overall RfD is expected to be protective of all other noncancer health effects in humans. *1) 2) & 3) The available evidence indicates there are effects across immune, developmental, cardiovascular, and hepatic organ systems at the same or approximately the same level of PFOA exposure. In fact, candidate RfDs within the immune, developmental, and cardiovascular outcomes are the same value (i.e. 3×10^{-8} mg/kg/day). Therefore, EPA has selected an overall RfD for PFOA of 3×10^{-8} mg/kg/day. The immune, developmental, and cardiovascular RfDs based on endpoints of decreased anti-tetanus and anti-diphtheria antibody concentrations in children, decreased birth weight, and increased total cholesterol, respectively, serve as co-critical effects for this RfD. The critical studies that serve as the basis of the RfD are all medium or high confidence epidemiological studies. The critical studies are supported by multiple other medium or high confidence studies in both humans and animal models and have health outcome databases for which EPA determined evidence indicates that oral PFOA exposure is associated with adverse effects. Additionally, the selected critical effects can lead to clinical outcomes in a sensitive lifestage (children) and therefore, the overall RfD is expected to be protective of all other noncancer health effects in humans. *4) Renal cell carcinoma (*Shearer et al. 2021): EPA selected the critical effect of renal cell carcinomas (RCC) in human males

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		reported by Shearer et al. (2021) as the basis of the overall CSF for PFOA. Shearer et al. (2021) is a well-conducted, multicenter case-control epidemiological study nested within the National Cancer Institute's (NCI's) Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO) with median PFOA levels relevant to the general U.S. population. The CSF derived from Shearer et al. (2021) was selected as the overall CSF over the CSF derived from Vieira et al. (2013) due to multiple study design considerations. Specifically, Shearer et al. (2021) exhibited several preferred study attributes compared with the Vieira et al. (2013) study including specificity in the health outcome considered (RCC vs. any kidney cancer), the type of exposure assessment (serum biomarker vs. modelled exposure), the source population (multicenter vs. Ohio and West Virginia regions), and study size (324 cases and 324 matched controls vs. 59 cases and 7,585 registry-based controls). US EPA states it prioritised health outcomes and endpoints with the strongest overall weight of evidence, which were the outcomes with evidence *demonstrates* or evidence *indicates* integration judgements, based on the synthesis of the available human, animal and mechanistic evidence for points of departure (POD) derivation using systematic review methods. i)With respect to **hepatic effects**, US EPA prioritised studies that evaluated endpoints related to serum biomarkers of injury for quantitative analyses because the reported effects on these endpoints *were well-represented* within the database and were generally consistent across the available *medium* confidence studies. All five *medium* confidence studies of general population adults from the updated literature searches reported positive associations between PFOA serum concentrations and ALT, three of which reported statistically significant responses. Serum ALT measures were considered a reliable indicator of impaired liver function because increased serum ALT is indicative of leakage of ALT from damaged hepatocytes. US EPA (2024a) state "*it is also important to note that while evaluation of direct liver damage is possible in animal toxicological studies, it is difficult to obtain biopsy-confirmed histological data in humans. Therefore, liver injury in humans is typically assessed using serum biomarkers of hepatotoxicity.*" ii)With respect to **immunological effects**, *evidence indicates* elevated exposures to PFOA are associated with immunological effects in humans. Evidence of immunosuppression in children associated with exposure to PFOA reported by epidemiological studies was consistent across studies and endpoints. Specifically, epidemiological studies reported associations between PFOA exposure and reduced humoral immune response to routine childhood immunisations, including lower

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		levels of tetanus and diphtheria, HiB, and rubella antibody titres. Reductions in antibody response were observed at multiple timepoints during childhood (specifically ages between 3-19 years in these studies), for either prenatal or postnatal childhood PFOA exposure levels, and were consistent across studies in children populations from medium confidence studies. Therefore, reduced antibody response in children was selected as an endpoint for POD derivation. Measurement of antigen-specific antibodies following vaccination(s) is a measure of the overall ability of the immune system to respond to a challenge. The antigen-specific antibody response is useful for evaluating the entire cycle of adaptive immunity. The SAB's PFAS review panel noted that reduction in the level of antibodies produced in response to a vaccine represents a "*failure of the immune system to respond to a specific challenge and is considered an adverse immunological health outcome*". As noted by Dewitt et al. (2019; 2017; 2016a; cited in US EPA 2024a) and in comments from other subject matter experts on the SAB's PFAS review panel, the clinical manifestation of a disease after chemical exposure is not required for a chemical to be classified as an immunotoxic agent and the ability to measure clinical outcomes as a result of mild to moderate immune-suppression in response to chemical exposure in traditional epidemiological studies can be challenging. Specifically, the SAB noted that "*[d]ecreased antibody responses to vaccines is relevant to clinical health outcomes and likely to be predictive of risk of disease*". The WHO Guidance for immunotoxicity risk assessment for chemicals similarly recommends measures of vaccine response as a measure of immune effects as "*childhood vaccine failures represent a significant public health concern*". Decreases in antibody response, even at smaller magnitudes in individuals, are clinically relevant when extrapolated to the overall population. This response also translates across multiple species, including rodents, and extensive historical data indicate that suppression of antigen-specific antibody responses by exogenous agents is predictive of immunotoxicity. Overall, EPA prioritised studies reporting responses to tetanus and diphtheria because the responses were consistently observed across a large number of studies (medium and low confidence) in children from multiple populations for these two vaccine types. iii) **Cardiovascular effects**: *Evidence indicates* exposure to PFOA is associated with cardiovascular effects in humans. The majority of studies in adults in the general population, including high-exposure communities, reported positive associations between PFOA serum concentrations and serum lipids. EPA selected total cholesterol for quantitative assessments because the association was the most consistently observed in adults and the studies for TC were of higher confidence for outcome

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		measurements compared with LDL. Increased serum cholesterol is associated with changes in incidence of cardiovascular disease events such as myocardial infarction (MI, i.e. heart attack), ischemic stroke (IS), and cardiovascular mortality occurring in populations without prior CVD events. Additionally, disturbances in cholesterol homeostasis contribute to the pathology of non-alcoholic fatty liver disease (NAFLD) and to accumulation of lipids in hepatocytes. Increases in serum cholesterol, even at smaller magnitudes at the individual level, are clinically relevant when extrapolated to the overall population. This is because, at the population level, even small magnitude increases in serum cholesterol could shift the distribution of serum cholesterol in the overall population relative to the clinical cut-off, leading to an increased number of individuals at risk for cardiovascular disease. The SAB PFAS Panel agreed with this interpretation, stating that "*an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. While the clinical relevance of exposure to PFOA…cannot be predicted on an individual basis, the increased number of individuals within a population with clinically defined abnormal values is of public health concern.*" *iv)* **Developmental effects:** *Evidence indicates* that elevated exposure to PFOA is associated with developmental effects in humans. Studies demonstrating foetal growth restriction were prioritised for POD derivation. The majority of high and medium confidence epidemiological studies (17/25) reported associations between PFOA and decreased mean birth weight in infants. Studies on changes in standardised birth weight measures (i.e., z-scores) also reported some inverse associations in high and medium confidence studies. Endpoints characterising foetal growth restriction were included for POD derivation because multiple studies reported effects on these endpoints, particularly decreased birth weight, and reported generally consistent findings across high and medium confidence studies. Low birth weight (LBW) is clinically defined as birth weight less than 2,500 g (approximately 5.8 lbs) and can include babies born small for gestational age (SGA) (birth weight below the 10th percentile for gestational age, sex, and parity). LBW is widely considered a useful population level public health measure and is on the World Health Organization's (WHO's) global reference list of core health indicators. Decreases in birthweight, even at smaller magnitudes at the individual level, are clinically relevant when extrapolated to the overall population. This is because, at the population level, even small magnitude decreases in birthweight could shift the distribution of birthweight in the overall population relative to the clinical cut-off, leading to an increased number of individuals at risk for decreased birthweight and

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		subsequent effects related to decreased birthweight. The SAB PFAS Panel agreed with this interpretation, stating that "*an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. While the clinical relevance of exposure to PFOA…cannot be predicted on an individual basis, the increased number of individuals within a population with clinically defined abnormal values is of public health concern*".
	Critical study(ies) underpinning point of departure	Epidemiological studies: *1) Decreased serum anti-tetanus & anti-diphtheria antibodies in children (medium confidence) – Two studies*: Budtz-Jørgensen, E., and P. Grandjean. 2018. Application of benchmark analysis for mixed contaminant exposures: mutual adjustment of perfluoroalkylate substances associated with immunotoxicity. PLoS One 13(10):e0205388 and Timmermann, CAG; Pedersen, HS; Weihe, P; Bjerregaard, P; Nielsen, F; Heilmann, C; Grandjean, P. (2021)*. Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7-12 years exposed to marine pollutants, a cross sectional study. Environmental Research 203: 111712. ** Note this paper is now dated 2022 online*. *2) Decreased infant birth weight (high confidence):* Wikström S., Lin P. I., Lindh C. H., Shu H. and Bornehag C. G. (2020). Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatr Res 87(6): 1093-1099. *3) Increased total cholesterol in adults (medium confidence):* Dong Z., Wang H., Yu Y. Y., Li Y. B., Naidu R. and Liu Y. (2019). Using 2003-2014 U.S. NHANES data to determine the associations between per- and polyfluoroalkyl substances and cholesterol: Trend and implications. Ecotoxicol Environ Saf 173: 461-468. *4) Renal cell carcinoma (medium confidence):* Shearer J. J., Callahan C. L., Calafat A. M., Huang W.-Y., Jones R. R., Sabbisetti V. S., Freedman N. D., Sampson J. N., Silverman D. T., Purdue M. P. and Hofmann J. N. (2021). Serum Concentrations of Per- and Polyfluoroalkyl Substances and Risk of Renal Cell Carcinoma. JNCI: Journal of the National Cancer Institute 113(5): 580-587.
	Species for critical study(ies)	Epidemiological studies in adults and children. 1) *Decreased serum anti-tetanus & anti-diphtheria antibodies in children*: Budtz-Jørgensen and Grandjean 2018: PFOA concentrations at age five years (males and females) and

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		anti-tetanus antibody serum concentrations at age seven; Timmerman et al. 2021: PFOA concentrations and anti-tetanus antibody concentrations at ages 7–12 (male and female). 2) *Decreased infant birth weight*: PFOA serum concentrations in first and second trimesters. 3) *Increased total cholesterol in adults*: Male and female adults, 20-80 years of age. 4) *Renal cell carcinoma:* Male and female adults, 55-74 years of age.
	Point of departure type (e.g. NOAEL, LOAEL, $BMDL_{10}$, etc.)	• Lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in response equal to 0.5 SD from the control mean ($BMDL_{0.5SD}$). • Lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in response ($BMDL_{5RD}$). • POD human equivalent doses (POD_{HEDs}). • Reference Dose (RfD). • Baseline Risk (R_o).
	Point of departure value (include units)	*1) Decreased serum anti-tetanus & anti-diphtheria antibodies in children*: Budtz-Jørgensen and Grandjean (2018): 3.47 ng/mL ($BMDL_{0.5SD}$) converted to 3.05 $x10^{-7}$ mg/kg/day (POD_{HED}). Timmerman et al. (2021): 2.26 ng/mL ($BMDL_{0.5SD}$) converted to 3.34 $x10^{-7}$ mg/kg/day (POD_{HED}). *2) Decreased infant birth weight*: 2.2 ng/mL ($BMDL_{5RD}$) equivalent to 2.92 $x10^{-7}$ mg/kg/day (POD_{HED}). *3) Increased total cholesterol in adults*: 2.29 ng/mL ($BMDL_{5RD}$) equivalent to 2.75 $x10^{-7}$ mg/kg/day (POD_{HED}). *4) Renal cell carcinoma:* R_0 of 0.0202 × 90% = 0.0182.
	Uncertainty factor(s) & rationale	EPA applied a composite uncertainty factor (UF_C) of 10 to the POD_{HEDs} from selected epidemiological studies (UF_C = 10: Composite $UF_C = UF_A \times UF_H \times UF_S \times UF_L \times UF_D$): • UF_A = 1: A UF_A of 1 is applied to effects observed in epidemiological studies as the study population is humans. • UF_H = 10: A UF_H of 10 is applied when information is not available relative to variability in the human population. • UF_S = 1: A UF_S of 1 is applied when effects are observed in adult human populations that are assumed to have been exposed to a contaminant over the course of many years. A UF_S of 1 is applied for developmental effects because the developmental period is recognised as a susceptible lifestage when exposure during a time window of development is more

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		relevant to the induction of developmental effects than lifetime exposure. • UF_L = 1: A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL. • UF_D = 1: A UF_D of 1 is applied when the database for a contaminant contains a multitude of studies of adequate quality that encompass a comprehensive array of endpoints in various lifestages and populations and allow for a complete characterisation of the contaminant's toxicity. Note for RCC, the CSF_{serum} was calculated as the product of the upper 95% confidence limit of the dose-response slope (b) and R_0.
	Guideline value (include units)	1) *Decreased serum anti-tetanus & anti-diphtheria antibodies in children*: Budtz-Jørgensen and Grandjean 2018: RfD = 3.05 $x10^{-8}$ mg/kg/day rounded down to 3 $x10^{-8}$ mg/kg/day (0.03 ng/kg/d). Timmerman et al. (2021): RfD = 3.34 $x10^{-8}$ mg/kg/day rounded down to 3 $x10^{-8}$ mg/kg/day (0.03 ng/kg/d). 2) *Decreased infant birth weight*: RfD = 2.92 $x10^{-8}$ mg/kg/day rounded up to 3 $x10^{-8}$ mg/kg/day (0.03 ng/kg/d). 3) *Increased total cholesterol in adults*: RfD = 2.75 $x10^{-8}$ mg/kg/day rounded up to 3 $x10^{-8}$ mg/kg/day (0.03 ng/kg/d). 4) *Renal cell carcinoma:* = CSF_{Serum} of 3.52 x 10^{-3} $(ng/mL)^{-1}$ converted to a CSF of 0.0293 $(mg/kg/d)^{-1}$ = 29,300 $(mg/kg/day)^{-1}$. MCL: 4 ng/L, i.e. a practical quantification limit (PQL) (USEPA 2024c). Other relevant information: • Equations used: RfD = POD_{HED} ÷ UF_C, CSF = CSF_{Serum} ÷ the selected clearance value (equivalent to dividing by the change in external exposure that results in a 1 ng/mL increase in serum concentration at steady-state). • CSF represents an increase in Cancer Risk per 1 ng/(kg*d) Increase in Dose.
	Mode of action for critical health endpoint	1) *Decreased serum anti-tetanus & anti-diphtheria antibodies in children*: Mechanistic data available from *in vitro*, *in vivo*, and epidemiological studies were used to evaluate the aetiology and mode of action of PFOA-associated immunosuppression and other effects on the immune system. The pleotropic immunomodulatory effects of PFOA, including impaired vaccine responses, may reflect perturbed function of B and/or T cells. At the molecular level, dysregulation of the NF-κB pathway may contribute to the

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		immunosuppressive effects of PFOA. The NF-κB pathway facilitates initial T cell responses by supporting proliferation and regulating apoptosis, participates in the regulation of CD4+ T cell differentiation, and is involved in mediating inflammatory responses. Dysregulation of the NF-κB pathway by PFOA, potentially consequent to the induction of oxidative stress, may be a key component of the mechanism underlying PFOA-mediated immunosuppression. Reduced NF-κB activation and consequent elevation of apoptosis is consistent with increased apoptosis in multiple cell types, the reduction of pre/pro-B cell numbers, and dysregulation of pro-inflammatory cytokines and mediators of inflammation. NF-κB activation also facilitates the induction of apoptosis during negative selection of T cells in the thymus, which is essential for the deletion of T cells that recognise self. In contrast, NF-κB acts as a pro-survival factor during the negative selection of B cells. In human studies, PFOA exposure has been associated with autoimmune diseases including ulcerative colitis. Further mechanistic evidence is needed to determine the directionality of the effect of PFOA on NF-κB, which will inform the cell types that predominantly contribute to the aetiology of autoimmune diseases associated with PFOA exposure. 2) *Decreased infant birth weight*: In general, the observed effects suggest that the developing liver, developing heart, and placenta may be affected by PFOA at the molecular level (e.g. differential methylation of genes, gene expression changes), which may be reflected in developmental health effects. The effects tend to vary by sex and developmental timepoint of outcome evaluation. More research is needed to strengthen the association between PFOA exposure to any one of the several possible contributing factors, including fluctuations in transporter gene expression, epigenetic changes, oxidative stress, and PPARα pathway activation, particularly in the placenta. 3) *Increased total cholesterol in adults*: While the precise events that lead to steatosis have yet to be elucidated, the current studies conducted in animals and *in vitro* studies support the following key molecular and cellular events related to PFOA-mediated hepatoxicity specific to changes in lipid metabolism: (1) PFOA accumulation in liver activates nuclear receptors; (2) nuclear receptors, including PPARα, then alter expression of genes involved in lipid homeostasis and metabolism; (3) the products of the genes altered by activated nuclear receptors modify the lipid content of liver to favour triglyceride accumulation, and possibly also cholesterol accumulation; (4) altered lipid content in liver leads to accumulation of lipid

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		droplets promoting development of steatosis and other changes leading to liver dysfunction; and (5) alterations in lipid metabolism leads to alterations in serum levels of triglycerides and cholesterol. An intriguing possibility that may be concurrent to these events is direct binding of PFOA to ACACA and ACACB enzymes in a manner that interferes with fatty acid biosynthesis. Although this series of events is plausible, significant gaps remain in understanding this process, including how these events interface with other cellular processes such as cell growth and survival, oxidative stress, and others in understanding the mechanisms of PFOA-mediated hepatoxicity. 4) *Renal cell carcinoma:* The available mechanistic data continue to suggest that multiple MOAs could play a role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in human populations as well as animal models. The few available mechanistic studies focusing on PFOA-induced renal toxicity highlight several potential underlying mechanisms of PFOA exposure-induced renal tumorigenesis, including altered cell proliferation and apoptosis, epigenetic alterations, and oxidative stress. However, due to data limitations, it is difficult to distinguish which mechanism(s) are operative for PFOA-induced kidney cancer.
	Genotoxic carcinogen?	Overall, the evidence suggests that PFOA does not induce mutations or operate through a genotoxic mechanism, with the majority of the study data demonstrating a lack of genotoxic effect of PFOA in both *in vitro* and *in vivo* assays. A notable exception is aneuploidy and DNA fragmentation of sperm significantly associated with PFOA exposure. Notes on carcinogenicity: The evidence from medium confidence epidemiological studies is primarily based on the incidence of kidney and testicular cancer, as well as some evidence of increased breast cancer incidence in susceptible subpopulations. Other cancer types have been observed in humans, although the evidence for these is generally limited to low confidence studies. The evidence of carcinogenicity in animal models is provided in three high or medium confidence chronic oral animal bioassays in Sprague-Dawley rats which together identified neoplastic lesions of the liver, pancreas, and testes. The available mechanistic data suggest that multiple MOAs could play a role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in human populations as well as animal models.
	Identified sensitive sub-populations	The selected critical effects can lead to clinical outcomes in a sensitive lifestage (children).

Agency Report Reference: US EPA (2024c). 40 CFR Parts 141 and 142: PFAS National Primary Drinking Water Regulation, Final Rule. Federal Register, Vol. 89, No. 82. Friday April 26, 2024.

US EPA (2024d). 40 CFR Parts 141: PFAS National Primary Drinking Water Regulation, Final Rule; correction. Federal Register, Vol. 89, No. 113. Tuesday June 11, 2024.

Supporting Documentation: US EPA (2024a). FINAL Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts, United States Environmental Protection Agency. April 2024. EPA Document No. 815R24006. https://www.epa.gov/system/files/documents/2024-04/main_final-toxicity-assessment-for-pfoa_2024-04-09-refs-formatted.pdf.

		The RfD is protective of effects that may occur in sensitive populations (e.g. infants, children), as well as hepatic effects in adults that may result from PFOA exposure. There is uncertainty about whether there are susceptible populations, such as certain racial/ethnic groups, that might be more sensitive to the health effects of PFOA exposure because of either greater biological sensitivity or higher exposure to PFOA and/or other environmental chemicals.
	Any non-health-based considerations?	Yes. The MCL is based on a PQL.
Risk Summary	Any risks to human health from drinking water identified in agency document?	Ingestion of drinking water is a potentially significant source of exposure to PFOA. Serum PFOA concentrations are known to be elevated among individuals living in communities with drinking water contaminated from environmental discharges. Under UCMR 3, 36,972 samples from 4,920 PWSs were analysed. PFOA was found above the UCMR 3 minimum reporting level (20 ng/L) in 379 samples at 117 systems. State results show continued occurrence of PFOA in multiple geographic locations. PFOA concentrations ranged from 0.21 to 650 ng/L with a range of median concentrations from 1.27 to 5.61 ng/L. Glassmeyer et al. (2017, cited in US EPA 2024a) sampled source and treated drinking water from 29 drinking water treatment plants for a suite of emerging chemical and microbial contaminants, including 11 PFAS. In this study, PFOA was reported in source water at 76% of systems, at a median concentration of 6.32 ng/L and maximum concentration of 112 ng/L. Similarly, in treated drinking water, PFOA was detected in 76% of systems, with a median concentration of 4.15 ng/L and maximum concentration of 104 ng/L.
	Any emerging risks identified?	Mixture analysis remains an area of emerging research, and there is no scientific consensus yet for the best approach to account for exposure by co-occurring PFAS. Additionally, multipollutant analyses from studies included in this assessment did not provide direct evidence that associations between exposure to PFOA and health effects are confounded by or are fully attributable to confounding by co-occurring PFAS.
Any other relevant information that should be captured?		Individual MCLs (USEPA 2024c) a. Perfluorooctanoic acid (PFOA) MCL = 4.0 nanograms per liter or parts per trillion (ng/L or ppt) b. Perfluorooctane sulfonic acid (PFOS) MCL = 4.0 ng/L c. Perfluorohexane sulfonic acid (PFHxS) MCL = 10 ng/L d. Perfluorononanoic acid (PFNA) MCL = 10 ng/L

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	e. Hexafluoropropylene oxide dimer acid (HFPO–DA) MCL = 10 ng/L Hazard Index MCL to account for dose-additive health effects for mixtures that could include two or more of four PFAS (PFHxS, PFNA, HFPO–DA, and perfluorobutane sulfonic acid (PFBS)). The Hazard Index MCL defines when the combined levels of two or more of these four PFAS requires action. A PFAS mixture Hazard Index less than or equal to 1 (unitless) indicates a level at which no known or anticipated adverse effects on the health of persons occur and allows for an adequate margin of safety with respect to health risk associated with a mixture of PFAS in finished drinking water. A PFAS mixture Hazard Index greater than 1 (unitless) indicates an exceedance of the health protective level. To calculate the Hazard Index, a ratio is developed for each PFAS by dividing the measured level of the PFAS in drinking water by the level (in ng/L or ppt) below which adverse health effects are not likely to occur (i.e. the Health Based Water Concentration or HBWC). The HBWCs for each PFAS in the Hazard Index are: a. PFHxS = 10 ng/L or ppt b. PFNA = 10 ng/L c. HFPO–DA = 10 ng/L d. PFBS = 2,000 ng/L The individual PFAS ratios are then summed across the mixture to yield the Hazard Index MCL (USEPA 2024c). The EPA is also finalising individual MCLGs and is promulgating individual MCLs for PFHxS, PFNA, and HFPO–DA at 10 ng/L. In addition to the individual MCLs for PFHxS, PFNA, and HFPO–DA, in consideration of the known toxic effects, dose additive health concerns and occurrence and likely co-occurrence in drinking water of these three PFAS, as well as PFBS, the EPA is finalising a Hazard Index (HI) of 1 (unitless) as the MCLG and MCL for any mixture containing two or more of PFHxS, PFNA, HFPO–DA, and PFBS (USEPA 2024c). A potential source of uncertainty in epidemiologic studies examining associations between a particular PFAS and health outcomes is confounding by other co-occurring PFAS. In studies of PFOA, such confounding may occur if there are other PFAS that are moderately or highly correlated with PFOA, associated with the outcome of interest, and not on the causal pathway between PFOA and the outcome. Note: Confounding only considered for birth weight changes. The individual and Hazard Index MCLs are independently applicable for compliance purposes. Overall, there is no evidence that the consistently observed associations between exposures to PFOA and the four priority noncancer health outcomes are confounded by or are fully attributable to confounding by co-occurring PFAS.

Agency Report Reference: US EPA (2024c). 40 CFR Parts 141 and 142: PFAS National Primary Drinking Water Regulation, Final Rule. Federal Register, Vol. 89, No. 82. Friday April 26, 2024. US EPA (2024d). 40 CFR Parts 141: PFAS National Primary Drinking Water Regulation, Final Rule; correction. Federal Register, Vol. 89, No. 113. Tuesday June 11, 2024. **Supporting Documentation:** US EPA (2024a). FINAL Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts, United States Environmental Protection Agency. April 2024. EPA Document No. 815R24006. https://www.epa.gov/system/files/documents/2024-04/main_final-toxicity-assessment-for-pfoa_2024-04-09-refs-formatted.pdf.	
Assessed in Appendix B?	Yes

A.2.2 Burgoon et al. (2023)

Report Reference: Burgoon L. D., Clewell H. J., Cox T., Dekant W., Dell L. D., Deyo J. A., Dourson M. L., Gadagbui B. K., Goodrum P., Green L. C., Vijayavel K., Kline T. R., House-Knight T., Luster M. I., Manning T., Nathanail P., Pagone F., Richardson K., Severo-Peixe T., Sharma A., Smith J. S., Verma N. and Wright J. (2023). Range of the perfluorooctanoate (PFOA) safe dose for human health: An international collaboration. Regulatory Toxicology and Pharmacology 145: 105502.		
General Information	Date of data extraction	09 July 2024
	Authors	Burgoon L. D., Clewell H. J., Cox T., Dekant W., Dell L. D., Deyo J. A., Dourson M. L., Gadagbui B. K., Goodrum P., Green L. C., Vijayavel K., Kline T. R., House-Knight T., Luster M. I., Manning T., Nathanail P., Pagone F., Richardson K., Severo-Peixe T., Sharma A., Smith J. S., Verma N. and Wright J.
	Publication date	Available online 29 October 2023
	Literature search timeframe	Not relevant.
	Publication type	Journal Article
	Peer reviewed?	Multiple authors were involved in review and editing of the report.
	Country of origin	International collaboration (24 scientists from 8 countries)
	Source of funding	The development of research and subsequent publication was under the auspices of the Alliance for Risk Assessment (ARA). No funding was accepted from any organisation for this work.
	Possible conflicts of interest	Several of the authors have worked over a number of years for various sponsors on PFAS issues. However, no outside funding was accepted to do this work by the Alliance for Risk Assessment.
Health considerations	Guideline value type (e.g. oral TRV, drinking water guideline)	*1)* Serum PFOA benchmark concentration (BMC). *2)* No Observed Adverse Effect Level (NOAEL). *3)* Reference dose (RfD). *4)* Provisional Safe Dose.
	Exposure timeframe	Lifetime.
	Critical human health endpoint	Liver effects in monkeys and developmental and immunological effects in mice from five experimental studies as follows: *1)* Increased liver weight.

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		2) Dose-dependent growth deficits for gestation days 1–17 *3)* Lipid parameters/relative liver weight. *4)* Neonatal survival. *5)* Immune suppression.
	Justification provided by agency for critical endpoint	Existing human observational studies cannot be used reliably for developing the critical effect in the absence of mechanistic data relevant to humans at serum concentrations seen in the general public. Human data are not an acceptable basis of the safe dose. The overall uncertainty in the database, both epidemiology and experimental animal, is sufficient to give pause to the development of a credible critical effect for PFOA. However, in recognition of the importance of managing PFOA potential health risks, a provisional approach could be developed based on several experimental animal studies. After reviewing the available PFOA database, each of three teams of experts decided on different critical effects on which to derive a range of safe doses: • Team 1: Developmental effects in mice (Lau et al. 2006) although considered other studies including Loveless et al. (2006). • Team 2: Developmental/reproductive effects in mice (of Abbott et al. 2007) and the immunotoxicity in mice (DeWitt et al. 2016). This team remained of the opinion that the overall database was insufficient at this time to make a reliable judgment of critical effect. • Team 3: Liver effect in monkey considering they were most relevant due to comparability of PPARα activation for potential liver effects and general physiology with humans (Butenhoff et al. 2002). After discussion by all three teams, there was an agreement to develop a range of safe doses based on liver effects in monkeys and developmental and immunological effects in mice.
	Critical study(ies) underpinning point of departure	Five experimental animal studies as the basis of the provisional safe PFOA dose range: *1)* Butenhoff, J.L., Kennedy, G.L., Frame, S.R., O'Conner, J.C., York, R.G. (2004). The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology 196, 95–116. Note: POD from Green, L.C., Crouch, E.A.C., 2019. Comments on Massachusetts Department of Environmental Protection's (DEP's) Groundwater and Soil Standards for Perfluoroalkyl Substances (PFAS) in the Department's Proposed 2019 Amendments to the Massachusetts Contingency Plan. July 19.. *2)* Lau, C., Thibodeaux, J.R., Hanson, R.G., Narotsky, M.G., Rogers, J.M., Lindstrom, A.B., Strynar, M.J. (2006). Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol. Sci. 90, 510–518.

Report Reference: Burgoon L. D., Clewell H. J., Cox T., Dekant W., Dell L. D., Deyo J. A., Dourson M. L., Gadagbui B. K., Goodrum P., Green L. C., Vijayavel K., Kline T. R., House-Knight T., Luster M. I., Manning T., Nathanail P., Pagone F., Richardson K., Severo-Peixe T., Sharma A., Smith J. S., Verma N. and Wright J. (2023). Range of the perfluorooctanoate (PFOA) safe dose for human health: An international collaboration. Regulatory Toxicology and Pharmacology 145: 105502.		
		3) Loveless, S.E., Finlay, C., Everds, N.E., Frame, S.R., Gillies, P.J., O'Connor, J.C., et al. (2006). Comparative responses of rats and mice exposed to linear/branched, linear, or branched ammonium perfluorooctanoate (APFO). Toxicology 220 (2–3), 203–217. *4)* Abbott, B.D., Wolf, C.J., Schmid, J.E., Das, K.P., Zehr, R.D., Helfant, L., Nakayama, S., Lindstrom, A.B., Strynar, M.J., Lau, C. (2007). Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha. Toxicol. Sci. 98, 571–581. *5)* Dewitt, J.C., Williams, W.C., Creech, N.J., Luebke, R.W. (2016). Suppression of antigen- specific antibody responses in mice exposed to perfluorooctanoic acid: role of PPARα and T- and B-cell targeting. J. Immunot. 13, 38–45.
	Species for critical study(ies)	*1)* Monkey. *2)* Mouse. *3)* Mouse (male). *4)* Mouse. *5)* Mouse.
	Point of departure type (e.g. NOAEL, LOAEL, $BMDL_{10}$, etc.)	Point of departure from five experimental animal studies considered were: *1)* Serum BMC. *2)* NOAEL. *3)* Serum BMC. *4)* NOAEL. *5)* NOAEL.
	Point of departure value (include units)	Point of departure from five experimental animal studies considered were: *1)* 19 µg/mL (Serum BMC, increased liver weight). *2)* 23 µg/mL (NOAEL, dose-dependent growth deficits for gestation days 1–17). *3)* 4.35 µg/mL (Serum BMC, lipid parameters/relative liver weight). *4)* 0.3 mg/kg/day (10.4 µg/ml) (NOAEL, neonatal survival). *5)* 0.94 mg/kg-day (equivalent to 22 µg/mL) (NOAEL, immune suppression).
	Uncertainty factor(s) & rationale	PFOA has an enormous database, but still has some uncertainty, especially in choosing the critical effect largely due to the relevance to humans of mode(s) of action in animals. A factor of 3-fold for this area of uncertainty should be considered. The use of the average clearance value (either mean, median, mode or geometric versions of these) from the Zhang et al. (2013) human study should be used with any of the experimental animal points of departure if in µg/ml of serum, or by comparison with kinetic information from the relevant species if the points of

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		departure are in units of dose. Moreover, the Zhang et al. (2013) study also shows human variability that can be used to develop a data-derived value for within human toxicokinetics. The following Uncertainty Factors were considered for each safe serum dose estimated: • Monkey or Mouse to human toxicokinetic factor = 1 [Factor is not needed since BMD is based on serum concentration]. • Monkey or Mouse to human toxicodynamic factor = 2.5 [IPCS (2005) default or 3 U.S. Environmental Protection Agency EPA (2014) default]. • Human toxicodynamic factor = 3 [default of IPCS (2005) and U.S. Environmental Protection Agency EPA (2014)] • Human toxicokinetic factor = 8.4 [0.79 ml/day/kg arithmetic mean clearance of average group from Zhang et al. (2013, Table 2) ÷ 0.094 ml/day/kg arithmetic 95% lower bound clearance of sensitive group from Zhang et al. (2013, Table 2)]. • Database uncertainty factor = 1 (Although it could be argued that the small number of animals in the study justifies an additional uncertainty factor; the counter-argument is that these are primates). This is a total UF of 75.6 (=1 x 3 x 3 x 8.4 x 1).
	Guideline value (include units)	The five RfD serum concentrations estimated divided by the uncertainty factors (1 × 3 x 3 × 8.4 x 1) were as follows: *1)* RfD serum concentration = 0.25 µg/ml [19 µg/ml ÷ (1 × 3 x 3 × 8.4 x 1)]. *2)* RfD serum concentration = 0.30 µg/ml [23 µg/ml ÷ (1 × 3 x 3 × 8.4 x 1)]. *3)* RfD serum concentration = 0.058 µg/ml [4.35 µg/ml ÷ (1 × 3 x 3 × 8.4 x 1)]. *4)* RfD serum concentration = 0.14 µg/ml [10.4 µg/ml ÷ (1 × 3 x 3 × 8.4 x 1)]. *5)* RfD serum concentration = 0.29 µg/ml [22 µg/ml ÷ (1 × 3 x 3 × 8.4 x 1)]. The suggested provisional safe dose range of this international collaboration is 0.01–0.07 µg/kg-day. Five safe doses (RfD) were estimated using a factor of 0.23 ml/day/kg [geometric mean clearance from Zhang et al. (2013, Table 2) assuming steady state] as follows: *1)* RfD = 0.06 µg/kg-day (0.25 µg/ml x 0.23 ml/day/kg). *2)* RfD = 0.07 µg/kg-day (0.30 µg/ml x 0.23 ml/day/kg). *3)* RfD = 0.07 µg/kg-day (0.30 µg/ml x 0.23 ml/day/kg). *4)* RfD = 0.01 µg/kg-day (0.058 µg/ml x 0.23 ml/day/kg). *5)* RfD = 0.07 µg/kg-day (0.29 µg/ml x 0.23 ml/day/kg).

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	Mode of action for critical health endpoint	Several MOAs could be envisioned but not enough evidence exists to establish any one of these MOAs with certainty. There was general agreement that the most likely MOAs for PFOA involved fatty acid mimicry. Disruption of lipid and fatty acid processing in the liver (observed in rodents) has been shown to involve activation of multiple, related nuclear receptors including PPARα, PPARγ, CAR, FXR, LXR, and PXR. However, humans and rodents have been shown to have strikingly different responses.
	Genotoxic carcinogen?	• No information on genotoxicity. Notes on carcinogenicity: • With regard to the potential carcinogenicity of PFOA, there was general agreement that the EPA's proposed change in the categorisation of PFOA from "suggestive evidence" to "likely carcinogen" is not justified. The EPA's determination was based primarily on clear evidence of PFOA-induced liver tumours in rodents and variously published associations between PFOA concentrations and kidney cancer in humans, and the EPA identified a case-control study of renal cell carcinoma (RCC) nested within the screening arm of PLCO cancer screening trial study as particularly influential (Shearer et al. 2021). • Rodent liver tumours are observed only at doses associated with peroxisomal proliferation, a response of limited relevance to human exposures. And, on the opinion of the study authors, the relevant epidemiological studies have not adequately considered the potential for confounding by impaired renal function, which is associated with both PFOA clearance and kidney cancer. • With regard to kidney cancer, the authors of the study note that if PFOA were a genuine cause of this cancer-type in humans, then one might expect that the massive doses of PFOA used in the rodent (and monkey) bioassays would have also induced kidney tumours. Yet, they did not. • Kidney cancer is frequently associated with impaired renal function and alterations in renal function that resulted in decreased PFOA excretion would result in a consequent increased PFOA concentration in serum. • Pharmacokinetic confounding led to the observed associations (between PFOA and kidney cancer).
	Identified sensitive sub-populations	-
	Any non-health-based considerations?	-

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Risk Summary	Any risks to human health from drinking water identified in agency document?	Not specifically, although various authorities have developed safe doses and some of the studies evaluated were based on drinking water as the route of exposure.
	Any emerging risks identified?	-
Any other relevant information that should be captured?		After reviewing the plethora of relevant information, none of the teams independently considered the epidemiology data, composed primarily of observational studies, to be sufficient to determine a critical effect considering the lack of information regarding the mode of action(s). The results from these studies were considered not only potentially confounded, with confounding that was not readily quantified, but also to have serum concentrations from unidentified sources of exposure to PFOA that were not significantly different from background in most studies, making it difficult or impossible to assign a clear exposure- response association, much less causation. Finally, all three teams did not rely on several potentially relevant studies of PFOA, and after discussion, agreed that the two-generation study by Macon et al. (2011, as cited in Burgoon et al. 2023) was not considered reliable for development of a safe dose range because the statistics in this study appeared to be based on pups and not their mothers. Using pups as the basis of the assessment is not in accordance with relevant guidelines. In addition, neither Onischenko et al. (2011, cited in Burgoon et al. 2023) nor Koskela et al. (2017, cited in Burgoon et al. 2023) were used because of too few animals and limited doses used in these studies to generate a confident estimate of the NOAEL/LOAEL interface, and furthermore, it was not certain that the statistics were based on the maternal experimental animals. After these presentations, clarifying questions and discussion, the following consensus positions were developed as summarised in Table 2 and shown below: 1. Should human studies be used for the development of the critical effect? No, existing human observational studies cannot be used reliably for this purpose. For example, changes in cholesterol appear to have only a small effect at low doses and an opposite effect at higher doses. These studies may support the choice of critical effect with some of the experimental animal work, however. 2. Should vaccine responses be used for the development of the critical effect? No, existing human observational vaccine findings are not primary immune responses and of questionable clinical relevance. Based on epidemiological study results, it is premature to assume that a population shift in the distribution

Report Reference: Burgoon L. D., Clewell H. J., Cox T., Dekant W., Dell L. D., Deyo J. A., Dourson M. L., Gadagbui B. K., Goodrum P., Green L. C., Vijayavel K., Kline T. R., House-Knight T., Luster M. I., Manning T., Nathanail P., Pagone F., Richardson K., Severo-Peixe T., Sharma A., Smith J. S., Verma N. and Wright J. (2023). Range of the perfluorooctanoate (PFOA) safe dose for human health: An international collaboration. Regulatory Toxicology and Pharmacology 145: 105502.	
	of antibody concentrations – if one exists – results in increased risk of susceptibility to diseases. Moreover, higher dose worker exposures do not suggest immune responses. 3. Should experimental animal studies be used for the development of the critical effect? The overall uncertainty in the database, both epidemiology and experimental animal, is sufficient to give pause to the development of a credible critical effect for PFOA. This conclusion is similar to what WHO (2022) found and for the same or similar reasons. However, in recognition of the importance of managing PFOA potential health risk, and despite the overall difficulties in the experimental animal studies, a provisional approach was explored as follows: o Frank toxicity in both monkeys and rats has been observed in a dose related manner. We might be able to tie these effects into other liver and or developmental endpoints. One member volunteered to conduct a BMD approach on the relevant monkey and rodent studies and send this to all three teams for consideration (information available upon request). o One team member asked participants to critique and improve upon Green and Crouch (2019, as cited in Burgoon et al. 2023) who reviewed the basis of Massachusetts Department of Environmental Protection's Groundwater and Soil Standards for PFOA and PFOS and suggested an alternate animal test model and target endpoint (i.e. monkey liver toxicity) using a BMD approach. o PFOA is the fluorinated version of the naturally occurring caprylic acid. A big difference between these two chemicals is their half-lives in the human body. Considering whether potential long-term toxicity from caprylic acid matches any of the findings with PFOA may prove useful.
Assessed in Appendix B?	Yes

Appendix B Existing Guidance/Guideline Assessment Tables

Addendum to PFAS Evidence Evaluation for Australian Drinking Water Guidelines Chemical Fact Sheets

Addendum / Work Expansion for 2024 NHMRC PFAS Review of Australian Health-based Guideline Values

National Health and Medical Research Council

SLR Project No.: 640.031365.00001

17 October 2024

SLR

B.1 Criteria for assessing existing guidance or guidelines

Administrative and technical criteria for assessing existing guidance or guidelines

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

B.1.1 US EPA (2024b)

Agency Report Reference: *US EPA (2024b). FINAL Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts, United States Environmental Protection Agency. April 2024. EPA Document No. 815R24007.* https://www.epa.gov/system/files/documents/2024-04/main_final-toxicity-assessment-for-pfos_2024-04-09-refs-formatted_508c.pdf.

Criteria		Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	Y	-
	Are the administrative processes documented and publicly available?	Y	-

Criteria		Y/N/?/NA	Notes
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	The systematic review work included in this assessment was prepared in collaboration with ICF under the U.S. EPA Contracts EP-C-16-011 (Work Assignment Nos. 4-16 and 5-16) and PR-OW-21-00612 (TO-0060). This document was prepared by the Health and Ecological Criteria Division, Office of Science and Technology, Office of Water (OW) of the U.S. Environmental Protection Agency (EPA). The agency gratefully acknowledges the valuable contributions of EPA scientists from the OW, Office of Research and Development (ORD), the Office of Children's Health Protection (OCHP), and the Office of Land and Emergency Management (OLEM). The final toxicity assessment was peer reviewed by the EPA Science Advisory Board (SAB) PFAS Review Panel in November 2021 and underwent public comment in March 2023. It incorporated expert scientific recommendations received from the SAB in 2022 as well as feedback from the public comment period. There is a procedure to manage potential conflicts of interest.
	Are funding sources declared?	Y (0.5)	Funding sources not provided in report, but likely funded by the Federal Government of USA.
	Was there public consultation on this work? If so, provide details.	Y	Yes, draft was released for public comment.
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Yes, draft was released for public comment and reviewed by SAB in November 2021. This final toxicity assessment underwent public comment in March 2023. Peer review outcome is documented.

Criteria		Y/N/?/NA	Notes
	Was the guidance/advice developed or updated recently? Provide details.	Y	This final toxicity assessment was peer reviewed by the EPA Science SAB per- and PFAS Review Panel in November 2021 and underwent public comment in March 2023. It incorporated expert scientific recommendations received from the SAB in 2022 as well as feedback from the public comment period. This final assessment builds upon the literature review presented in the 2016 Health Effects Support Document for Perfluorooctane Sulfonic Acid (PFOS) (hereafter referred to as the 2016 PFOS HESD) and is an update of the SAB review draft, Proposed Approaches to the Derivation of a Draft Maximum Contaminant level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water and the subsequent Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water.
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Y	Methodology and results of the health effects systematic review and toxicokinetics methods are detailed in Sections 2.1 and 3.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Y	The evidence integration was conducted according to guidance outlined in the IRIS Handbook and the Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (Anionic and Acid Forms) IRIS Assessments. The evidence integration included evidence stream evaluation, in which the qualitative summaries on the strength of evidence from studies in animals and humans were evaluated, and subsequent inference across all evidence streams. Human relevance of animal models as well as mechanistic evidence to inform mode of action were considered.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	Y	Documented in Sections 2.1 and 3 of the report.
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	NA	Unpublished data do not seem to be mentioned.

Criteria		Y/N/?/NA	Notes
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	Y	Yes, documented in Section 2.1.2. The authors undertook a title and abstract screen, excluding overlapping epidemiological studies, and noted why reports were excluded.
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	NA	Although other reviews are cited, US EPA used their own independent assessment to come to conclusions.
	Can grey literature such as government reports and policy documents be included?	Y	-
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	Y	Detailed throughout the report
	Evidence search		
	Are databases and other sources of evidence specified?	Y	Yes, refer to Section 2.1.1.
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	Y	The following publicly available databases were searched for literature containing the chemical search terms outlined in Appendix A: Web of Science™ (WoS) (Thomson Reuters), • PubMed® (National Library of Medicine), • ToxLine (incorporated into PubMed post 2019), and • TSCATS (Toxic Substances Control Act Test Submissions). For the second data stream, other review efforts and searches of publicly available sources were used to identify relevant studies (see Appendix A); studies cited in assessments published by other U.S. federal, international, and/or U.S. state agencies, studies identified during mechanistic or toxicokinetic evidence synthesis, studies identified by the SAB in their final report dated August 23, 2022, and studies submitted through public comment by May 2023. For the third data stream, EPA relied on epidemiological and animal toxicological literature synthesised in the 2016 PFOS HESD to identify studies relevant to the five priority health outcomes.

Criteria		Y/N/?/NA	Notes
	Is it specified what date range the literature search covers? Is there a justification?	Y	Four separate searches from 2019 (back to 2013), 2020, 2021, and 2022.
	Are search terms and/or search strings specified?	Y	Search terms in Appendix A.
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	Y	The US EPA used populations, exposures, comparators, and outcomes (PECO) criteria to screen the literature identified from the literature sources outlined above in order to prioritise studies for dose-response assessment and to identify studies containing supplemental information such as mechanistic studies that could inform the mode of action analyses. The PECO criteria used for screening the health effects, toxicokinetic, and mechanistic literature are provided in Appendix A (not attached to the reviewed document).
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	Y	All studies were evaluated for risk of bias, selective reporting, and sensitivity following the Methods in Appendix A.
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	Y	Yes, full details provided in report.
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	Y	Yes, confidence is assigned to the levels of evidence and an overall weight of evidence is examined to describe certainty in the evidence.
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	Y	This is detailed for different types of studies (epidemiological or animal toxicological studies).
	Are the parameter value assumptions documented and explained?	Y	Conversion factors for points of departure and conversions to the reference doses (RfDs) were provided as well as for CSF from animal studies. Note that modelling from individual studies for selected points of departure was provided in Appendix A.

Criteria		Y/N/?/NA	Notes
	Are the mathematical workings/algorithms clearly documented and explained?	Y	Basic mathematical workings are provided in the main report. Note that modelling from individual studies for selected points of departure was provided in Appendix A.
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	NA	The maximum contaminant level (MCL) for PFOS is based on a Practical Quantification Limit of 4 ng/L (USEPA 2024c). Attainability and practicality of using a PQL was also considered (USEPA 2024c) with suggestions from public comments QC failures that will necessitate repeat sample analysis, increased cost, and reduced laboratory capacity whilst others commented lower PQLs can be achieved.
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	Y	Detailed discussions of mechanistic data and mode of actions were discussed for each effect considered (not solely critical effects).
	What processes are used when expert judgement is required and applied? Is the process documented and published?	?	Unclear.
	Is dose response modelling (e.g. BMDL) routinely used?	Y	Yes, where possible.
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	Y (1/2)	Low-dose linear extrapolation is used for any chemicals causing cancer. Cancer-based values have been derived by US EPA. Since PFAS are understood not to act via a genotoxic mode of action for eliciting cancer, this part of the methodology is not consistent with Australian science policy. Therefore, this criterion has been assigned a '1/2'.
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	Y	Typically 1 in a million.

Summary:

Total # of 'Must-Have' criteria met (or not applicable): 19/20 = 95%

Total # of 'Should-Have' criteria met (or not applicable): 9/10 = 90%

Total # of 'May-Have' criteria met (or not applicable): 2/2 = 100%

B.1.2 US EPA (2024a)

Agency Report Reference: *US EPA (2024a). FINAL Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts, United States Environmental Protection Agency. April 2024. EPA Document No. 815R24006.*
https://www.epa.gov/system/files/documents/2024-04/main_final-toxicity-assessment-for-pfoa_2024-04-09-refs-formatted.pdf.

Criteria		Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	Y	-
	Are the administrative processes documented and publicly available?	Y	-

Criteria		Y/N/?/NA	Notes
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	The systematic review work included in this assessment was prepared in collaboration with ICF under the U.S. EPA Contracts EP-C-16-011 (Work Assignment Nos. 4-16 and 5-16) and PR-OW-21-00612 (TO-0060). This document was prepared by the Health and Ecological Criteria Division, Office of Science and Technology, Office of Water (OW) of the U.S. Environmental Protection Agency (EPA). The agency gratefully acknowledges the valuable contributions of EPA scientists from the OW, Office of Research and Development (ORD), the Office of Children's Health Protection (OCHP), and the Office of Land and Emergency Management (OLEM). The final toxicity assessment was peer reviewed by the EPA Science Advisory Board (SAB) PFAS Review Panel in November 2021 and underwent public comment in March 2023. It incorporated expert scientific recommendations received from the SAB in 2022 as well as feedback from the public comment period. There is a procedure to manage potential conflicts of interest.
	Are funding sources declared?	Y (0.5)	Funding sources not provided in report, but likely funded by the Federal Government of USA.
	Was there public consultation on this work? If so, provide details.	Y	Yes, draft was released for public comment and reviewed by SAB in November 2021. This final toxicity assessment underwent public comment in March 2023. Peer review outcome is documented.
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	This document has been reviewed in accordance with U.S. Environmental Protection Agency (EPA) policy and approved for publication. This final toxicity assessment was peer reviewed by the EPA Science Advisory Board (SAB) PFAS Review Panel in November 2021.

Criteria		Y/N/?/NA	Notes
	Was the guidance/advice developed or updated recently? Provide details.	Y	This final toxicity assessment was peer reviewed by the EPA SAB PFAS Review Panel in November 2021 and underwent public comment in March 2023. It incorporated expert scientific recommendations received from the SAB in 2022 as well as feedback from the public comment period. This final assessment builds upon the literature review presented in the 2016 Health Effects Support Document for Perfluorooctanoic Acid (PFOA) (hereafter referred to as the 2016 PFOA HESD) and is an update of the SAB review draft, Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water, and the subsequent Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water.
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Y	Methodology and results of the health effects systematic review and toxicokinetics methods are detailed in Sections 2.1 and 3.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Y	The evidence integration was conducted according to guidance outlined in the IRIS Handbook and the Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (Anionic and Acid Forms) IRIS Assessments. The evidence integration included evidence stream evaluation, in which the qualitative summaries on the strength of evidence from studies in animals and humans were evaluated, and subsequent inference across all evidence streams. Human relevance of animal models as well as mechanistic evidence to inform mode of action were considered.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	Y	Documented in Sections 2.1 and 3 of the report.
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	NA	Unpublished data do not seem to be mentioned.

Criteria		Y/N/?/NA	Notes
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	Y	Yes, documented in Section 2.1.2. The authors undertook a title and abstract screen, excluding overlapping epidemiological studies, and noted why reports were excluded.
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	NA	Although other reviews are cited, US EPA used their own independent assessment to come to conclusions.
	Can grey literature such as government reports and policy documents be included?	Y	-
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	Y	Detailed throughout the report.
	Evidence search		
	Are databases and other sources of evidence specified?	Y	Yes, refer to Section 2.1.1.
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	Y	The following publicly available databases were searched for literature containing the chemical search terms outlined in Appendix A (U.S. EPA, 2024a): Web of Science™ (WoS) (Thomson Reuters), • PubMed® (National Library of Medicine), • ToxLine (incorporated into PubMed post 2019), and • TSCATS (Toxic Substances Control Act Test Submissions). For the second data stream, other review efforts and searches of publicly available sources were used to identify relevant studies (see Appendix A, U.S. EPA, 2024a); studies cited in assessments published by other U.S. federal, international, and/or U.S. state agencies, studies identified during mechanistic or toxicokinetic evidence synthesis, studies identified by the SAB in their final report dated August 23, 2022, and studies submitted through public comment by May 2023. For the third data stream, EPA relied on epidemiological and animal toxicological literature synthesised in the 2016 PFOS HESD to identify studies relevant to the five priority health outcomes.

Criteria		Y/N/?/NA	Notes
	Is it specified what date range the literature search covers? Is there a justification?	Y	Four separate searches from 2019 (back to 2013), 2020, 2021, and 2022.
	Are search terms and/or search strings specified?	Y	Search terms in Appendix A.
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	Y	The US EPA used populations, exposures, comparators, and outcomes (PECO) criteria to screen the literature identified from the literature sources outlined above in order to prioritise studies for dose-response assessment and to identify studies containing supplemental information such as mechanistic studies that could inform the mode of action analyses. The PECO criteria used for screening the health effects, toxicokinetic, and mechanistic literature are provided in Appendix A (not attached to the reviewed document).
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	Y	All studies were evaluated for risk of bias, selective reporting, and sensitivity following the Methods in Appendix A.
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	Y	Yes, full details provided in report.
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	Y	Yes, confidence is assigned to the levels of evidence and an overall weight of evidence is examined to describe certainty in the evidence.
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	Y	This is detailed for different types of studies (epidemiological or animal toxicological studies).
	Are the parameter value assumptions documented and explained?	Y	Conversion factors for points of departure and conversions to the reference doses (RfDs) were provided. Note that modelling from individual studies for selected points of departure was provided in Appendix A.

Criteria		Y/N/?/NA	Notes
	Are the mathematical workings/algorithms clearly documented and explained?	Y	Basic mathematical workings are provided in the main report. Note that modelling from individual studies for selected points of departure was provided in Appendix A.
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	Y	The maximum contaminant level (MCL) for PFOA is based on a Practical Quantification Limit of 4 ng/L (USEPA 2024c). Attainability and practicality of using a PQL was also considered (USEPA 2024c) with suggestions from public comments QC failures that will necessitate repeat sample analysis, increased cost, and reduced laboratory capacity whilst others commented lower PQLs can be achieved.
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	Y	Detailed discussions of mechanistic data and mode of actions were provided for each effect considered (not solely critical effects).
	What processes are used when expert judgement is required and applied? Is the process documented and published?	?	Unclear.
	Is dose response modelling (e.g. BMDL) routinely used?	Y	Yes, where possible.
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	1/2	Low-dose linear extrapolation is used for any chemicals causing cancer. Two cancer-based values have been derived by US EPA. Since PFAS are understood not to act via a genotoxic mode of action for eliciting cancer, this part of the methodology is not consistent with Australian science policy. Therefore, this criterion has been assigned a '1/2'.
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	Y	Typically 1 in a million.

Summary:

Total # of 'Must-Have' criteria met (or not applicable): 19/20 = 95%

Total # of 'Should-Have' criteria met (or not applicable): 9/10 = 90%

Total # of 'May-Have' criteria met (or not applicable): 2/2 = 100%

B.1.3 Burgoon et al. (2023)

Report Reference: *Burgoon L. D., Clewell H. J., Cox T., Dekant W., Dell L. D., Deyo J. A., Dourson M. L., Gadagbui B. K., Goodrum P., Green L. C., Vijayavel K., Kline T. R., House-Knight T., Luster M. I., Manning T., Nathanail P., Pagone F., Richardson K., Severo-Peixe T., Sharma A., Smith J. S., Verma N. and Wright J. (2023). Range of the perfluorooctanoate (PFOA) safe dose for human health: An international collaboration. Regulatory Toxicology and Pharmacology 145: 105502.*

Criteria		Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	Y	The report was prepared under the Alliance for Risk Assessment (ARA), a collaboration of organisations that fosters the development of technical chemical risk assessment products and services. The ARA put out a call to participate in a project to derive safe doses for PFOA and PFOS. A committee was established for PFOA, held regular meetings, and evaluated available scientific data relied upon by other international organisations.
	Are the administrative processes documented and publicly available?	N	No available information online.
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	The international collaboration had an advisory committee and potential conflicts of interest were identified. ARA has a steering committee which is made up of professionals in different segments of the risk assessment community and brings with them years of experience protecting public health.
	Are funding sources declared?	Y	No funding was accepted from any organisation for this work. ARA receives donations to carry out its projects. None were disclosed in the journal article.
	Was there public consultation on this work? If so, provide details.	N	None disclosed

Criteria		Y/N/?/NA	Notes
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y (1/2)	There is at least one committee peer reviewing the project. Additionally, the journal (Regulatory Toxicology and Pharmacology) follows a single anonymised review process. Submissions are initially assessed by journal editors to determine suitability for publication in this journal. If the submission is deemed suitable, it will typically be sent to a minimum of two reviewers to assess the scientific quality. The results of the internal or journal peer review were not published.
	Was the guidance/advice developed or updated recently? Provide details.	Y	The advice was recently developed (in 2023).
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Y	The method of how the review was conducted is documented.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Y	Yes. The focus was on PFOA related studies relied upon by international organisations when deriving criteria for PFOA.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	NA	No review was conducted. Instead, the paper is reliant on reviews of studies conducted by a range of International Agencies.
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	NA	Unpublished data are not specifically mentioned nor are data (published or unpublished) discussed. Instead, the paper relies on descriptions of data and evaluations by other international organisations.
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	Y	Yes. PFOA related studies relied upon were those relied upon and described by international organisations when deriving criteria for PFOA.
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	Y	Although assessments of other organisation reviews are cited and relied upon in this journal article, the authors did use their own independent assessment to come to conclusions.
	Can grey literature such as government reports and policy documents be included?	NA	-

Criteria		Y/N/?/NA	Notes
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	Y	The authors provide discussions on mode of action and validity of different studies evaluated.
	Evidence search		
	Are databases and other sources of evidence specified?	Y	The journal article relied upon reviews by select international organisations.
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	It is not clear how the Advisory Committee selected the relevant publications (i.e. international organisational reviews) for the journal article. The journal article is reliant on international organisation reviews that did carry out literature searches.
	Is it specified what date range the literature search covers? Is there a justification?	N	It is not clear how the Advisory Committee selected the relevant publications (i.e. international organisational reviews) for the journal article.
	Are search terms and/or search strings specified?	N	It is not clear how the Advisory Committee selected the relevant publications (i.e. international organisational reviews) for the journal article.
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	It is not specifically stated how the relevant publications and international organisations were selected.
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	The journal article relied upon individual studies considered relevant by other agencies.
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	Y	Yes, there were regular meetings to discuss the data and it is described in a logical / sequential manner in the journal article.

Criteria		Y/N/?/NA	Notes
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	Y	Yes, although they do not provide confidence in the levels of evidence, the journal article includes review of the available studies, relevance of the associated effects, uses a weight of evidence approach to evaluate study findings, and makes specific conclusions on the studies evaluated. Formal certainty ratings are not derived/reported.
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	Y	Uncertainty factors are appropriately justified/explained.
	Are the parameter value assumptions documented and explained?	Y	Yes. Factors such as clearance etc. are selected from studies and appropriately justified.
	Are the mathematical workings/algorithms clearly documented and explained?	Y	The journal article does not calculate departure points so only some simple math multiplying established departure points by uncertainty factors and serum reference doses by clearance factors. The math is shown when the simple calculations are performed.
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	NA	-
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	Y	Although there is very little information on mechanistic/mode of action studies in the document, the journal article includes a concise discussion of mode of action.
	What processes are used when expert judgement is required and applied? Is the process documented and published?	Y	Outlined in the journal article is a process whereby they have three teams separately evaluating the same data to identify the decision making in independent reviews. There were also regularly scheduled meetings to discuss the review and findings.
	Is dose response modelling (e.g. BMDL) routinely used?	Y	Yes, where dose response modelling was relied upon in the evaluated studies, the results of this modelling were used.

Criteria		Y/N/?/NA	Notes
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	Y	The position taken in the journal article was that carcinogenicity of PFOA observed in studies was not relevant to humans or there were confounding factors. As such, carcinogenicity and low dose extrapolation (or data /studies reliant upon low dose extrapolation) were not considered necessary/relevant.
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	NA	There was general agreement that the US EPA's proposed change in the categorisation of PFOA from "suggestive evidence" to "likely carcinogen" is not justified.
Summary: Total # of 'Must-Have' criteria met (or not applicable): 16/20 = 80% Total # of 'Should-Have' criteria met (or not applicable): 7.5/10 = 75% Total # of 'May-Have' criteria met (or not applicable): 1/2 = 50%			

Appendix C Discussions on PFAS Mixture Assessment

Addendum to PFAS Evidence Evaluation for Australian Drinking Water Guidelines Chemical Fact Sheets

Addendum / Work Expansion for 2024 NHMRC PFAS Review of Australian Health-based Guideline Values

National Health and Medical Research Council

SLR Project No.: 640.031365.00001

17 October 2024

SLR

C.1 Discussions for PFAS Mixture Assessment

C.1.1 US EPA (2024e, 2024f, 2024g)

In 2021, the US EPA released a draft framework for estimating potential non-cancer risk associated with PFAS mixtures (US EPA 2021, 2022) which was released for public comment in 2023 (US EPA 2023) and later finalised in 2024 (US EPA 2024e). The following three different approaches for assessing PFAS mixtures were proposed and included a detailed discussion of the benefits and cons of each for users of the Framework to decide which approach was most appropriate to use.

1 The Hazard Index (HI) approach: Considered likely to be the most health protective approach as it is based on the most sensitive health outcome for each PFAS in a PFAS mixture.

2 The Relative Potency Factor (RPF) approach provides a mixture toxicity estimate by scaling the potency of component chemicals for a common health effect. This approach is considered more data intensive than the HI approach and typically data must meet two requirements, i) PFAS must share a mode of action or critical effect and ii) PFAS must have similar dose response functions.

3 The mixture benchmark dose (M-BMD) approach uses a dose addition model-based equation for the mixture. This approach provides more accurate predictions of a mixture effect even if the slopes of the dose response curves differ among the chemicals.

The US EPA considered the MOA data to be limited/lacking therefore relied upon the "*similarity of toxicological endpoint/effect/adverse outcome*" when identifying an appropriate approach to use.

In 2022, US EPA released a technical fact sheet (USEPA 2022) which adopted the HI approach to assess the potential non-cancer risk for a mixture of four PFAS, i.e. PFOA, PFOS, GenX chemicals, and PFBS using the equation shown below:

$$HI = \left(\frac{[PFOA]_{Water}}{[PFOA]_{HA}}\right) + \left(\frac{[PFOS]_{Water}}{[PFOS]_{HA}}\right) + \left(\frac{[GenX]_{Water}}{[GenX]_{HA}}\right) + \left(\frac{[PFBS]_{Water}}{[PFBS]_{HA}}\right)$$

Where:

- HI = hazard index;
- $[PFAS_{Water}]$ = concentration for a given PFAS in water;
- $[PFAS_{HA}]$ = the Health Advisories (HA) value for a given PFAS.

The $[PFAS_{HA}]$ in the technical fact sheet were 0.004 ng/L for PFOA, 0.02 ng/L for PFOS, 10 ng/L for GenX chemicals, and 2,000 ng/L for PFBS. It was noted that "*If water sampling results show the presence of PFOA, PFOS, or levels of GenX chemicals or PFBS in drinking water above **the health advisory levels**, water systems should notify their state drinking water safety agency (or EPA in jurisdictions for which EPA is the primary drinking water safety agency) and consult with the relevant agency on the best approach to conduct additional sampling*" (US EPA 2022).

In the Final PFAS National Primary Drinking Water Regulation, the US EPA released Final MCL (enforceable levels) of 4 ng/L each for PFOA and PFOS, 10 ng/L for PFHxS, PFNA,

and GenX chemicals, and 2,000 ng/L for PFBS (US EPA 2024c, d, g)[27]. The final HI approach was announced for a mixture of two or more of four PFAS (PFHxS, PFNA, GenX chemicals, and PFBS) with a HI to be calculated using the following equation (US EPA 2024g):

$$HI = \left(\frac{[PFHxS]_{Water}}{10}\right) + \left(\frac{[PFNA]_{Water}}{10}\right) + \left(\frac{[GenX]_{Water}}{10}\right) + \left(\frac{[PFBS]_{Water}}{2{,}000}\right)$$

Where

- HI = hazard index;
- [$PFAS_{Water}$] = concentration for a given PFAS in water in units of ng/L.

It is evident that in the updated HI approach that PFOS and PFOA are no longer included whereas two new PFAS are included, PFHxS and PFNA. The exclusion of PFOA and PFOS from the updated HI approach may simply be because the MCL for PFOA and PFOS are based on PQLs rather than a HA. Compliance with the Hazard Index MCL is determined by a running annual average of four HI estimated from quarterly samples collected over the past year (US EPA 2024g).

The HI approach as adopted by the US EPA is likely to be the most health protective but it is also limited in application as only four PFAS are considered and PFOS and PFOA are considered separately. Even though the US EPA framework allows for the use of the RPF approach or M-BMD approach they have not yet been proposed for assessing mixtures by US EPA.

C.1.2 European Union (EU 2020)

The updated (recast) European Union (EU) Drinking Water Directive (EU 2020), which took effect on 12 January 2021, legislated the following two limits for PFAS mixtures considered "minimum requirements for parametric values used to assess the quality of water intended for human consumption":

- 'Sum of PFAS' of 0.1 µg/L (100 ng/L): This is a subset of 'PFAS Total' substances that contain a perfluoro-alkyl moiety with three or more carbons (i.e. $–C_nF_2n–$, n ≥ 3) or a perfluoroalkyl-ether moiety with two or more carbons (i. e. $–C_nF_{2n}OC_mF2_m–$, n and m ≥ 1), i.e. 20 measurable PFAS [28].
- 'PFAS Total' of 0.5 µg/L (500 ng/L): 'PFAS Total' means the totality of per- and polyfluoroalkyl substances[29].

The basis of these limits was not enunciated in the EU Drinking Water Directive. Nonetheless, the 'Total PFAS' limit is the same value proposed by WHO (2022) except that

[27] The basis of the HA for PFOA, PFOS, PFHXS, PFNA, PFBS, and GenX chemicals were previously considered in the 2024 PFAS Review.

[28] Sum of PFAS includes: Perfluorobutanoic acid (PFBA), Perfluoropentanoic acid (PFPA), Perfluorohexanoic acid (PFHxA), Perfluoroheptanoic acid (PFHpA), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluoroundecanoic acid (PFUnDA), Perfluorododecanoic acid (PFDoDA), Perfluorotridecanoic acid (PFTrDA), Perfluorobutane sulfonic acid (PFBS), Perfluoropentane sulfonic acid (PFPS), Perfluorohexane sulfonic acid (PFHxS), Perfluoroheptane sulfonic acid (PFHpS), Perfluorooctane sulfonic acid (PFOS), Perfluorononane sulfonic acid (PFNS), Perfluorodecane sulfonic acid (PFDS), Perfluoroundecane sulfonic acid, Perfluorododecane sulfonic acid, Perfluorotridecane sulfonic acid.

[29] This parametric value shall only apply once technical guidelines for monitoring this parameter are developed in accordance with Article 13(7). Member States may then decide to use either one or both of the parameters 'PFAS Total' or 'Sum of PFAS' (EU 2020).

the WHO value is for 30 measurable PFAS whereas the EU Drinking Water Directive refers to the totality of PFAS (**Section C.1.7**). The lower Sum of PFAS limit (100 ng/L) is for a subgroup of measurable PFAS that doesn't include fluorotelomer PFAS and precursors to other PFAS. It is unlikely that either of these limits are health-based and instead they are assumed to be based on practical considerations.

The grouped approach adopted by the EU (and WHO) has the advantage of providing a means for water providers to assess mixtures of measurable PFAS in water, something that is not currently achievable with the methods described in the US EPA framework due to the distinct lack of toxicological data (US EPA 2024c). However, it is not health based and there is no reasoning/justification provided as to whether such a limit would prove to be health effective. It is also unclear how the totality of PFAS (Total PFAS) will be measured. Nonetheless, it is stated in the EU Drinking Water Directive for PFAS Total and Sum of PFAS that "*By 12 January 2024, the Commission shall establish technical guidelines regarding methods of analysis for monitoring of per- and polyfluoroalkyl substances […], including detection limits, parametric values and frequency of sampling*" and that "*by 12 January 2024, Member States shall take the measures necessary to ensure that water intended for human consumption complies with the parametric values set out in Part B of Annex I*" (EU 2020).

C.1.3 European Commission (EC 2022)

The Scientific Committee on Health, Environmental and Emerging Risks (SCHEER), a committee of the European Commission (EC), released an opinion on the appropriate Quality Standard (QS) to adopt for groundwater. The committee "*does not agree with a group QS of 0.5 µgL^{-1} for Total PFAS*" legislated by the EU (see **Section C.1.2**) due to the unavailability of an analytical method that can measure PFAS in its entirety (EC 2022). Further, instead of adopting a Sum of PFAS limit of 100 ng/L for a "Group of 10" PFAS (a subset of the 20 PFAS recast in EU Drinking Water Directive), SCHEER has proposed ("*strongly suggested*") to use the surface water QS of 4.4 ng/L for PFOA equivalents derived using a "*relative potency approach*" based on PFOA equivalents (EC 2022).

The surface water QS relies upon the Total Weekly Intake (TWI) of 4.4 ng/kg/week (equivalent to a TDI of 0.693 ng/kg/d) derived by the 'CONTAM Panel' of European Food Safety Authority (EFSA) for the sum of four PFAS (∑PFAS4), i.e. the sum of PFOA, PFOS, PFNA, and PFHxS (EFSA 2020). The ∑PFAS4 were found to contribute most to the PFAS levels observed in human serum, share toxicokinetic properties in humans and show similar accumulation and long half-lives. Also, in terms of effects, these compounds in general show the same effects when studied in animals. As a pragmatic approach, the CONTAM Panel assumed by default equal potencies for effects of these four PFAS on immune outcomes. The CONTAM Panel noted that this TWI is protective for the other potential critical endpoints such as increase in serum cholesterol, reduced birth weight and high serum levels of ALT considered in the previous Opinion on PFOS and PFOA (EFSA CONTAM Panel, EFSA 2018).

The TWI is based on a $BMDL_{10}$ of 17.5 ng/mL for the ∑PFAS4 derived using 10% decreased antibody titre following diphtheria vaccination in 1-year old children. Taking into account 1 year of breastfeeding and transfer of PFAS in breast milk to the infant, the equivalent serum concentration in mothers was determined by physiologically based pharmacokinetic (PBPK) modelling to be 6.9 ng/mL at 35 years of age. This corresponds to a dose of 0.63 ng/kg bw/day (or 4.4 ng/kg bw/week). No uncertainty factor was applied, because the $BMDL_{10}$ is based on infants which are expected to be a sensitive population group. In addition, a decreased vaccination response is considered a risk factor for disease rather than a disease. Although not explicitly shown in EC (2022), the surface water QS of 4.4 ng/L was derived using a TDI of 0.693 ng/kg/d, a RSC of 20% (0.2), drinking water consumption of 2

L/d, and body weight of 70 kg, i.e. surface water QS = 4.4 ng/L =0.693 ng/kg/d x 70 kg x 0.2 ÷ 2 L/d.

EC (2022) refer to the approach taken as an "RPF Approach". Although it is understood that the TDI selection was justified with RPF for liver function, it was calculated using a benchmark dose approach for the mixture, i.e. it appears to be a M-BMD Approach as described in the US EPA Framework (USEPA 2024d).

The suggested QS by EC (2022) permits the assessment of an additional PFAS not regulated in Australia (PFNA) and considers the ∑PFAS4 as a combined group. However, the approach adopted is limited in that it is only applicable to four PFAS (there are many other PFAS measured in environmental waters and drinking water) and the basis of the TWI is questionable[30].

C.1.4 Health Canada (HC 2018a, 2018b, 2022, 2023)

In 2018, Health Canada (HC) proposed a HI approach to assess mixtures of PFOA and PFOS using the recently developed DWG given the HI approach is health protective and these two PFAS are the predominant PFAS detected in Canadian waters (HC 2018a, 20218b). The HI approach was not considered for other PFAS due to the lack of toxicological data although HC had derived screening values for a further nine PFAS (PFBA, PFPeA, PFHxA, PFHpA, PFNA, PFBS, PFHxS, 6:2 FTS and 8:2 FTS). The HI for PFOA and PFOS is calculated as follows:

$$HI = \frac{PFOS_{concentration}}{MAC_{PFOS}} + \frac{PFOA_{concentration}}{MAC_{PFOA}}$$

$$or\ \frac{PFOS_{concentration}}{0.6\mu g/L} + \frac{PFOA_{concentration}}{0.2\mu g/L}$$

Where:

- $PFAS_{Concentration}$ = water concentration for an individual PFAS (µg/L).
- MAC_{PFAS} = maximum acceptable concentration for an individual PFAS (µg/L).

A practical approach was proposed in 2023 for the Sum of PFAS as HC proposed a drinking water objective of 30 ng/L for the sum of measurable PFAS (HC 2022, HC 2023)[31]. The purpose of the objective is to reduce exposure to PFAS through drinking water as a precautionary measure while formal guidelines are being formalised. The objective is based on the following practical considerations.

- The levels of PFAS found in Canadian waters.
- The technology available to remove PFAS from drinking water.
- The lowest levels of PFAS that can be measured in water using validated methods.
- The lowest concentration that can be achieved from a technical standpoint for a larger number of PFAS to reduce potential exposure to PFAS in drinking water.

The result of a non-detect is considered to have a value of zero when summing measurable PFAS. A health-based approach was not adopted in part due to rapidly evolving science,

[30] Statistically significant associations were found between the PFOA in the plasma of one-year-old breastfed children and decreased levels of vaccine antibodies against influenza, tetanus, and diphtheria but not for PFOS, PFNA, or PFHxS. There was also no relationship observed between the PFAS in plasma and the number of infections within the study group.

[31] The sum of measurable PFAS refers to "*the full list of substances in either the United States Environmental Protection Agency (U.S. EPA) Method 533 or U.S. EPA Method 537.1, or both*" (HC 2023).

lack of consensus on critical effects, and varying approaches to hazard and risk assessment (amongst Canadian jurisdictions) (HC 2022). Furthermore, people are being exposed to multiple PFAS simultaneously with little known regarding the potential hazard associated with exposure to these mixtures and a substance-by-substance assessment of the TRVs for each PFAS is not a sustainable approach for managing PFAS in drinking water (HC 2023).

C.1.5 Maine Department of Human Health Services (Maine DHHS 2021)

In 2021, the Maine Legislature established a new interim State drinking water standard of 20 ng/L for the combined sum of six different PFAS: PFOA, PFOS, PFHxS, PFNA, PFHpA and PFDA (Maine DHHS 2021). The basis of this DWG and the approach to six PFAS was not located in the publicly available literature. Nonetheless, the approach, DWG and six PFAS are the same as the approach with read across toxicity as established by Mass DEP and described below (**Section C.1.6**).

Maine DHHS recently announced in April 2024 that they are evaluating the new federal standard for PFAS including the updated Hazard Index approach derived by US EPA with the intention to "*propose a final federally-aligned State standard through the rule making process. As of now, the current interim standard of 20 ppt for six PFAS compounds (alone or in combination) is still in effect*" (Maine DHHS 2024). The new federal standard for PFAS is as described in **Section C.1.1**.

The relative merits of US EPA's Hazard Index approach and Mass DEP RPS Approach are discussed in the relevant sections.

C.1.6 Massachusetts Department of Environmental Protection (Mass DEP 2022, Mass DEP 2019)

In a letter to public water suppliers, the Massachusetts Department of Environmental Protection (Mass DEP) established a drinking water Maximum Contaminant Limit (MCL) of 20 ng/L (20 ppt) for the sum of six PFAS substances (the "PFAS6"); PFOS, PFOA, PFHxS, PFNA, PFHpA, and PFDA (Mass DEP 2022). The basis of the MCL for the PFAS6 is not provided in this letter[32]. Additional information related to the derivation of the PFAS6 MCL was found on the Mass DEP website for PFAS[33].

Mass DEP outlined the origins of a revised MCL for an updated PFAS subgroup that include the PFAS6 in their 2019 technical support document (Mass DEP 2019) extending previous work in 2018 which included a subgroup of five PFAS (PFOA, PFOS, PFNA, PFHxS, and PFHpA) [34]. The PFAS subgroup considered were those PFAS with plus or minus two carbons (C6-C10 compounds) compared with PFOA and PFOS, i.e. PFHxA, PFHpA, PFNA, PFDA, PFHxS, PFHpS, PFNS, and PFDS. Three of these PFAS (PFNS, PFDS, and PFHpS) were immediately excluded from the sub-group as, in 2018, they were not included as USEPA Method 537.1 analytes (Mass DEP 2019). PFHxA was also excluded from the

[32] It is noted that the revised MCL for the PFAS6 (20 ng/L) is not equivalent to the EPA DWG quoted in the letter including; the Interim Health Advisory for PFOA (0.004 ng/L), Interim Health Advisory for PFOS (0.02 ng/L), Final Health Advisory for GenX chemicals (HFPO)-replacement chemical for PFOA (10 ng/L), or Final Health Advisory for PFBS (2,000 ng/L).

[33] Mass DEP website last accessed 11 July 2024 at this location: https://www.mass.gov/info-details/per-and-polyfluoroalkyl-substances-pfas#drinking-water-standards-and-health-information-

[34] Mass DEP previously established an (ORSG) of 70 ng/L in 2018 for the 2018 subgroup of five closely related PFAS (including PFOA, PFOS, PFNA, PFHxS, and PFHpA, Mass DEP 2019). The ORSG is higher than the PFAS6 MCL as Mass DEP included an additional uncertainty factor of $10^{1/2}$ to the RfD derivation for PFOS and PFOA in the latter 2019 reevaluation.

PFAS6 as it was determined it has a much shorter half-life and is substantially less toxic than PFAS in this subgroup (Mass DEP 2019). PFDA was retained as Mass DEP undertook an evaluation of this PFAS and found it shared similar toxicity endpoints and potencies with other PFAS in this subgroup. As such, the PFAS6 includes the five previously considered PFAS in the previous 2018 subgroup and PFDA. This practice of grouping like PFAS together is referred to as a "subgroup approach".

The PFAS6 MCL of 20 ng/L is based on Mass DEP Office of Research and Standards (ORS) reference dose (RfD) for PFOA and PFOS of $5x10^{-6}$ mg/kg/day. The RfD is based on the lowest of the range of eleven of twelve RfDs reported by USEPA (ranging from $2x10^{-5}$ to $5x10^{-5}$ mg/kg/d) from two developmental toxicity/multigenerational toxicity studies from which the following was identified.

- A serum LOAEL of 38.0 mg/L for pup ossification and accelerated puberty (GD 1-17) in CD1 mice from Lau et al. (2006) converted to a POD_{HED} of 5.3 µg/kg/d and the application of an uncertainty factor of 300 (UF_H = 10, UF_A = 3, UF_L = 10).
- A NOAEL of 6.26 mg/L for reduced pup body weight in Sprague Dawley rat from Luebker et al. (2005a) converted to a POD_{HED} of 0.51 µg/kg/d and the application of an uncertainty factor of 30 (UF_H = 10, UF_A = 3).

Mass DEP applied an additional uncertainty actor of 3 ($10^{1/2}$) to account for effects (developmental mammary and liver effects for the Lau et al. 2006 study and immune effects for the Luebker et al. 2005 study) being caused by these compounds at lower doses than relied upon in the US EPA 2016 assessment. Hence the RfD adopted by Mass DEP was $5x10^{-6}$ mg/kg/d (=$2x10^{-5}$ mg/kg/d ÷ 3).

Mass DEP justified their subgroup approach by undertaking toxicological evaluations for each of the PFAS6 and deriving RPFs alongside previously derived RPFs (from Zeilmaker et al. 2018, Luz et al. 2019, as cited in Mass DEP 2019) as shown in **Table C.1** below.

Table C.1 PFAS Relative Potency to PFOA Reported in Mass DEP 2019

PFAS6	Zeilmaker et al. (2018) (1)	Luz et al. (2019) (2)	Mass DEP 2019 (3, 4)
PFOA	1	1	1
PFOS	2	4	1 to 4
PFNA	10	2	0.6 – 3
PFHxS	0.6	0.5	0.2 – 0.8
PFDA	4 ≤ RPF ≤ 10	2	1 - 2
PFHpA	0.01 ≤ RPF ≤ 1	-	-

(1) Potential RPFs were developed for several PFAS based on liver toxicity endpoint.

(2) RPFs were calculated for seven PFAS and a range of effects (hepatocellular hypertrophy, liver weight, kidney weight, cholesterol, body weight and reticulocyte count) based on 28-day bioassays (NTP 2018) using BMDLs as applied doses.

(3) RPFs derived for free thyroxine (fT4) and relative liver weight from NTP (2018) using modelled averaged Bayesian Benchmark Dose (BBMD) associated with the benchmark response (BMR), e.g. 20% decrease from control ($BBMD_{20}$) and 5% decrease from control ($BBMD_{05}$).

(4) The range shown is for four values calculated using serum or human equivalent doses for fT4 ($BBMD_{20}$) and relative liver weights ($BBMD_{05}$).

The derivation of the "drinking water value" by Mass DEP as "*described below:*

$$Drinking\ Water\ Value = \frac{RfD \times RSC}{Water\ Consumption\ Rate\ per\ kg\ body\ weight}$$

Where:

- *RfD = 5 x 10^{-6} mg/kg-day*
- *Water consumption rate for lactating woman = 0.054 L/kg-day*
- *Relative Source Contribution Factor (RSC) = 0.2*

$$Drinking\ Water\ Value = \frac{5 \times 10^{-6} mg/kg - d \times 0.2}{0.054 L/kg - d}$$

$$= 0.0000185\ mg/L$$

$$= 0.00002\ mg/L$$

or 20 ng/L (20 ppt), rounded to one significant figure.

When these six compounds occur alone, together, or in any combination, the sum of their concentrations should be compared to 0.00002 mg/L." (Mass DEP 2019, page 35).

Note that Mass DEP identified pregnant women, nursing mothers and infants as sensitive groups.

Mass DEP refer to the methodology taken as an RPF Approach. However, they did not use the RPFs to adjust for concentrations or the individual PFAS RfDs even though the RPF ranged from 0.2 to 2 and other RPFs derived in the literature with the same data ranged from 0.01 to 10 (refer to **Table C-1** above). It could be argued that the TRVs did not need to be adjusted, i.e. a RPF of 1 is applicable for each of these PFAS considered to have equal toxicity, i.e. these PFAS are "*equipotent"* (Mass DEP 2019). However, Mass DEP only consider liver effects and did not calculate RPFs for other effects (including critical effects that their PFOS and PFOA guidelines are based on). Therefore, the RPF Approach as referred to by Mass DEP is more akin to a Surrogate Approach supported by RPFs for liver effects. For this reason, the approach adopted by Mass DEP will be referred to as a Surrogate Approach in this Addendum report.

Mass DEP identified Vermont and Connecticut as adopting a similar approach to PFAS mixtures which essentially considers the PFAS subgroup to be "equipotent" (Mass DEP 2019). Mass DEP (2019) found PFAS "*cancer data is concerning*" and may consider an MCL goal of zero in the future. Mass DEP is currently working with public water suppliers with sources above the PFAS6 MCL to lower the concentration of PFAS6 in their water (Mass DEP 2022).

The sub-group approach developed by Mass DEP has merit due to the paucity of toxicological data for many PFAS. Using read across with similar chemical properties, physical properties, and toxicities is an approach used for other chemical classes (e.g. polychlorinated biphenyls, dioxins, petroleum hydrocarbons etc.). In this instance, Mass DEP undertook toxicological evaluations for the PFAS6 and used RPF based on liver toxicity to justify their subgroup approach for them. However, a complicating factor is that the RPFs are derived for liver toxicity whereas the RfDs utilised to derive the MCL are based on developmental effects. Ideally, RPFs would be derived for a range of effects including those on which the critical effects are based.

C.1.7 World Health Organisation (WHO 2022, 2023).

WHO provisional Guideline Value (pGV)

WHO (2022), a draft background document offered for public consultation from 29 September to 11 November 2022, adopts a practical approach to adopting a DWG for 'Total PFAS'. WHO have indicated that they will be undertaking a more comprehensive review on PFAS including further examination of whether international health-based GV can be established (WHO 2023).

WHO (2022) proposed a combined provisional guideline value (pGV) of 0.5 µg/L (500 ng/L) for total PFAS as an effective means to managing PFAS as a class and reducing exposure to these substances based on the following considerations:

- Approximately 30 PFAS (including PFOS and PFOA) "*are currently measurable by available methods*".
- PFOS and PFOA are likely to co-occur together with other PFAS (i.e. as a mixture) in the environment[35].
- Available data indicate that 0.5 µg/L for Total PFAS should be achievable[36].
- Water suppliers should make every effort to achieving overall levels as low as reasonably practical.

As is evident from the above considerations the pGV for Total PFAS is not a health-based DWG. Instead, it is based on practical considerations aimed at reducing exposures or undertaking activities that are achievable ("*especially for resource limited countries and contexts that do not have these systems in place or do not have the ability to consistently operate them effectively* […] *and […] there is limited benefit in establishing requirements that cannot practically be achieved*", WHO 2023).

It is further noted that the pGV for each of PFOS and PFOA (0.1 µg/L or 100 ng/L) should not be exceeded when calculating the combined pGV. The pGV for PFOS and PFOA are also based on practical considerations, i.e. achievable concentrations following treatment with high pressure membrane filtration and upper-bound concentrations detected in drinking-water sources have mostly been in the low µg/L range.

Other Approaches Identified by WHO

There were two additional approaches identified in the WHO (2022) document from other international agencies that employed a mixtures-based approach. The Danish Ministry of the Environment included PFOSA with PFOS and PFOA in a HI approach, whereas the Swedish National Food Agency included 9 additional PFAS in addition to PFOS and PFOA in a surrogate approach[37], refer to **Table C.2** below. In both additional approaches the PFOS HBV was used for the other PFOSA by the Danish and nine additional PFAS by the Swedish.

[35] Many PFAS demonstrate high persistence, accumulation potential and/or hazards to the environment and/or human health.

[36] Most studies undertaken on real drinking water systems often have low concentrations of PFAS mixtures in the inlet, often well below 0.5 µg/L and high-pressure membrane processes or GAC would be expected to reduce total PFAS concentrations to below 0.5 µg/L (WHO 2022).

[37] In a 2014 publication, Livsmedelsverket (2014) stated in an earlier publication for seven PFAS that it was "assumed *that the constituent PFAAs have the same toxicity as PFOS*" (translated from Swedish using Google Translate). This is considered akin to a surrogate approach.

The Swedish and Denmark approaches have since been updated in line with the RPF approach from the European Commission (EC 2022) and Sum of PFAS approach from the European Union (EU 2020).

Table C.2 Other Approaches Identified by WHO [1]

Organisation	PFOS HBV	PFOA HBV	Combined Approach
Danish Ministry of the Environment (2015) (Superseded in 2021)	0.1 (0.2)	0.3 (0.6)	PFOA (conc. µg/L) / 0.3 µg/L + PFOS (conc. µg/L) / 0.1 µg/L + PFOSA (conc. µg/L) / 0.1 µg/L < 1
Swedish National Food Agency (2014) (Superseded in 2021)[5]	0.09 (0.2)	-	0.09 µg/L for total PFAS (PFBS, PFHxS, PFOS, 6:2 FTS and PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA)

HBV = Health-based values, Value in brackets is given as the "PFOS WHO Eq GV" [2].

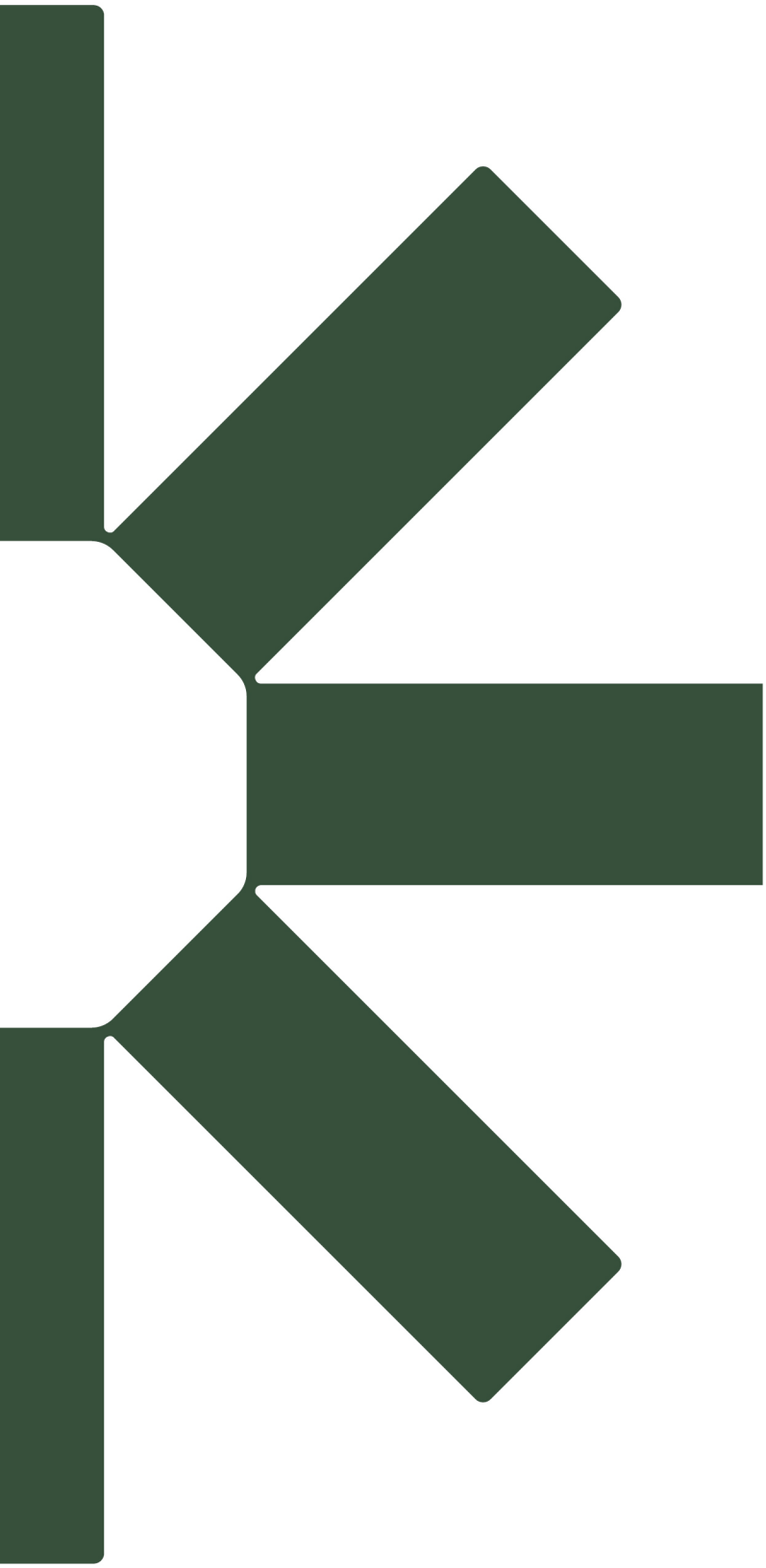
(1) Only the approaches that considered more constituents than PFOS, PFOA and PFHxS are shown.
(2) WHO equivalent values based on HBV, human body weight of 60 kg, adult drinking water intake of 2 L/day, and allocation factor of 20%, unless otherwise stated.

(3) Danish Ministry of the Environment (2015) HBVs based on drinking water intake rate of 0.03 L/kg bw/day and allocation factor of 10%.

(4) Swedish National Food Agency (2014) HBVs based on infant body weight of 4.2 kg, drinking water intake rate for infants (0.7 L/day), and allocation factor of 10%.

(5) In 2021, Denmark adopted a guideline value of 2 ng/L for ∑PFAS4 (DHI 2021).

(6) In 2023, Sweden adopted the RPF approach for ∑PFAS4 from the European Commission (EC 2022) and the Sum of PFAS limit of 100 ng/L for PFAS-21 from the European Union (EU 2020), refer to Life Source (2023).



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