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 Report

National Health and Medical Research Council

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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 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). There is currently no Australian drinking water guideline or existing Fact Sheet for perfluorobutane sulfonic acid (PFBS) and hexafluoropropylene oxide ammonium salt plus hexafluoropropylene oxide dimer acid (also termed GenX Chemicals).

The National Health and Medical Research Council (NHMRC) have contracted SLR Consulting Australia Pty Ltd (SLR) to identify existing sources of guidance or guidelines on the impact of exposure to these five select PFAS in drinking water at levels higher or lower than the current Australian Drinking Water Guidelines health-based guideline values (where these exist) on human health outcomes.

An evidence scan to inform an update to the existing supporting information provided in the current Fact Sheet was also requested to be undertaken. This included levels detected in Australian drinking water, analytical/detection, monitoring and treatment guidance.

This evidence review has been undertaken in line with a new methodological framework intended to implement best practice methods for evidence evaluations as per the NHMRC Standards for Guidelines.

This Evaluation Report summarises the evaluation undertaken for the five select PFAS and concludes by identifying potential drinking water guideline values for adoption/adaption in the Australian context. The methodology of the review is also provided in more detail in an accompanying Technical Report.

The volume of information found in the literature search undertaken in August 2023 and needing to be assessed was very large. Due to resource constraints and with agreement from NHMRC with advice from the Water Quality Advisory Committee, critical evaluation of studies was prioritised to those studies that had not been previously reviewed and/or considered by an Australian agency for guidance/guideline value development. The latest review by an Australian jurisdiction in which guidance values were derived for three of the PFAS under consideration (PFOS+PFHxS and PFOA) was the document from Food Standards Australia New Zealand (FSANZ 2017b). This forms the basis of the current toxicity reference values (TRVs) for PFOS/PFHxS and PFOA which were used by NHMRC to derive the current guideline values in drinking water for these chemicals. FSANZ (2021) also published a review of immunomodulation effects, in which the jurisdiction reviewed a number of studies, findings of which are used to support discussions in this report on relevant PFAS.

The candidate drinking water guidelines (DWGs) for potential adoption/adaptation of suitable information for each of the five PFAS are provided in **Sections 6** to **10** of this report, with the conclusions presented in **Section 11**. 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 for some chemicals. In summary, the following options for guideline values were proposed.

- PFOS – the current Australian health-based DWG of 70 ng/L is still considered to be appropriate.
- PFHxS – a guideline value of 34 ng/L was considered as being potentially suitable (and conservative) for PFHxS on its own, as was the current Australian DWG value of 70 ng/L for the sum of PFOS + PFHxS. In practice this means it is considered

reasonable to retain the existing guideline value of 70 ng/L as the sum of PFOS+PFHxS, with PFHxS not exceeding 34 ng/L.

- PFBS – guideline values ranging from 1,041 to 2,939 ng/L in drinking water were considered as being appropriate and conservative. This would be a new DWG for this chemical.
- PFOA – guideline values ranging from 9.5 to 70 ng/L in drinking water were considered as being potentially appropriate and conservative, as is the current Australian guideline value of 560 ng/L. However, due to various reasons outlined in **Sections 9.2.1** to **9.2.5**, the confidence in the candidate guideline values (9.5 to 70 ng/L) is considered very low to low. It is therefore suggested the information is not of high enough quality to warrant revision of the current Australian guideline value for PFOA (560 ng/L), for which the confidence in the underpinning study is high.
- GenX Chemicals – there is currently insufficient evidence to derive a health-based DWG for GenX Chemicals. However, a concentration of potential concern of 263 ng/L could be derived based on the limited toxicity data available. There is currently no existing DWG for GenX Chemicals.

From the available information gathered on exposure to the five PFAS of interest in Australian distributed drinking waters and the information gathered to inform supporting information in the Fact Sheet, all DWG options would be readily measurable with current commercial analytical techniques. Although existing treatment technologies do not appear to be particularly effective at removing PFAS from water, DWG options would be achievable if uncontaminated[1] source waters are utilised. However, the DWG options may not be achievable for local drinking water supplies in contaminated areas without addition of a PFAS-removal treatment step or use of an alternative water supply.

Based on concentrations identified in existing water quality data in the Australian context, it is unlikely that PFOS, PFHxS, PFBS and PFOA will present a human health risk from drinking water in uncontaminated regions of Australia. No concentrations of GenX Chemicals in drinking water were identified in the Australian context, so it is unknown if the candidate DWG proposed for GenX Chemicals will be above or below what is found in Australian drinking water. Additional research is required to identify if GenX Chemicals are found in Australian drinking water and at what levels.

[1] Here uncontaminated means locations that are not directly affected by a point source of PFAS. Contaminated locations include locations where historical use of PFAS-containing firefighting foam has occurred. It is recognised that PFAS are widespread in the environment and small amounts of PFAS may still be found in uncontaminated locations.

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Acronyms and Abbreviations

Acronym	Definition
∑	Sum
ADHD	Attention Deficit Hyperactivity Disorder
AICIS	Australian Industrial Chemicals Introduction Scheme
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
APFO	Ammonium Perfluorooctanoate
APVMA	Australian Pesticides and Veterinary Medicines Authority
AST	Aspartate Aminotransferase
ATSDR	US Agency for Toxic Substances and Disease Registry
BfR	German Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment)
BMDL	Lower Benchmark Dose
BUN	Blood Urea Nitrogen
CAR	Constitutive Androgen Receptor
CDC	US Centre for Disease Control
CI	Confidence Interval
CNT	Carbon Nanotube
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
DWG	Drinking Water Guideline
E2	Oestradiol
EFSA	European Food Safety Authority
ESI^-	Negative Electrospray Ionisation
F1	First Filial Generation
F2	Second Filial Generation
FSANZ	Food Standards Australia New Zealand
GAC	Granular Activated Carbon
GC/MS	Gas Chromatography Mass Spectrometry
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
HBWC	Health-Based Water Concentration
HED	Human Equivalent Dose

Acronym	Definition
HDL	High Density Lipoprotein
Hib	*Haemophilus influenza T*ype b
HPLC	High Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectrometry
IgG1	Immunoglobulin G1
IgM	Immunoglobulin M
IL-4	Interleukin 4
IU/L	International Units per Litre
iTRAQ	Isobaric Tags for Relative and Absolute Quantitation
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K+ PFBS	PFBS Potassium Salt
LOAEL	Lowest Observed Adverse Effect Level
LOEL	Lowest Observed Effect Level
LOR	Limit of Reporting
MDH	Minnesota Department of Health
MPART	Michigan PFAS Action Response Team
MS/MS	Tandem Mass Spectrometer
NHMRC	National Health and Medical Research Council
NJDEP	New Jersey Department of Environmental Protection
NOAEL	No Observed Adverse Effect Level
OEHHA	Californian Office of Environmental Health and Hazard Assessment
OR	Odds Ratio
PBPK	Physiologically Based Pharmacokinetic
PCB	Polychlorinated Biphenyl
PFAS	Per- and Poly-fluoroalkylated Substances
PFBS	Perfluorobutane sulfonic acid (CAS No. 375-73-5).
PFHxS	Perfluorohexane sulfonic acid (CAS No. 355-46-4)
PFHxSK	PFHxS Potassium Salt
PFOA	Perfluorooctanoic acid (CAS No. 335-67-1)
PFOS	Perfluorooctane sulfonic acid (CAS No. 1763-23-1)
PND	Postnatal Day
POD	Point of Departure
PPARα	Peroxisome Proliferator-Activated Receptor Alpha
PPS	Preputial Separation
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QTof-MS	Quadrupole Time of Flight Mass Spectroscopy

Acronym	Definition
RIVM	Dutch National Institute for Public Health and the Environment
RPF	Relative Potency Factor
RSC	Relative Source Contribution
SD	Standard Deviation
SDH	Sorbitol Dehydrogenase
SLR	SLR Consulting Australia Pty Ltd
SRBC	Sheep Red Blood Cell
SWRCB	California State Water Resources Control Board
T3	Triiodothyronine
T4	Thyroxine
TG	Test Guideline
Tg	Triglyceride
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.
TOF Assay	Total Organic Fluorine Assay
TOP Assay	Total Oxidisable Precursor Assay
TRV	Toxicity Reference Value
TSH	Thyroid Stimulating Hormone
UF	Uncertainty Factor
UPLC	Ultraperformance Liquid Chromatography
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

1.0 Introduction and Background

An Australian drinking water guideline and existing Fact Sheet[2] are available for three per- and polyfluoroalkyl substances (PFAS): 70 ng/L for perfluorooctane sulfonic acid + perfluorohexane sulfonic acid (PFOS, CAS No. 1763-23-1 + PFHxS, CAS No. 355-46-4) and 560 ng/L for perfluorooctanoic acid (PFOA, CAS No. 335-67-1). There is currently no Australian drinking water guideline or existing Fact Sheet for perfluorobutane sulfonic acid (PFBS, CAS No. 375-73-5) and hexafluoropropylene oxide ammonium salt (CAS No 62037-80-3) plus hexafluoropropylene oxide dimer acid (CAS No 13252-13-6) (also termed GenX Chemicals).

The National Health and Medical Research Council (NHMRC) have contracted SLR Consulting Australia Pty Ltd (SLR) to identify existing sources of guidance or guidelines on the impact of exposure to these five select PFAS in drinking water at levels higher or lower than the current health-based guideline values (where these exist) on human health outcomes.

An evidence scan to inform an update to the existing supporting information (e.g. levels detected in Australian drinking water, analysis/detection, monitoring and treatment guidance) provided in the Fact Sheet was also requested to be undertaken. The findings of this evaluation will be used by NHMRC to develop and/or update public health advice and/or health-based guideline values (if required) for inclusion in the *Australian Drinking Water Guidelines* (2011) (the Guidelines). The evidence reviews undertaken by SLR were governed by a newly designed methodological framework intended to implement best practice methods for evidence evaluations as per the *2016 NHMRC Standards for Guidelines*. For each PFAS, SLR was asked to:

- Customise and apply the 'Research Protocol' template provided by NHMRC to answer research questions.
- Produce a Technical Report and an Evaluation Report for five select PFAS.
    - The Technical Report is to capture the details and methods used to undertake each review.
    - The Evaluation Report is to interpret, synthesise and summarise the existing guidance and evidence pertaining to the research questions.

These tasks were performed in consultation with NHMRC's Water Quality Advisory Committee (the Committee) and NHMRC.

For the five select PFAS, the requirements of the evaluation were as follows:

1 Screen any existing guidance/guidelines[3] (if available).

2 Collate and review any useful supporting information for modification/expansion of the existing PFAS (PFOS+PFHxS and PFOA) chemical Fact Sheet.

[2] A single Fact Sheet currently exists for PFOS+PFHxS and PFOA (NHMRC and NRMMC 2011); advice on new chemicals would either be included in the same Fact Sheet or new Fact Sheets developed as required if determined by NHMRC with advice from the Committee.

[3] 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.

The report herein is the Evaluation Report for the five PFAS evaluated (PFOS, PFOA, PFHxS, PFBS and GenX Chemicals). A combined Evaluation Report was produced since there was a large cross-over between the information for the various PFAS evaluated.

1.1 Objectives

The overarching objective of this review is to identify relevant information from existing guidance/guidelines on the impact of exposure to each of the five select PFAS (i.e. PFOS, PFHxS, PFOA, PFBS, and GenX Chemicals) in drinking water on human health outcomes.

Another objective of the review is to undertake an evidence scan to inform any modification/expansion of supporting information (e.g. monitoring and treatment guidance) that is provided in the existing PFAS Fact Sheet.

2.0 Research Questions

Research questions for this review were drafted by SLR and peer reviewed and agreed upon by the Committee and NHMRC prior to conducting the literature searches. The research questions guiding the review are provided in **Table 2-1**.

Table 2-1 Research Questions for Evidence Evaluation of Health-Related Advice and Supporting Information in Fact Sheets for Five PFAS

#	Research Questions
Health-Related Advice	
Health-based guideline value	
1	What level of PFOS, PFOA, PFHxS, PFBS and GenX Chemicals in drinking water causes 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	What other recent guideline values exist?
5	If there are existing guidance/guideline values, are the proposed option/s for health-based guideline values relevant to the Australian context?
6	How were they derived and are there any uncertainties with the key studies or the approaches used?
7	Are they suitable to adopt/adapt?
Health considerations	
8	What are the key adverse health hazards from exposure to PFOS, PFOA, PFHxS, PFBS and GenX Chemicals in Australian drinking water?
Typical Australian water levels or exposure profile	
9	What are the typical levels in Australian drinking water supplies, considering distributed drinking water and households using their own borewater, rainwater or surface water for drinking? (1)
10	Do they vary around the country or under certain conditions e.g. drought?
11	What other factors should be considered (e.g. differences between groundwater versus surface water sources)?
Risk summary	

#	Research Questions
12	What are the risks to human health from exposure to PFOS, PFOA, PFHxS, PFBS and GenX Chemicals in Australian drinking water?
13	Is there evidence of any emerging risks that are not mentioned in the current Fact Sheet that require review or further research?
Supporting information in the Fact Sheet	
General description	
14	Is the general description in the Fact Sheet current for all 5 PFAS under review?
15	What are the chemicals used for and how might people be exposed?
16	How do the chemicals end up in drinking water and in what form?
Measurement	
17	Is the measurement information in the Fact Sheet current?
18	What are the current analytical methods used to measure/detect the concentration of the specified chemicals in water?
19	What are the limits of quantification or limit of reporting for these chemicals in drinking water?
20	What are the indicators of the risks?
21	How can we measure this exposure?
Treatment options	
22	Is the information on treatment of drinking water in the Fact Sheet current?
23	What are the available options for removing the specified chemicals from drinking water?
Risk management options	
24	What are the current practices to minimise or manage the risks identified?
(1) Due to resource constraints and with agreement from NHMRC with advice from the Committee, data gathering for this research question focused on distributed water from uncontaminated locations (i.e. locations not directly affected by a point source of PFAS); only a few publications were consulted to inform PFAS concentrations in residential/private bore water in proximity to contaminated sites and bore water used for drinking in proximity to fire stations.	

3.0 Methodology Overview

As part of the review, a number of literature searches were undertaken to target specific information relevant to answering the research questions. They consisted of the following:

- A targeted literature search undertaken in August 2023 of existing health-based guidance/guidelines. Jurisdictions included in this search were those previously identified by ToxConsult (2019) as providing reliable information and meeting a large proportion of pre-determined technical and administrative criteria as per the Assessment Tool in the Technical Report. They included the World Health Organization (WHO) including the Joint FAO/WHO Expert Committee on Food Additives (JECFA), European Food Safety Authority (EFSA), United States Environmental Protection Agency (US EPA), US Agency for Toxic Substances and Disease Registry (ATSDR), Californian Office of Health and Hazard Assessment (OEHHA), Food Standards Australia New Zealand (FSANZ), and the Australian Pesticides and Veterinary Medicine Authority (APVMA).

- As it was known prior to undertaking the search that other jurisdictions (not identified in the first dot point above) had also recently derived guidance/guideline values for the five PFAS under consideration, a number of additional jurisdictions were included in the search. These were Health Canada, Dutch National Institute for Public Health and the Environment (RIVM), German Bundesinstitut für Risikobewertung (BfR – Federal Institute for Risk Assessment), US Centre for Disease Control (CDC), Australian Industrial Chemicals Introduction Scheme (AICIS), and various US state health departments including Minnesota, Washington, Maine, Alabama, Alaska, Connecticut, Vermont, New Jersey, Michigan and Massachusetts.
- An additional evidence scan of recent publicly available literature for supporting information in the Fact Sheet (e.g. general description, uses, measurement techniques and limits of reporting in drinking water, treatment options, etc.).

Results were subjected to the following steps in order to identify the most relevant information:

- A preliminary title screen where titles of results were scanned by a researcher and a decision recorded regarding relevance of the result; and
- A content screen where full text content of reports/reviews/articles selected to be included from the preliminary title screen step were reviewed in relation to the research questions by a subject expert to determine which to include in data extraction.

Relevant data were extracted by populating various pre-constructed tables which focused on data needed to answer the research questions. Synthesis was conducted by presenting summarised extracted data in tabular format for each individual research question. For each candidate jurisdiction's guidance/guideline value identified for the five PFAS included in this report, an evaluation of existing jurisdiction Guidelines was undertaken with respect to a defined list of administrative and technical criteria (previously defined by ToxConsult 2019 and NHMRC) using an Assessment Tool. The reader is referred to the accompanying Technical Report for the detailed methodology, records of the literature screening process (including all records that were excluded) and all data extraction, and Assessment Tool tables.

The volume of information found and needing to be assessed was very large. Due to resource constraints and with agreement from NHMRC with advice from the Committee, critical evaluation of studies underpinning existing guideline values in this Evaluation Report was prioritised to those studies that had not been previously reviewed and/or considered by an Australian agency for guidance/guideline value development. The latest review by an Australian jurisdiction in which guidance values were derived for three of the PFAS under consideration (PFOS+PFHxS and PFOA) was the Food Standards Australia New Zealand (FSANZ 2017b)[4] document. This forms the basis of the current toxicity reference values (TRVs) for PFOS/PFHxS and PFOA which have been used by NHMRC to derive the current guideline values in drinking water for these chemicals. FSANZ (2021) also published a review of immunomodulation effects, in which the jurisdiction reviewed a number of studies, findings of which are used to support discussions in this report on relevant PFAS. This agreed amendment to the scope of the Evaluation Report is captured in an addendum to the Research Protocol (see **Section 3.4** in Technical Report). This Evaluation Report provides the following.

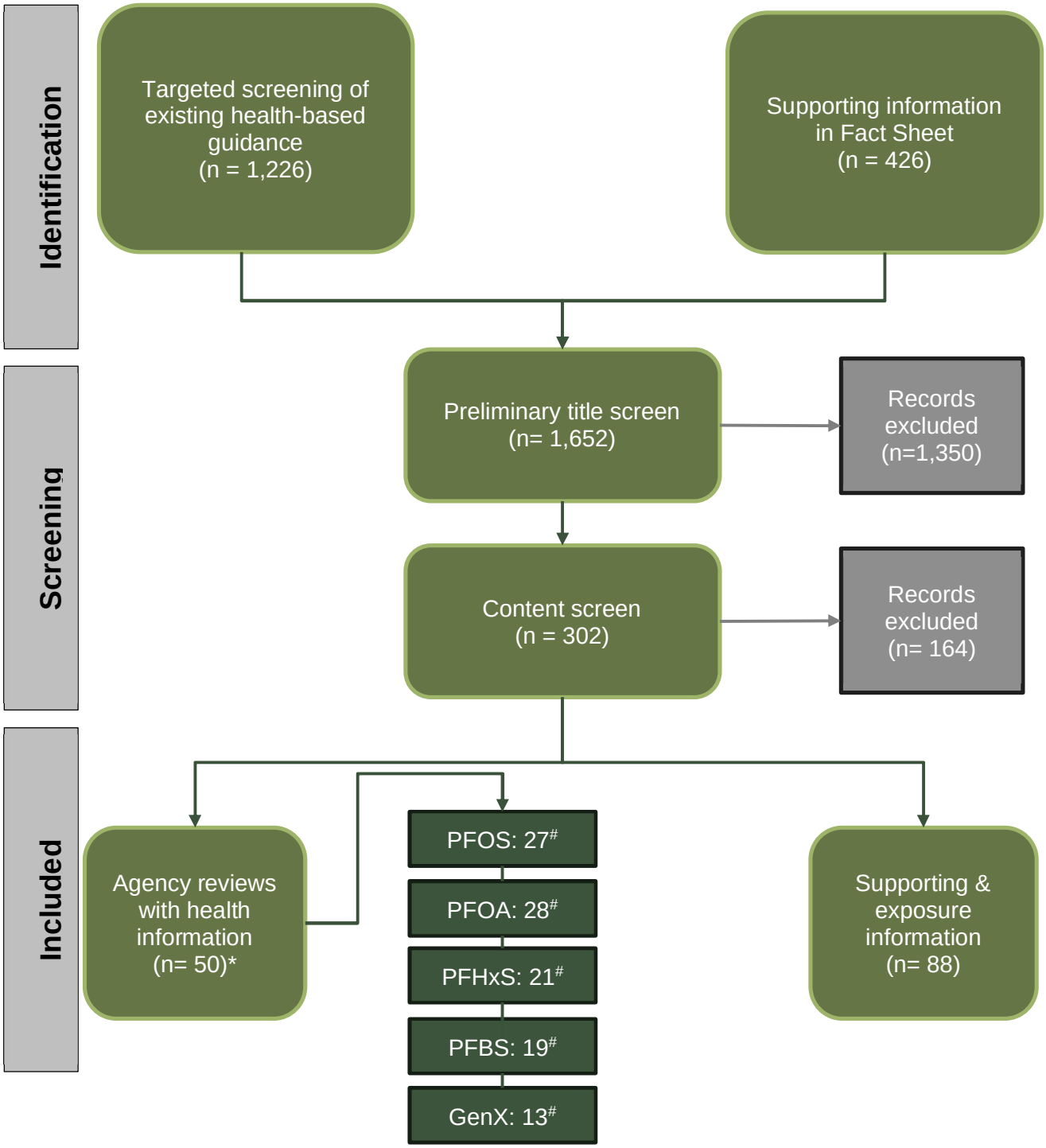
[4] Based on an evaluation of the 'must-have', 'should-have' and 'may-have' administrative and technical criteria specified in the Assessment Tool in the Research Protocol, it is concluded that the FSANZ (2017b) guidance would be suitable for adoption/adaptation (see details in Technical Report).

- A tabular summary of the various guidance/guideline values found in the literature review (and for which data extraction summaries are provided in the Technical Report). This tabular summary in **Section 5.0** provides colour coding for the health endpoints on which the guidance/guideline values are based.
- The full list of critical studies underpinning each of the guidance values derived by various national and international jurisdictions is shown in **Appendix A** of this Evaluation Report, along with an indication of whether or not the critical study had been previously evaluated / considered by FSANZ (2017b, 2021).
- Discussions/critical evaluation of those studies underpinning existing guidance values not previously considered in the FSANZ (2017b) review. These are the studies marked with a cross (i.e. '×') in **Appendix A** in the column denoted 'FSANZ (2017b)'. In line with the agreed change to the scope of the Evaluation Report, critical review of guidance values in this Evaluation Report was limited to the following.
    - All the GenX Chemicals and PFBS guidance values (as these two PFAS were not previously considered by an Australian agency).
    - For PFOS:
        - Values derived by US EPA (critical study: Budtz-Jørgensen and Grandjean 2018).
        - Value for PFOS, PFOA, PFHxS derived by EFSA (critical study: Abraham et al. 2020).[5]
    - For PFOA:
        - Value derived by OEHHA (critical studies: Gallo et al. 2012; Li et al. 2017).
        - Value derived by ATSDR (critical study: Koskela et al. 2016).
        - Value derived by New Jersey Department of Environmental Protection (NJDEP) (critical study: Loveless et al. 2006).
        - Value derived by Michigan PFAS Action Response Team (MPART) (critical studies: Onishchenko et al. 2011, Koskela et al. 2016).
        - Value for PFOS, PFOA, PFHxS derived by EFSA (critical study: Abraham et al. 2020).
        - Value for PFOA derived by US EPA (critical study: Budtz-Jørgensen and Grandjean 2018).
    - For PFHxS:
        - Value for PFOS, PFOA, PFHxS derived by EFSA (critical study: Abraham et al. 2020).
        - Value derived by Minnesota Department of Health (MDH), MPART and OEHHA (critical study: NTP 2022).
- A brief summary of supporting information was provided in the Evaluation Report, with further detail provided in the Technical Report if required by NHMRC and the Committee.

Figure 1 shows an overview of the literature search process followed for the five PFAS included in this review. This is presented as a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram that describes the study selection process and numbers of records at each stage of screening (Moher et al. 2009).

[5] Note this study has since been evaluated by FSANZ (2021); the FSANZ (2021) evaluation of the study was primarily relied upon along with information from other jurisdictions that have considered use of this information in derivation of their guidance/guideline values.

Figure 3-1 Overview of literature search process followed for the five PFAS



*Some reviews derived guidance/guideline values for more than one PFAS.
This value indicates the number of agency reviews that data was extracted from for each individual PFAS as shown in Appendix B of the Technical Report. Not all agency reviews had guideline values/guidance as some were used for supporting information only. Due to resource constraints and with agreement from NHMRC with advice from the Committee, critical evaluation of studies underpinning existing guideline values in this Evaluation Report was prioritised to those studies that had not been previously reviewed and/or considered by an Australian agency for guidance/guideline value development (see **Appendix A**).

This report provides the summary of the findings (**Section 4.0**), a tabular summary of all existing guidance/guideline values sourced in the literature search (**Section 5.0**), a discussion of the results for each PFAS (**Section 6.0** to **10.0**), and a conclusion (**Section 11.0**). 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 adaptation of existing guidance values was considered, these guidance values may already incorporate the safety factor shown in the denominator of 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

The targeted screening of existing health-based guidance identified multiple existing health-based guidance/guideline values for the five PFAS included in this evaluation in the literature consulted. Responses to research questions were informed by the data extractions from the guidance/guideline documents found in the literature reviewed.

Detailed summary findings tables for each research question are provided in the Technical Report. In this Evaluation Report, the research question tables have been condensed to highlight differences between the various agency guidance/guideline documentation and other studies where they have been identified.

4.1 Health-based aspects

Research questions 1-8 all cover health-based aspects of the review; this is considered to be the most important information in the Fact Sheet. **Table 4-1** provides a synthesis of the results.

Table 4-1 Summary of findings from data extraction for health-based aspects

#	Research Questions	Response	
1	What level of PFOS, PFOA, PFHxS, PFBS and GenX Chemicals in drinking water causes adverse health effects?	PFOS	Overt adverse health effects from drinking water exposure to PFOS in humans have not been explicitly recorded in any of the jurisdictional reviews. However, numerous jurisdictions have derived DWGs (also summarised in **Section 5.0** and the Technical Report) based on different critical health endpoints (some of which are clearly adverse but others which are not necessarily adverse) in animal studies and human epidemiological studies. The DWGs include the following (listed in ascending order): • **0.02 ng/L** (interim health advisory) based on draft TRV of 0.0079 ng/kg/day and a DWG goal of 4 ng/L based on minimum reporting level (US EPA 2022c, e; 2021b). • **0.4 ng/L** (for cancer effects) and **7 ng/L** (for non-cancer effects) in California (OEHHA 2019a). Note as the cancer DWG was below the limit of reporting (LoR) at the time for PFOS (and PFOA), the State Water Resources Control Board (SWRCB) set the DWGs at the lowest levels at which PFOA and PFOS could be reliably detected in drinking water at the time. • **1 ng/L** (cancer) and **2 ng/L** (non-cancer) in California based on cancer slope factor of 15.6 $(mg/kg\text{-}day)^{-1}$ and TRV of 0.64 ng/kg/day (OEHHA 2023a). • **4 ng/L** for PFOA + PFOS based on minimum reporting level (US EPA 2022d, 2022e), also adopted by Minnesota (MDH 2023a). • **10 ng/L** in Connecticut (derivation not provided) (CDPH 2023a). • **10 ng/L** in New Jersey (NJDEP 2019b) as an interim criterion derived from TRV of 1.8 ng/kg/day. • **14 ng/L** (child) and **52 ng/L** (adult) using intermediate-duration (14d-365d) TRVs derived in the draft ATSDR (2018a) toxicological profile, superseded by the final report from ATSDR (2021a). No updated guidance regarding DWG has since been released. • **15 ng/L** derived by Minnesota (MDH 2020a) using toxicokinetic model in infants and a relative source contribution of 50% for the peak 'reference' serum concentration in the US population during infancy, which produces steady state serum concentrations at approximately 20% of the 'reference' serum concentration (i.e. existing background serum). Also adopted by State of Washington (WSDH 2019a, 2022b, 2023a). • **16 ng/L** derived by Michigan (MPART 2019a) which is a level calculated to be at a point where no or minimal risk exists for people drinking water with PFOS. It is based on a reference level in the US population rather than a health endpoint.

#	Research Questions	Response	
			• **20 ng/L** as sum of six PFAS (PFOS, PFOA, PFHxS, PFNA, PFHpA, and PFDA) (basis unclear) in Massachusetts (Mass DEP 2022a) and Maine (Maine DHHS 2021a). • **70 ng/L** for PFOS + PFHxS in Australia (DOH 2017) derived using FSANZ (2017b) TRV of 20 ng/kg/day. • **100 ng/L** in EU for sum of PFAS (a subset of 'PFAS Total' substances that contain a perfluoro-alkyl moiety with three or more carbons or a perfluoroalkylether moiety with two or more carbons) and 500 ng/L for PFAS total (totality of PFAS detected with available analytical methods and monitoring guidelines) (EU 2020, EC 2022). No basis provided. • **100 ng/L** or **500 ng/L** for Total PFAS on basis of practical considerations (not health-based) (WHO 2022). • **600 ng/L** in Canada (HC 2018a) based on TRV of 60 ng/kg/day.
		PFHxS	Overt adverse health effects from drinking water exposure to PFHxS in humans have not been explicitly recorded in any of the jurisdictional reviews. However, numerous jurisdictions have derived DWGs (also summarised in **Section 5.0** and the Technical Report) based on different critical health endpoints (some of which are clearly adverse but others which are not necessarily adverse) in animal studies and human epidemiological studies. The DWGs include the following (listed in ascending order): • **2 ng/L** (non-cancer) in California based on TRV of 2.4 ng/kg/day (OEHHA 2022a). Guidelines for other endpoints of **11 ng/L** and **60 ng/L** were also derived. • **4 ng/L** for PFOA + PFOS based on minimum reporting level (US EPA 2022d, 2022e), also adopted by Minnesota (MDH 2023a). The proposal is that four PFAS (PFBS, PFHxS, GenX and PFNA) be evaluated in combination with each other, using an approach called a Hazard Index. A hazard index is calculated by comparing a measured drinking water value with a standard, but it is unclear what standard MDH (2023a) recommend be used for PFHxS. • **20 ng/L** as sum of six PFAS (PFOS, PFOA, PFHxS, PFNA, PFHpA, and PFDA) (basis unclear) in Massachusetts (Mass DEP 2022a) and Maine (Maine DHHS 2021a). • **47 ng/L** derived by Minnesota (MDH 2020b) using toxicokinetic model in infants and a relative source contribution of 50% for the peak 'reference' serum concentration in the US population during infancy, which produces steady state serum concentrations at approximately 20% of the 'reference' serum concentration (i.e. existing background serum). • **49 ng/L** in Connecticut (derivation not provided) (CDPH 2023a). • **51 ng/L** derived by Michigan (MPART 2019a) which is a level calculated to be at a point where no or minimal risk exists for people drinking water with PFHxS. It is based on a reference level in the US population rather than a health endpoint.

#	Research Questions	Response	
			• **65 ng/L** derived by State of Washington (WSDH 2019a, 2022b, 2023a) using the TRV derived by MDH (2020b). • **70 ng/L** for PFOS + PFHxS in Australia (DOH 2017) derived using FSANZ (2017b) TRV of 20 ng/kg/day. • **100 ng/L** in EU for sum of PFAS (a subset of 'PFAS Total' substances that contain a perfluoro-alkyl moiety with three or more carbons or a perfluoroalkylether moiety with two or more carbons) and 500 ng/L for PFAS total (totality of PFAS detected with available analytical methods and monitoring guidelines) (EU 2020, EC 2022). No basis provided. • **140 ng/L** (child) and **517 ng/L** (adult) using intermediate-duration (14d-365d) TRVs derived in the draft ATSDR (2018a) toxicological profile, superseded by the final report from ATSDR (2021a). No updated guidance regarding DWG has since been released. • **600 ng/L** in Canada (HC 2019a) likely adopted from the value for PFOS based on TRV of 60 ng/kg/day.
		PFBS	Overt adverse health effects from drinking water exposure to PFBS in humans have not been explicitly recorded in any of the jurisdictional reviews. However, numerous jurisdictions have derived DWGs (also summarised in **Section 5.0** and the Technical Report) based on the same critical health endpoint (decreased thyroxine hormone levels) in animal studies. The DWGs include the following (listed in ascending order): • **2 ng/L** ('action level') in Alaska, no basis provided (Alaska DEC 2019a). • **4 ng/L** for PFOA + PFOS based on minimum reporting level (US EPA 2022d, 2022e), also adopted by Minnesota (MDH 2023a). The proposal is that four PFAS (PFBS, PFHxS, GenX and PFNA) be evaluated in combination with each other, using an approach called a Hazard Index. A hazard index is calculated by comparing a measured drinking water value with a standard, but it is unclear what standard MDH (2023a) recommend be used for PFBS. • **100 ng/L** in EU for sum of PFAS (a subset of 'PFAS Total' substances that contain a perfluoro-alkyl moiety with three or more carbons or a perfluoroalkylether moiety with two or more carbons) and 500 ng/L for PFAS total (totality of PFAS detected with available analytical methods and monitoring guidelines) (EU 2020, EC 2022). No basis provided. • **100 ng/L** (non-cancer) in Minnesota, derived from a TRV of 84 ng/kg/day (MDH 2022e, g). • **345 ng/L** in Washington State (WSDH 2019a, 2023a, 2022b) derived using a TRV of 300 ng/kg/day. • **420 ng/L** in Michigan (MPART 2019a) derived using TRV of 300 ng/kg/day. • **500 ng/L** in California (OEHHA 2021d) derived using TRV of 600 ng/kg/day. • **760 ng/L** in Connecticut (derivation not provided) (CDPH 2023a). • **2,000 ng/L** in Massachusetts, derivation not provided (Mass DEP 2022a).

#	Research Questions	Response	
			• **2,000 ng/L** (interim) derived by US EPA (2021c, 2022c, k) using a TRV of 300 ng/kg/day. • **15,000 ng/L** in Canada (screening value), basis not provided (HC 2019a).
		PFOA	Overt adverse health effects from drinking water exposure to PFOA in humans have not been explicitly recorded in any of the jurisdictional reviews. However, numerous jurisdictions have derived DWGs (also summarised in **Section 5.0** and the Technical Report) based on different critical health endpoints (some of which are clearly adverse but others which are not necessarily adverse) in animal studies and human epidemiological studies. The DWGs include the following (listed in ascending order): • **0.004 ng/L** (interim health advisory) based on draft TRV of 0.0015 ng/kg/day and a DWG goal of 4 ng/L based on minimum reporting level (US EPA 2022c, d; 2021a). • **0.007 ng/L** (cancer) and **3 ng/L** (non-cancer) in California based on cancer slope factor of 2,600 $(mg/kg\text{-}day)^{-1}$ and TRV of 0.87 ng/kg/day (OEHHA 2023a). • **0.1 ng/L** (for cancer effects) and **2 ng/L** (for non-cancer effects) in California (OEHHA 2019a). Note as the cancer DWG was below the LoR for PFOA (and PFOS) at the time, the State Water Resources Control Board (SWRCB) set the DWGs at the lowest levels at which PFOA and PFOS could be reliably detected in drinking water at the time. • **4 ng/L** for PFOA + PFOS based on minimum reporting level (US EPA 2022d, 2022e), also adopted by Minnesota (MDH 2023a). • **8 ng/L** derived by Michigan (MPART 2019a) which is a level calculated to be at a point where no or minimal risk exists for people drinking water with PFOA. It is based on a reference level in the US population rather than a health endpoint. • **10 ng/L** in New Jersey (NJDEP 2019a) as an interim criterion derived from TRV of 2 ng/kg/day. • **10 ng/L** in State of Washington (WSDH 2019a, 2022b, 2023a) based on TRV from ATSDR (2021a) of 3 ng/kg/day. • **16 ng/L** in Connecticut (derivation not provided) (CDPH 2023a). • **21 ng/L** (child) and **78 ng/L** (adult) using intermediate-duration (14d-365d) TRVs derived in the draft ATSDR (2018a) toxicological profile, superseded by the final report from ATSDR (2021a). No updated guidance regarding DWG has since been released. • **20 ng/L** as sum of six PFAS (PFOS, PFOA, PFHxS, PFNA, PFHpA, and PFDA) (basis unclear) in Massachusetts (Mass DEP 2022a) and Maine (Maine DHHS 2021a).

#	Research Questions	Response	
			• **35 ng/L** derived by Minnesota (MDH 2022d, f) using toxicokinetic model in infants and a relative source contribution of 50% for the peak 'reference' serum concentration in the US population during infancy, which produces steady state serum concentrations at approximately 20% of the 'reference' serum concentration (i.e. existing background serum). • **100 ng/L** in EU for sum of PFAS (a subset of 'PFAS Total' substances that contain a perfluoro-alkyl moiety with three or more carbons or a perfluoroalkylether moiety with two or more carbons) and 500 ng/L for PFAS total (totality of PFAS detected with available analytical methods and monitoring guidelines) (EU 2020, EC 2022). No basis provided. • **100 ng/L** or 500 ng/L for Total PFAS on basis of practical considerations (not health-based) (WHO 2022). • **200 ng/L** in Canada (HC 2018b) based on TRV of 21 ng/kg/day. • **560 ng/L** in Australia (DOH 2017) derived using FSANZ (2017b) TRV of 160 ng/kg/day.
		GenX Chemicals	Overt adverse health effects from drinking water exposure to GenX Chemicals in humans have not been explicitly recorded in any of the jurisdictional reviews. However, numerous jurisdictions have derived DWGs (also summarised in **Section 5.0** and the Technical Report) based on the same critical health endpoint (increased absolute and relative liver weight and histopathological changes in the liver) in a mouse study. The DWGs include the following (listed in ascending order): • **10 ng/L** derived by US EPA (2021e, 2022c, j; adopted by WSDH 2022b, 2023a) using a TRV of 3 ng/kg/day. • **10 ng/L** in Massachusetts, derivation not provided, but likely adopted from US EPA (Mass DEP 2022a). • **19 ng/L** in Connecticut (derivation not provided) (CDPH 2023a). • **20 ng/L** in New Jersey (interim) derived from a TRV of 3 ng/kg/day adopted from US EPA (2021e) (NJDEP 2023a). • **100 ng/L** in EU for sum of PFAS (a subset of 'PFAS Total' substances that contain a perfluoro-alkyl moiety with three or more carbons or a perfluoroalkylether moiety with two or more carbons) and **500 ng/L** for PFAS total (totality of PFAS detected with available analytical methods and monitoring guidelines) (EU 2020, EC 2022). No basis provided. • **140 ng/L** (health goal) in North Carolina, derivation not provided (NC DHHS 2017). • **370 ng/L** in Michigan (MPART 2019a) derived using TRV of 77 ng/kg/day.
2	What is the critical human health	PFOS	Where DWGs have used a health-based derivation, critical health endpoints vary depending on jurisdiction. Other jurisdictions may have only derived TRVs but no DWGs. These are summarised in the tabular summaries of existing

#	Research Questions	Response	
	endpoint that determines this value?		guidance/guideline values in **Section 5.0**, with more detail provided in the Technical Report. An overview of the critical effects underpinning these values is as follows. • Developmental toxicity in rodent studies including delayed eye opening and decreased pup body weight (ATSDR 2018a, 2021a; FSANZ 2017b). • Increase in total blood cholesterol levels (BfR 2019a, OEHHA 2023a) and decreased antibody formation following certain childhood vaccines in humans (BfR 2019a, EFSA 2020a, US EPA 2021b). • Increased liver weight and hepatocellular hypertrophy in rat study (HC 2018a). • Increased interleukin-4 (IL-4) and decreased sheep red blood cell (SRBC) specific Immunoglobulin M (IgM) levels in mice (MDH 2020a, WSDH 2019, 2022b, 2023a). • Suppression of plaque forming cell response and increase in liver mass in mice (MPART 2019a, NJDEP 2019b, OEHHA 2019a). • Hepatocellular adenomas in male rats, and hepatocellular adenomas/carcinomas in female rats (OEHHA 2019a, 2023a).
		PFHxS	Where DWGs have used a health-based derivation, critical health endpoints vary depending on jurisdiction. Other jurisdictions may have only derived TRVs but no DWGs. These are summarised in the tabular summaries of existing guidance/guideline values in **Section 5.0**, with more detail provided in the Technical Report. An overview of the critical effects underpinning these values is as follows. • Thyroid follicular epithelial hypertrophy/hyperplasia in a reproductive/developmental toxicity study with rats (ATSDR 2018a, 2021a). • Developmental toxicity in rodent studies with PFOS (FSANZ 2017b). • Decreased thyroxine (T4) levels in rats (MDH 2020b, MPART 2019a, OEHHA 2022a, WSDH 2019, 2022b, 2023a). • Increased relative liver weight in female rats and decreased litter size in mice (OEHHA 2022a). • Decreased antibody formation following tetanus vaccines in children (US EPA 2023).
		PFBS	Where DWGs or TRVs have been derived for PFBS, the jurisdictions have agreed that the most sensitive health endpoint is decreased total thyroxine (T4) in rats. Differences in resulting guidance/guideline values (summarised in tabular form in **Section 5.0**) are due largely to differences in choice of uncertainty factors and/or assumptions used for deriving the guideline values. The detail is provided in the Technical Report and also discussed in **Section 8.0**.

#	Research Questions	Response	
		PFOA	Where DWGs have used a health-based derivation, critical health endpoints vary depending on jurisdiction. Other jurisdictions may have only derived TRVs but no DWGs. These are summarised in the tabular summaries of existing guidance/guideline values in **Section 5.0**, with more detail provided in the Technical Report. An overview of the critical effects underpinning these values is as follows. • Skeletal alterations in adult mouse offspring and/or decreased foetal mouse body weight (ATSDR 2018a, 2021a; FSANZ 2017b; WSDH 2019a, 2022b, 2023a). • Delayed ossification, accelerated preputial separation (PPS) in male mice offspring, trend for decreased pup body weight, and increased maternal liver weight (MDH 2022f). • Developmental delays (decreased number of inactive periods, altered novelty induced activity and skeletal alteration such as bone morphology and bone cell differentiation in the femurs and tibias) of mice (MPART 2019a). • Increased liver weight in male mice (NJDEP 2019a). • Increase in total blood cholesterol levels (BfR 2019a) and decreased antibody formation following certain childhood vaccines in humans (EFSA 2020a, US EPA 2021a). • Hepatocellular hypertrophy in rat study (HC 2018b). • Increased risk of kidney cancer and increased alanine aminotransferase (ALT) in humans (OEHHA 2023a).
		GenX Chemicals	Where DWGs or TRVs have been derived for GenX Chemicals, the jurisdictions have agreed that the most sensitive health endpoint is liver effects (increased absolute and relative weight and histopathologic findings, i.e. liver single cell necrosis in parental mice) from an unpublished Reproduction/ Developmental Toxicity Study in Mice (DuPont 2010). Differences in resulting guidance/guideline values (summarised in tabular form in **Section 5.0**) are due largely to differences in choice of uncertainty factors and/or assumptions used for deriving the guideline values. The detail is provided in the Technical Report and also discussed in **Section 10**.
3	What are the justifications for choosing this endpoint?	PFOS	Detailed justification for each endpoint is provided in the Technical Report. Some of the common themes and/or discrepancies between jurisdictions are summarised as follows. • The most sensitive targets of PFOS toxicity in laboratory animals are similar to those identified in longer term epidemiological studies (ATSDR 2021a). • Based on observations in animals and humans, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) decided to combine its assessment on the sum of four PFAS, i.e. PFOA, PFNA, PFHxS and PFOS as these four PFAS contribute most to the levels observed in human serum, share toxicokinetic properties in humans and show similar accumulation and long half-lives (EFSA 2020a). Also, in terms of effects, these

#	Research Questions	Response	
			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. RIVM (2021a) critiqued this assumption made by EFSA (2020a) as they stated no statistically significant associations were observed between PFOS levels and Hib, tetanus IgG1, and diphtheria antibodies in the critical study selected for TRV development by EFSA (i.e. Abraham et al. 2020). Nor were such associations observed for the other two PFAS (PFNA and PFHxS). Multivariate analysis, correcting for polychlorinated biphenyls (PCBs), also revealed a significant influence of PFOA exposure (and not PFOS, PFNA, or PFHxS) on antibody levels. The study reported that an association was only found between PFOA and the effect on the immune system. Knowing that PFAS are not equipotent for other effects (for example liver effects), RIVM (2021a) considers it plausible that various PFAS are also not equipotent for their immune effects. Hence for PFAS not included in the EFSA-4, RIVM (2021a) suggested using relative potency factors (RPFs) for liver effects from Bil et al. (2021) to adapt TRVs for these. • FSANZ (2017b) chose the NOAELs from four studies for a range of effects and converted these to a health-based TRV. The lowest TRV calculated from the study by Luebker et al. (2005b) was selected. A literature review commissioned by FSANZ concluded that the weight of evidence from the available animal studies indicates that PFOS can adversely modulate immune system responsiveness (Drew and Hagan 2016). However, there are significant uncertainties regarding species sensitivity, strain sensitivity and the influence of route of administration on immune system modulation by PFOS that have yet to be resolved. As a result, it was concluded it is not possible to determine a reliable NOAEL or LOAEL for adverse effects on immune function for use in a quantitative risk assessment of PFOS at the time. Drew and Hagan (2016) concluded that the epidemiology data available do not provide compelling evidence for increased incidence of disease associated with PFOS effects on immune function. • HC (2018a) state that epidemiological studies have shown associations between exposure to PFOS and multiple non-cancer health outcomes, such as reproductive, developmental, and immunological effects. However, these studies cannot be used to derive the non-cancer TRVs for PFOS due to their limitations, including in terms of study design, bias and confounders. Immune system effects in animal studies were excluded from the quantitative risk assessment due to inconsistencies in NOAELs and LOAELs among studies and uncertainty of the importance of observed effects to human health. Of note for discussion of clinical importance in humans is the Grandjean et al. (2012) study, which demonstrated that despite decreased vaccine-specific immunoglobulin response in PFOS-exposed children, the number of children with immunoglobulin levels below the clinically protective level was low. In humans, evidence of immunosuppression is inconsistent - associations are observed between PFOS levels and decreases in antibodies against some (but not all) illnesses, and the influence of PFOS exposure on clinical immunosuppression (i.e. incidence of illnesses) appears to be more tenuous. Therefore, although low PFOS doses appear to be associated with

#	Research Questions	Response	
			immunosuppression, the data are not considered to be presently reliable for use as a key study for the PFOS assessment. • MPART (2019a) noted for all of the PFAS examined, points of departure were selected from studies with laboratory animal models. This approach does not negate findings associated with epidemiological studies, but reflects that humans experience uncontrolled and imperfectly documented rather than controlled, precisely measured exposures. Additionally, these points of departure reflect adverse health effects that occur at low doses and that are supported by the weight-of-evidence across endpoints and between findings in humans and laboratory animal models. • Similarly, while OEHHA (2019a) reviewed human epidemiology studies focusing on liver toxicity, immunotoxicity, and thyroid toxicity, an epidemiological analysis was not presented in their document because there were no studies that could be used for point of departure (POD) determination and dose-response assessment. Nonetheless, the epidemiology data suggest that there are associations between PFOA and/or PFOS and suppressed antibody response and increased liver enzymes. These epidemiological data are supportive of the animal toxicology data used to derive the TRVs for noncancer effects. The epidemiology data on thyroid hormone levels are inconsistent and, at times, contradictory. The recent immunotoxicity studies of PFOS are much less sensitive than the Dong et al. (2009) study (the critical study chosen for TRV derivation by OEHHA). Thus, these recent immunotoxicity studies are not considered as critical studies for POD derivation. • US EPA (2022c, e; 2021b) concluded decreased immune response to vaccination was observed after exposure during a sensitive developmental life stage, and it yields the lowest POD among the candidate PODs. Other candidate TRVs were derived based on other health effects (e.g. development/growth) observed in epidemiology studies; all of the candidate TRVs are associated with low daily oral exposure doses, ranging from 0.1 to 0.001 ng/kg bw/day. • WHO (2022) opted for a pragmatic DWG rather than a health-based DWG. Although the reduced antibody response following vaccination has been considered by some jurisdictions as the most robust end point based on epidemiological data, it is unclear whether this correlation results in increased rates of infection and hence the clinical implications are uncertain. Although animal data would generally be utilised in the absence of adequate human data for risk assessment purposes, there are also areas of uncertainty around the suitability of animal studies for assessing the effects to human health for PFOS and PFOA, including interspecies differences in kinetic parameters such as elimination half-life and clearance rate. Additionally, diverging estimates of the human half-life of PFOA may also add uncertainty to animal-to-human dosimetric adjustments, as well as physiologically based pharmacokinetic (PBPK)-based conversions of human plasma PFAS concentrations to external doses. Finally, the uncertainty and lack of consensus in the critical health end point to derive a TRV is evident from the diverse range of endpoints utilised by other agencies to derive TRVs, and the resulting range in

#	Research Questions	Response	
			proposed drinking-water values. Although the values derived by several different organisations vary significantly, all have margins of safety. Data analysis also shows that science on PFAS is evolving very rapidly in various areas.
		PFHxS	Detailed justification for each endpoint is provided in the Technical Report. Some of the common themes and/or discrepancies between jurisdictions are summarised as follows. • ACGIH (2021a) indicated that since the liver effects due to PFHxS in animal studies were not considered relevant to humans, the lowest LOAEL identified for PFHxS was 1 mg/kg/day for decreases in the number of pups per litter identified in a study by Chang et al. (2018). The investigators noted that the toxicological significance of this alteration was uncertain because there was no clear dose-response and no alterations in the number of implantation sites, number of viable pups, or pup to implant ratios. A similar conclusion with respect to the Chang et al. (2018) study was also made by MPART (2019a). Thus, the Butenhoff et al. (2009) study, which reported thyroid effects in male rats at a LOAEL of 3 mg/kg/day, with a NOAEL of 1 mg/kg/day, was selected as the principal study. Other jurisdictions (e.g. MDH 2020b, WSDH 2019, 2022b, 2023a; MPART 2019a, OEHHA 2022a) agree that alterations in serum thyroid hormone levels in animal studies due to PFHxS exposure appears to be a sensitive effect. • With respect to the epidemiological data, ACGIH (2021a) conclude there are sufficient data to identify possible sensitive targets for many of the perfluoroalkyls; however, there are two major limitations to establishing dose-response relationships for these effects and using the epidemiological studies to derive TRVs: accurate identification of environmental exposure levels producing increased risk for adverse effects (exposure estimates and routes of exposure) and likely co-exposure to mixtures of perfluoroalkyls. Other limitations include the cross-sectional design of the majority of epidemiological studies and the potential that reverse causality contributes to the observed associations. The epidemiological databases for several perfluoroalkyls provide valuable information on hazard identification; however, uncertainties regarding doses associated with adverse effects and possible interactions between compounds preclude use of these data to derive TRVs. MPART (2019a) agree with this conclusion. • For PFHxS, FSANZ (2017b) concluded that there was not enough toxicological and epidemiological information to justify establishing a tolerable daily intake. However, as a precaution, and for the purposes of site investigations, the PFOS tolerable daily intake should apply to PFHxS. In practice, this means that the level of PFHxS exposure should be added to the level of PFOS exposure; and this combined level be compared to the tolerable daily intake for PFOS. • The justification/comments provided for PFOS by EFSA (2020a) and RIVM (2021a) also applies to PFHxS. • According to the US EPA (2023), the immune organ-/system-specific TRV is based on the lowest overall human equivalent POD; therefore, the selected TRV based on decreased serum anti-tetanus antibody concentration in

#	Research Questions	Response	
			children (a susceptible life stage for this effect) is considered protective of the observed health effects associated with lifetime PFHxS exposure.
		PFBS	Detailed justification for each endpoint is provided in the Technical Report. Some of the common themes and/or discrepancies between jurisdictions are summarised as follows. • Selection of total T4 as the critical effect is based on several key considerations that account for cross-species correlations in thyroid physiology and hormone dynamics particularly within the context of a developmental life stage (MPART 2019a). The Workgroup evaluated available agency decision documents and selected the study associated with the draft USEPA (2018) PFBS toxicity value based on thyroid effects. The kidney effects identified in the draft USEPA (2018) toxicity assessment were identified as a potentially compensatory response. The thyroid effects were identified as having greater functional significance. • OEHHA (2021d) determined four studies to be of acceptable quality, adequate data reporting, and sufficient sensitivity for health-protective concentration derivation. They included two subchronic oral studies, a two-generation reproductive toxicity study in rats, and a developmental toxicity study. Thyroid hormone disruption from the Feng et al. (2017) and NTP (2022) studies were the most sensitive endpoints in the PFBS animal toxicity database, and both were considered for health-protective concentration derivation. OEHHA (2021d) derived a TRV using the mouse study rather than the rat study due to uncertainties of kinetics in the rat. • According to the US EPA (2021c, 2022c, k), the hazards of potential concern for oral PFBS exposure include thyroid, developmental, and kidney effects. Overall, the evidence supports a hazard for thyroid, developmental, and kidney effects based on the evidence from animal studies. The limited evidence for thyroid or renal effects in human studies is equivocal, and no studies evaluating developmental effects following PFBS exposure in humans were available. Thus, data in humans were not considered further, and the available animal studies that evaluated these effects are considered in the derivation of oral TRVs. • For all of the PFAS examined, points of departure were selected from studies with laboratory animal models. This approach does not negate findings associated with epidemiological studies, but reflects that humans experience uncontrolled and imperfectly documented rather than controlled, precisely measured exposures. Additionally, these points of departure reflect adverse health effects that occur at low doses and that are supported by the weight-of-evidence across endpoints and between findings in humans and laboratory animal models (MPART 2019a).
		PFOA	Detailed justification for each endpoint is provided in the Technical Report. Some of the common themes and/or discrepancies between jurisdictions are summarised as follows. • Intermediate-duration oral studies of PFOA in animals indicate that the liver, immune system, reproductive system, and the developing organism are the primary targets of toxicity because adverse outcomes were

#	Research Questions	Response	
			observed at lower doses than other effects and have been consistently observed across studies (ATSDR 2021a). Although these studies identified the lowest LOAEL values, not all were considered suitable as the basis of an intermediate-duration oral TRV. Increases in liver weight, hepatocellular hypertrophy, and alterations in serum lipid levels, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving a TRV by ATSDR (2021a). • ATSDR (2021a) also concluded there are sufficient epidemiological data to identify possible sensitive targets for many of the perfluoroalkyls; however, there are two major limitations to establishing dose-response relationships for these effects and using the epidemiological studies to derive TRVs: accurate identification of environmental exposure levels producing increased risk for adverse effects (exposure estimates and routes of exposure) and likely co-exposure to mixtures of perfluoroalkyls. Other limitations include the cross-sectional design of the majority of epidemiological studies and the potential that reverse causality contributes to the observed associations. The epidemiological databases for several perfluoroalkyls provide valuable information on hazard identification; however, uncertainties regarding doses associated with adverse effects and possible interactions between compounds preclude use of these data to derive TRVs. • WSDH (2019a, 2022b, 2023a) agreed with ATSDR (2021a) since developmental endpoints yielded health protective values that were as low as or lower than liver injury and immunotoxicity endpoints and the fact that there are sufficient supporting toxicity data demonstrating PFOA's developmental toxicity in fish, rats, mice, and monkeys. • Based on observations in animals and humans, the EFSA (2020a) CONTAM Panel decided to combine its assessment on the sum of four PFAS, i.e. PFOA, PFNA, PFHxS and PFOS as these four PFAS contribute most to the 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. • FSANZ (2017b) chose four NOAELs from three studies for a range of health endpoints and converted these to a TRV. TRVs were calculated with the lowest TRV selected based on the lowest NOAEL from the study by Lau et al. (2006). PFOA is a peroxisome proliferator-activated receptor alpha (PPARα) agonist; that is, it induces peroxisome proliferation. PPARα agonists typically cause hepatocellular hypertrophy and markedly increased liver weight in rodents, although primates are refractory to this response. Increased liver weight in rodents in response to a PPARα agonist, in the absence of hepatocellular degeneration or necrosis, is usually regarded as an adaptive response and not predictive of human toxicity (Hall et al. 2012). FSANZ has not interpreted increase in absolute and/or relative liver weight in rodents, in the absence of hepatocellular degeneration or necrosis, as an adverse effect for the purpose of identifying a NOAEL or LOAEL. Similarly, FSANZ has not

#	Research Questions	Response	
			interpreted increased absolute liver weight in a small number of monkeys (Butenhoff et al. 2002) as an adverse effect because there was no significant effect on relative liver weight, and no histological evidence of hepatocellular hypertrophy or liver lesions. Consequently, the NOAELs and LOAELs identified by FSANZ for some studies differ from those of regulatory agencies that identify increased liver weight as an adverse effect. Currently available epidemiology data are insufficient to establish a cause-and-effect relationship between PFOA exposure and clinically relevant immunomodulatory effects in humans. • HC (2018b) disagreed with this. They indicated that in animals, non-cancer effects observed at the lowest levels of exposure include reproductive and developmental effects, liver effects and changes in serum lipid levels. For various reasons, the most appropriate endpoint to derive a TRV for PFOA was considered to be hepatocellular hypertrophy (liver effects) in rats, occurring at the same levels as the changes in serum lipid levels. HC (2018b) also stated that epidemiological studies have shown associations between exposure to PFOA and multiple non-cancer health outcomes, such as dysfunctions of the immunological system and alterations in birth weight and lipid levels. However, these studies cannot be used to derive the non-cancer TRVs for PFOA due to limitations in terms of design, bias, confounding, and possibility of chance findings. • MDH (2022f) provides very detailed justification for why they selected decreased postnatal growth leading to developmental effects (e.g. lower pup body weight, delayed eye opening, delayed vaginal opening, and accelerated preputial separation) as the basis of the TRV. In brief: o Effects of thyroid hormone levels in animal studies were observed at higher serum concentrations. o Associations between PFOA exposure and risk of infectious diseases (as a marker of immune suppression) have not been consistently seen in epidemiological studies, although there was some indication of effect modification by gender (i.e. associations seen in female children but not in male children). Three studies examined associations between maternal and/or child serum PFOA levels and vaccine response (measured by antibody levels) in children and adults. The study in adults reported that a reduction in antibody response to one of the three influenza strains tested after receiving the flu vaccine was associated with increasing levels of serum PFOA. While decreased vaccine response was associated with PFOA levels in these studies, similar results were also observed with other perfluorinated chemicals and, therefore, could not be attributed specifically to PFOA. Several animal studies demonstrate effects on the spleen and on thymus weights as well as decreased immune response. These effects were observed at serum concentrations similar to the critical study LOAEL. The immune system is listed as one of the co-critical effects. o No clear effects of PFOA on male fertility endpoints have been identified. Increased full litter resorptions and increased stillbirths were observed in pregnant mice exposed at serum concentrations >700-fold higher than the serum concentration corresponding to the TRV.

#	Research Questions	Response	
			o The human data pertaining to neurotoxicity (including neurodevelopmental effects) of PFOA are limited, but do not indicate the presence of associations between PFOA and a variety of outcomes. Epidemiology studies of children found a weak statistical association between serum PFOA and parental reports of Attention Deficit Hyperactivity Disorder (ADHD). Data in animals suggest the need for additional neurotoxicity studies to fully understand the neurological effects of PFOA. • According to NJDEP (2019a), increased relative liver weight is a well-established effect of PFOA that is more sensitive than most other toxicological effects such as immune system toxicity and most reproductive / developmental effects. • According to OEHHA (2019a), Li et al. (2017) generated a LOAEL of 0.05 mg/kg-day (administered dose) for changes in mitochondrial membrane potential, increases in biomarkers of apoptosis, and increased oxidative DNA damage in the liver of female mice. This LOAEL corresponds to a serum concentration of 0.97 mg/L, which is lower than the POD of 4.35 mg/L based on increased relative liver weight in male mice (Loveless et al. 2006) that formed the basis for an interim TRV. The NOAELs/LOAELs (based on administered dose) determined from recent immunotoxicity studies are substantially higher than the LOAEL of 0.05 mg/kg-day for liver toxicity from the Li et al. (2017) study, which was selected as a critical study for development of a noncancer TRV by the agency. • According to US EPA (2022c, d; 2021a) decreased immune response to vaccination was observed after exposure during a sensitive developmental life stage and yields the lowest POD among the candidate PODs. Other candidate TRVs were derived based on other health effects (e.g. development/growth) observed in epidemiology studies; all of the candidate TRVs are associated with low daily oral exposure doses, ranging from 1 to 0.001 ng/kg bw/day. • WHO (2022) opted for a pragmatic DWG rather than a health-based DWG. Although the reduced antibody response following vaccination has been considered by some jurisdictions as the most robust end point based on epidemiological data, it is unclear whether this correlation results in increased rates of infection and hence the clinical implications are uncertain. Although animal data would generally be utilised in the absence of adequate human data for risk assessment purposes, there are also areas of uncertainty around the suitability of animal studies for assessing the effects to human health for PFOS and PFOA, including interspecies differences in kinetic parameters such as elimination half-life and clearance rate. Additionally, diverging estimates of the human half-life of PFOA may also add uncertainty to animal-to-human dosimetric adjustments, as well as PBPK-based conversions of human plasma PFAS concentrations to external doses. Finally, the uncertainty and lack of consensus in the critical health end point to derive a TRV is evident from the diverse range of endpoints utilised by other agencies to derive TRVs, and the resulting range in proposed drinking water values. Although

#	Research Questions	Response	
			the values derived by several different organisations vary significantly, all have margins of safety. Data analysis also shows that science on PFAS is evolving very rapidly in various areas.
		GenX Chemicals	Detailed justification for each endpoint is provided in the Technical Report. Some of the common themes and/or discrepancies between jurisdictions are summarised as follows. • MPART (2019a) states that for all of the PFAS examined, PODs were selected from studies with laboratory animal models. This approach does not negate findings associated with epidemiological studies, but reflects that humans experience uncontrolled and imperfectly documented rather than controlled, precisely measured exposures. Additionally, these points of departure reflect adverse health effects that occur at low doses and that are supported by the weight-of-evidence across endpoints and between findings in humans and laboratory animal models. The Workgroup noted that while primarily industry-funded studies are the only ones available, they followed recognised testing guidelines and/or were published following external peer-review. These studies appear to be sufficient for developing values for GenX Chemicals. • US EPA (2021e, 2022c, j) concluded the available toxicity studies demonstrate that the liver is particularly sensitive to GenX Chemicals-induced toxicity. US EPA determined that the constellation of liver lesions observed in the rodent are relevant to human health and not a result of PPARα-induced cell proliferation unique to rodents. WSDH (2023a, 2022b) adopted the US EPA value. • NJDEP (2023a) reviewed the basis of the US EPA TRV of 3 ng/kg/day for GenX Chemicals and concluded it to be scientifically justified and health protective.
4	What other recent guideline values exist?	The various guideline values retrieved as part of the literature search undertaken are provided in the response to Research Question 1 and Question 6.	
5	If there are existing guidance/guideline values, are the proposed option/s for health-based guideline values relevant to the Australian context?	The cancer-derived DWGs derived by some agencies (e.g. OEHHA 2019a, 2023a) are not derived consistent with Australian science policy, 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). Also refer to detailed discussions in **Sections 6.0** to **10.0** of this Evaluation Report.	

#	Research Questions	Response	
6	How were they derived and are there any uncertainties with the key studies or the approaches used?	The derivation of the various DWGs and TRVs has been described in detail in the Technical Report. The derivation approach, along with level of uncertainty factors and toxicokinetic modelling employed, varies by jurisdiction. The focus of the critical evaluation of studies and derivations for the various PFAS in **Sections 6.0** to **10.0** is on those guidance/guideline values which are based on critical studies that have not previously been evaluated and/or considered by FSANZ (2017b) in their review and derivation of TRVs for PFOS + PFHxS and PFOA. The reader is referred to the Technical Report for detail as well as the discussion section for each relevant PFAS in this Evaluation Report. The various TRVs derived by the different jurisdictions and the underlying critical study (in ascending order) are as follows.	
		PFOS	• **0.0079 ng/kg/day** (DRAFT, US EPA 2022c, e; 2021b) (critical studies in humans: Grandjean et al. 2012 and Budtz-Jørgensen and Grandjean 2018). • **0.63 ng/kg/day** (i.e. 4.4 ng/kg/week) as sum of PFOA, PFNA, PFHxS and PFOS (EFSA 2020a, adopted by RIVM 2021a) (critical study in humans: Abraham et al. 2020). • **0.64 ng/kg/day** (OEHHA 2023a) (critical study in humans: Steenland et al. 2009). • **1.8 ng/kg/day** (NJDEP 2019b, OEHHA 2019a) (critical study in mice: Dong et al. 2009). • **2 ng/kg/day** (ATSDR 2021a) (critical study in rats: Luebker et al. 2005a). • **2.89 ng/kg/day** (MPART 2019a) (critical study in mice: Dong et al. 2009). • **3.1 ng/kg/day** [MDH 2020a; adopted by WSDH (2019, 2022b, 2023a)] (critical study in mice: Dong et al. 2011). • **20 ng/kg/day** (FSANZ 2017b) (critical study in rats: Luebker et al. 2005b). • **60 ng/kg/day** (HC 2018a) (critical study in rats: Butenhoff et al. 2012b).
		PFHxS	• **0.0004 ng/kg/day** (DRAFT, US EPA 2023) (critical studies in humans: Grandjean et al. 2012 and Budtz-Jørgensen and Grandjean 2018). • **0.63 ng/kg/day** (i.e. 4.4 ng/kg/week) as sum of PFOA, PFNA, PFHxS and PFOS (EFSA 2020a, adopted by RIVM 2021a) (critical study in humans: Abraham et al. 2020). • **2.4 ng/kg/day** (OEHHA 2022a) (critical study in rats: NTP 2022). Also derived candidate TRVs for two other endpoints of **2.9 ng/kg/day** (increased liver weight, critical study in rats: NTP 2022) and **14.3 ng/kg/day** (decreased live pups per litter, critical study in mice: Chang et al. 2018). • **9.7 ng/kg/day** [MDH 2020b, MPART 2019a, WSDH (2019, 2022b, 2023a)] (critical study in rats: NTP 2018). • **20 ng/kg/day** (FSANZ 2017b) (critical study in rats for PFOS: Luebker et al. 2005b). • **20 ng/kg/day** (ATSDR 2021a) (critical study in rats: Butenhoff et al. 2009).

#	Research Questions	Response	
		PFBS	• **84 ng/kg/day** (MDH 2022e, g) (critical study in rats: NTP 2022). • **300 ng/kg/day** (MPART 2019a, US EPA 2022c, k; 2021c; WSDH 2019a, 2023a, 2022b) (critical study in mice: Feng et al. 2017). • **600 ng/kg/day** (OEHHA 2021d) (critical study in mice: Feng et al. 2017).
		PFOA	• **0.0015 ng/kg/day** (DRAFT, US EPA 2022c, d; 2021a) (critical studies in humans: Grandjean et al. 2012 and Budtz-Jørgensen and Grandjean 2018). • **0.45 ng/kg/day** (OEHHA 2019a) (critical study in mice: Li et al. 2017) • **0.63 ng/kg/day** (i.e. 4.4 ng/kg/week) as sum of PFOA, PFNA, PFHxS and PFOS (EFSA 2020a, adopted by RIVM 2021a) (critical study in humans: Abraham et al. 2020). • **0.87 ng/kg/day** (OEHHA 2023a) (critical study in humans: Gallo et al. 2012). • **2 ng/kg/day** (NJDEP 2019a) (critical study in mice: Loveless et al. 2006). • **3 ng/kg/day** (ATSDR 2021a; adopted by WSDH 2019a, 2022b, 2023a) (critical study in mice: Koskela et al. 2016). • **3.9 ng/kg/day** (MPART 2019a) (critical studies in mice: Onishchenko et al. 2011, Koskela et al. 2016). • **18 ng/kg/day** (MDH 2022f) (critical study in mice: Lau et al. 2006). • **21 ng/kg/day** (HC 2018b) (critical study in rats: Perkins et al. 2004). • **160 ng/kg/day** (FSANZ 2017b) (critical study in mice: Lau et al. 2006).
		GenX Chemicals	• **3 ng/kg/day** (US EPA 2021e, 2022c, j; adopted by NJDEP 2023a, WSDH 2023a, 2022b) (critical study in mice: DuPont 2010, unpublished). • **77 ng/kg/day** (MPART 2019a) (critical study in mice: DuPont 2010, unpublished).
7	Are they suitable to adopt/adapt?	For each health-based guidance value derived by the various jurisdictions for the five PFAS under consideration, an evaluation of the technical and administrative processes was undertaken in line with pre-determined criteria (see Assessment Tool in Technical Report, **Appendix D**). Based on this evaluation, a general sense with respect to suitability for potential adoption/adaptation can be gauged. • For PFOS, several jurisdictions met a high degree (i.e. ~>60%) of 'must-have' and 'should-have' criteria. These included ATSDR (2021a), EFSA (2020a), FSANZ (2017b), NJDEP (2019b), OEHHA (2023a), and US EPA (2022c, e; 2021b). • For PFHxS, jurisdictions for which derivation reports met a high degree (i.e. ~>60%) of 'must-have' and 'should-have' criteria included ATSDR (2021a), FSANZ (2017b), OEHHA (2022a) and US EPA (2023).	

#	Research Questions	Response
		• For PFBS, jurisdictions for which derivation reports met a high degree (i.e. ~>60%) of 'must-have' and 'should-have' criteria included US EPA (2022c, k; 2021c) and potentially OEHHA (2021d). • For PFOA, jurisdictions for which derivation reports met a high degree (i.e. ~>60%) of 'must-have' and 'should-have' criteria included ATSDR (2021a), EFSA (2020a), FSANZ (2017b), NJDEP (2019a), OEHHA (2023a), and US EPA (2022c, d; 2021a). • For GenX Chemicals, the only jurisdiction for which the derivation report met a high degree (i.e. ~>60%) of 'must-have' and 'should-have' criteria was US EPA (2021e). However, the reader is also referred to the critical detailed discussions on suitability for adoption/adaptation in **Sections 6.0** to **10.0** herein for each individual PFAS. It was concluded in **Section 11.0** that a number of guidance values may be suitable for adoption/adaptation. The resulting adapted candidate DWGs are provided in **Table 11-1**.
8	What are the key adverse health hazards from exposure to PFOS, PFOA, PFHxS, PFBS and GenX Chemicals in Australian drinking water?	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 response to Research Question 2.

4.2 Exposure-related aspects

Another important aspect of the Fact Sheet covers exposure-related considerations. This is important for consideration of whether exposures by Australians to the chemicals evaluated are potentially approaching a health-based guidance value that will be used for deriving a candidate DWG. It is also important for considerations of whether typical levels of the chemical considered in Australian drinking water supplies would adhere to any derived DWG. Research questions 9-11 cover exposure-related aspects of the review. For these aspects, drinking water quality reports from various water corporations around Australia were consulted in addition to the jurisdictional reviews sourced as part of the health-based review and the supporting information. **Table 4-2** provides a synthesis of the results.

Table 4-2 Summary of findings from data extraction for exposure-related research questions

#	Research Questions	Findings	
9	What are the typical levels in Australian drinking water supplies, considering distributed drinking water and households using their own borewater, rainwater or surface water for drinking?	PFOS	Up to 6 ng/L in Queensland raw water catchments (QAEHS 2018a, 2018b) and Sydney distributed drinking water (Sydney Water 2023) but up to 16 ng/L in Australia according to WHO (2022). PFOS+PFHxS concentration was at 90% of the Australian DWG in one bore in a drinking water borefield supplying Esperance, Western Australia (WCWA 2019, 2020). Once this apparent PFOS/PFHxS contamination was identified, this bore was no longer used (WCWA 2023). Maximum concentrations in residential/private bores for domestic purposes (including drinking) surrounding various contaminated sites or in proximity to fire stations can be much higher (i.e. 80 to 136,000 ng/L) (GHD 2018, AECOM 2017, 2017b; BSC 2021).
		PFHxS	Up to 5 ng/L in Queensland raw water catchments (QAEHS 2018a, 2018b), Sydney distributed drinking water (Sydney Water 2023) and Western Australia distributed drinking water (WCWA 2023). PFOS+PFHxS concentration was at 90% of the Australian DWG in one bore in a drinking water borefield supplying Esperance, Western Australia (WCWA 2019, 2020). Once this apparent PFOS/PFHxS contamination was identified, this bore was no longer used (WCWA 2023). Maximum concentrations in residential/private bores for domestic purposes (including drinking) surrounding various contaminated sites or in proximity to fire stations can be much higher (i.e. 130 to 54,300 ng/L) (GHD 2018, AECOM 2017, 2017b; BSC 2021).
		PFBS	Up to 2.2 ng/L in Queensland (QAEHS 2018a, 2018b) raw water catchments. Maximum concentrations in residential/private bores for domestic purposes (including drinking) surrounding various contaminated sites can be much higher (i.e. 40 to 6,520 ng/L) (GHD 2018, AECOM 2017, 2017b).

#	Research Questions	Findings	
		PFOA	Up to 10 ng/L in raw water catchments and distributed drinking water in various Australian jurisdictions (QAEHS 2018a, 2018b, Sydney Water 2023, WHO 2022, WCWA 2023). Maximum concentrations in residential/private bores for domestic purposes (including drinking) surrounding various contaminated sites or in proximity to fire stations can be much higher (i.e. 20 to 10,500 ng/L) (GHD 2018, AECOM 2017, 2017b; BSC 2021).
		GenX Chemicals	No information regarding GenX Chemicals levels in Australian drinking water was identified from literature retrieved.
10	Do they vary around the country or under certain conditions e.g. drought?	From limited amount of literature identified in the public domain and reviewed, the levels of PFOS, PFOA and PFHxS in distributed drinking water from Queensland, Sydney and Western Australia were generally less than 10 ng/L (also refer to the response to Research Question 9 in the Technical Report, **Section 4.3**). Distributed drinking water concentrations for PFOS+PFHxS and PFOA appear to be within the range quoted within the previous Australian Fact Sheet in the Guidelines and, along with PFBS, are at the low end of those concentrations observed in various international jurisdictions (including the US and parts of Europe). However, concentrations in bore water used for domestic purposes (including drinking) in close proximity to contaminated sites can be much higher than in distributed drinking water. No information regarding GenX Chemicals levels in Australian drinking water was identified from literature retrieved.	
11	What other factors should be considered (e.g. differences between groundwater versus surface water sources)?	The main factor to consider for exposure to PFAS in drinking water is whether drinking water infrastructure is located in the vicinity of potentially contaminating activities (HC 2018a, NJDEP 2019b, OEHHA 2023a, WHO 2022) as identified in response to Research Question 20 (refer to **Section 4.5** in Technical Report). In addition, 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), PFAS may be present above respective health-based DWGs.	

4.3 Risk-based aspects

Research questions 12 and 13 are risk-based considerations. The publications subjected to detailed data extraction were also consulted to answer these questions. **Table 4-3** presents a summary of the findings.

Table 4-3 Summary of findings from data extraction for risk-based research questions

#	Research Questions	Findings
12	What are the risks to human health from	Provided drinking water catchments are protected from PFAS contamination and alternative water supplies are available if

#	Research Questions	Findings
	exposure to PFOS, PFOA, PFHxS, PFBS and GenX Chemicals in Australian drinking water?	PFAS contamination is identified, risk of harm from exposure to PFOS+PFHxS, PFOA and PFBS in distributed drinking water in Australia appears to be relatively low based on the limited PFAS distributed drinking water data (measured concentrations <10 ng/L, refer to Research Question 9 above) when these values are compared to the existing and/or candidate drinking water guidelines for these PFAS (see also **Section 11.0)**. 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), one or more of these five PFAS may be present above respective health-based DWGs. This does not automatically mean there is a risk of harm to human health due to the fact the DWGs are based on doses which resulted in no adverse effects in animal studies coupled with large uncertainty factors and small relative source contribution to drinking water. However, it does mean additional investigation into the potential risks to human health from drinking water is warranted in such cases.
13	Is there evidence of any emerging risks that are not mentioned in the current Fact Sheet that require review or further research?	There is clearly uncertainty with respect to the critical human health endpoints selected by various jurisdictions around the World (particularly with respect to PFOS and PFOA) which has resulted in wide divergence in derived guidance and guideline values (see also **Section 5.0** and detailed discussions in **Section 6.0** to **10.0**). Further research is required to relate some of the endpoints found to be associated with PFAS exposure in epidemiological studies (e.g. decreased antibody response to certain vaccines in children) to adverse effects. It is currently unclear whether these findings translate to a clear increase in infection and/or disease (see also discussion in **Section 6.2**). Other emerging risks are discovery of more PFAS contaminated sites and the use of more marginal water supplies (which may be found to contain PFAS contamination). In SLR's experience, other PFAS such as PFBA and PFHxA are commonly detected in environmental media (e.g. water, soil, etc.) in Australia. An approach to the assessment of these PFAS, as well as PFAS not routinely monitored for in Australia, would be of benefit.

4.4 Supporting information

Supporting information in Fact Sheets for chemicals in the Guidelines typically consist of (NHMRC and NRMMC 2011) a brief general description (i.e. uses of PFAS, sources in drinking water), typical values in Australian drinking water, treatment of drinking water, and measurement (i.e. analytical) considerations. The remaining Research questions 14-24 cover the supporting information of the review. For these aspects, in addition to consulting the previously mentioned sources (e.g. the drinking water quality reports from various water corporations and utilities around Australia, the health-based jurisdictional literature identified in the targeted search), additional targeted searches were undertaken (for details, refer to Technical Report). **Table 4-4** provides a summary of the results.

Table 4-4 Summary of findings from data extraction for supporting information

#	Research Questions	Findings
14	Is the general description in the Fact Sheet current for all 5 PFAS under review?	Yes. The general description for PFOS, PFHxS, and PFOA in the current Fact Sheet appears current based on the responses to the research questions in this table below. It is also relevant for PFBS and GenX Chemicals. From the articles reviewed that comment on sources and provide a general description, it is apparent that PFAS are used in numerous industrial applications and formulated within manufactured goods. There are point sources and diffuse sources of PFAS resulting in their releases to the environment. There is no need to update the current general description.
15	What are the chemicals used for and how might people be exposed?	PFAS are in numerous industrial applications and manufactured goods. This includes food packaging, firefighting foams, non-stick cookware, clothes and protective coatings for fabrics and carpets, electronics, mist suppressors, and/or fluoropolymer manufacturing. People are predominantly exposed from food. Drinking water can be a significant source of PFAS in areas surrounding sites with contaminating activities.
16	How do the chemicals end up in drinking water and in what form?	PFAS can end up in drinking water through nonpoint sources such as runoff and groundwater infiltration or from point sources from sites with contaminating activities (such as firefighting training grounds, industrial facilities, and municipal and industrial wastewater treatment plant effluent, or even through atmospheric deposition). The form (or type) of PFAS present will be dependent on the contaminating activity. There may be many types of PFAS present including those not routinely measured by Australian laboratories.
17	Is the measurement information in the Fact Sheet current?	Yes. The measurement information for PFOS, PFHxS, and PFOA in the Fact Sheet appears current based on the responses to Research Question 18 in this table below. High performance liquid chromatography (HPLC) (sometimes replaced with ultraperformance liquid chromatography or UPLC) coupled to a tandem mass spectrometer (MS/MS) is the most common routine method used for PFAS analysis in the journal articles reviewed and by Australian commercial laboratories (NMI 2023, SGS 2023, ALS 2023, Eurofins 2023). This information is also relevant to PFBS and GenX Chemicals. It is noted that GenX Chemicals are not routinely measured by Australian laboratories and have only recently been added to analytical schedules offered by some commercial laboratories. Specific PFAS analytical methods are not cited in the Fact Sheet. Commercial laboratories are basing their in-house methods on USEPA Methods 533, 537.1 and 1633 and/or US DoD QSM 5.3.
18	What are the current analytical methods used to measure/detect the concentration of the specified chemicals in water?	HPLC equipped with a tandem mass spectrometer (MS/MS) operated in negative electrospray ionisation (ESI^-) is the most common method used for PFAS analysis. For lower levels of detection and identification of unknown PFAS, more specialised mass spectrometry may be used such as high-resolution mass spectrometry (HRMS) and quadrupole time of flight mass spectroscopy (QToF-MS). Gas chromatography mass spectrometry (GC/MS) is sometimes used for volatile PFAS, and non-PFAS specific test methods include total oxidisable precursor assay (TOP assay), and total organic fluorine assay (TOF assay) as combustion ion chromatography.

#	Research Questions	Findings
19	What are the limits of quantification or limit of reporting for these chemicals in drinking water?	Reporting limits for PFOS, PFHxS, PFBS, PFOA and GenX Chemicals are as follows: • 1 to 20 ng/L for standard analysis • 0.2 to 2 ng/L for low or trace analysis. • 0.1 ng/L for ultra trace analysis. Reporting limits are laboratory dependant and only one of four laboratories offered ultra trace analysis. More specialised mass spectrometry can result in lower reporting limits (pg/L levels).
20	What are the indicators of the risks?	Three important indicators of risk are PFAS levels in food, water and human serum. Currently, there is a dearth of data in Australia for these PFAS risk indicators. Risk of harm from exposure to PFAS in available distributed drinking water data (<10 ng/L for PFOS + PFHxS and <5 ng/L for PFOA, refer to relevant Research Question 9 above) appears to be relatively low based on measured concentrations when they are compared to the existing Australian DWGs for these PFAS (PFOS+PFHxS: 70 ng/L and PFOA: 560 ng/L). There is currently no relevant guideline value for PFBS or GenX Chemicals, nor is there drinking water data in Australia for GenX Chemicals. However, there are many sites of PFAS contamination in water bodies 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), one or more of these five PFAS may be present above respective health-based DWGs in these cases. Food is often the major source of PFAS exposure (see responses to this Research Question below). PFAS serum concentrations can be used as another (potentially improved) measure of dose from PFAS exposure. However, this testing involves consistent, ongoing participation and consent to be reliable and there is limited Australian data. In SLR's experience, other PFAS such as PFBA and PFHxA are commonly detected in environmental media (e.g. water, soil, etc.) in Australia. An approach to the assessment of these PFAS, as well as PFAS not routinely monitored for in Australia, would be of benefit when considering indicators of risk.
21	How can we measure this exposure?	Exposure can be estimated from PFAS directly measured in water and different foodstuffs or from biomonitoring data. PFAS in these media can be directly measured using standard HPLC-MS/MS methods as outlined in response to Research Question 19 in this table above. In SLR's experience, water quality data and biomonitoring data for PFAS are collected routinely to monitor for PFAS exposure by some international jurisdictions. This is not undertaken routinely in Australia except on an *ad-hoc* (as needed) basis in areas with contaminated sites. Currently, minimal information is available in Australia to estimate exposure to PFAS by Australians and, when estimated, it is often supported by read across data from other jurisdictions (typically from the US, but also Canada and some European locations).

#	Research Questions	Findings
22	Is the information on treatment of drinking water in the Fact Sheet current?	The treatment information for PFOS, PFHxS, and PFOA in the current Fact Sheet appears current based on the responses to Research Question 23 below. Multiple reviewed articles note that standard/traditional treatment processes at drinking water treatment plants are ineffective at removing PFAS. In some cases, PFAS concentrations in drinking water have been found to be higher than raw water in the United States and Japan (Xiao 2022). Granular activated carbon (GAC), ion exchange resins, reverse osmosis and nanofiltration are common treatment options being employed for PFAS removal, however most have shortcomings with respect to power consumption, PFAS specificity etc. in line with the treatment information provided in the current Fact Sheet. Alternate methods are being investigated. There is no identified need to change the treatment information provided in the current Fact Sheet.
23	What are the available options for removing the specified chemicals from drinking water?	Commonly employed drinking water treatment methods include: • Ion Exchange Resins. • Granular activated carbon (GAC), modified activated carbon products, and biochars. • Reverse Osmosis. • Nanofiltration/membranes (carbon nanotubes or CNT etc.). These methods have been used to various success but have different strengths and weaknesses (PFAS specificity, short breakthrough times, expense etc.). There are various other treatment methods also being used including destructive treatments (advanced oxidation processes, electrochemical oxidation, incinerations, sono-chemical, biodegradation, photolysis, reductive degradation, microwave enhanced Fenton process etc.).
24	What are the current practices to minimise or manage the risks identified?	Water treatment is one practice used to manage risks associated with PFAS exposure. In areas contaminated with PFAS, a common and immediate public health response is to prevent people from drinking PFAS contaminated water. This can be done by restricting use of contaminated raw water sources, sourcing water from alternate (uncontaminated) areas, providing new connections to distributed drinking water, providing an in-premise filtration system and/or supplying bottled water.

DWG = Drinking Water Guideline. LOR = Limit of Reporting. HPLC = High performance liquid chromatography. UPLC = Ultraperformance liquid chromatography. MS/MS = tandem mass spectrometer. GAC = Granular activated carbon. GC/MS = Gas Chromatography Mass Spectrometry. ESI^- = electrospray ionisation. HRMS = high-resolution mass spectrometry. TOP = Total Oxidisable Precursor Assay. TOF Assay = Total Organic Fluorine Assay.

5.0 Tabular summary of existing guidance/guideline values

It is noted guidance/guideline values differ quite markedly depending on the jurisdiction and there is discrepancy with respect to what jurisdictions consider to be the critical health endpoint. Guidance/guideline values have also been reducing rapidly over time as new studies and new interpretation of the data emerge in the publicly available literature. To gain a visual appreciation of the differences and the lowering of values over time, **Table 5-1** and **Table 5-2** provide tabular summaries of the guidance and guideline values, respectively, derived by the various jurisdictions for PFOS, PFHxS, PFBS, PFOA and GenX Chemicals. It is noted only the most recently derived guidance/guideline values were subjected to data extraction in the Technical Report. Older values are nevertheless provided in the tables and

were sourced from the various jurisdictional websites for historical context. The summary tables are colour coded according to the critical health endpoint that underpins the guidance/guideline value.

From **Table 5-1**, it is evident there is much disparity between the critical health endpoints chosen by the various jurisdictions for derivation of guidance values for PFOS and PFOA, and less so for PFHxS, whereas for PFBS and GenX Chemicals most jurisdictions that have derived a guidance value seem to concur with respect to the critical health endpoint (although the latter is possibly due to the relative lack of available studies for deriving guidance/guideline values). There is even more variability between resulting guideline values, as shown in **Table 5-2**, due to some not being health-based and there being wide variation in the assumptions used to derive them. Due to the large variability in guideline values, it was deemed most informative to focus on critically reviewing available guidance values.

Table 5-1 Summary of existing guidance values for the five PFAS included in this report (ng/kg/day)

PFAS	Year	WHO	EFSA	RIVM	BfR/TWK	Health Canada	FSANZ	ATSDR	MDH	WSDH	MPART	NJDEP	OEHHA	US EPA
PFOS	2006				83									
	2008		150											770
	2011					83								
	2014													30
	2016					60				20				20
	2017						20 (e)							
	2018		**1.8**	25				2 (d)						
	2019				**1.8 (f)**					3.1	2.89	1.8		
	2020	- (a)	**0.63 (b, g)**						3.1					
	2021			**0.63 (b, g)**									1.8	
	2022													**0.0079 (DRAFT) (g)**
	2023												**0.64**	
PFOA	2006				100									
	2008		1,500											1,900
	2011					100			4,200					
	2014													20
	2016			12.5						20				20
	2018		**0.8**	12.5		21	160	3 (d, g)						20
	2019				**0.8 (f)**					3	3.9 (g)	2 (g)		
	2020	- (a)	**0.63 (b, g)**										0.45	
	2021			**0.63 (b, g)**									**0.87 (g)**	
	2022								18					**0.0015 (DRAFT) (g)**
	2023													

PFAS	Year	WHO	EFSA	RIVM	BfR/TWK	Health Canada	FSANZ	ATSDR	MDH	WSDH	MPART	NJDEP	OEHHA	US EPA
PFHxS	2017						20 (e)							
	2018			20.8				20 (d)						
	2019										9.7 (g)			
	2020		0.63 (b, g)						9.7 (g)					
	2021			0.63 (b, g)										2
	2022									9.7 (g)			2.4 (g)	
	2023													0.0004 (DRAFT) (g)
PFBS	2011													
	2018			12,500 (e)										10,000
	2019										300 (g)			
	2020													
	2021												600 (g)	300 (g)
	2022								84 (g)	300 (g)				
	2023													
GenX Chemicals	2018													80
	2019a										77 (g)	3 (g)		
	2019b			208 (e)										
	2020													
	2021													3 (g)
	2022													
	2023													

PFAS	Year	WHO	EFSA	RIVM	BfR/TWK	Health Canada	FSANZ	ATSDR	MDH	WSDH	MPART	NJDEP	OEHHA	US EPA

WHO = World Health Organization. EFSA = European Food Safety Authority. RIVM = Rijksinstituut voor Volksgezondheid en Milieu (Dutch National Institute for Public Health and the Environment). BfR/TWK = Bundesinstituts für Risikobewertung (German Federal Institute for Risk Assessment)/Trinkwasserkommission (Drinking Water Commission). FSANZ = Food Standards Australia New Zealand. ATSDR = Agency for Toxic Substances and Disease Registry. MDH = Minnesota Department of Health. WSDH = Washington State Department of Health. MPART = Michigan PFAS Action Response Team. NJDEP = New Jersey Department of Environmental Protection. OEHHA = California Office of Environmental Health and Hazard Assessment. US EPA = United States Environmental Protection Agency.

(a) A TRV was not derived by WHO (2022a). A DWG was derived based on pragmatism.
(b) Health-based guidance value from EFSA (2020a) applies to the sum of four PFAS, i.e. ∑PFOA, PFNA, PFHxS and PFOS.
(c) A TRV for PFOS was conservatively adopted for PFHxS. The TRV is applied as a sum (PFOS+PFHxS).
(d) The TRV from ATSDR is based on intermediate exposure timeframe (14 - <365 days).
(e) Based on a relative potency factor of 0.06 for GenX, 0.001 for PFBS and a TRV of 12.5 ng/kg/day (for PFOA). From 2021, the TRV for PFOA (summed with three other PFAS) may be substantially lower (0.63 ng/kg/day).
(f) It is presumed by SLR that BfR adopted the updated tolerable weekly intake in 2020 from EFSA (2020a) given that BfR adopted the 2018 value from EFSA (2018, as quoted in BfR 2019a).
(g) These guidance values are based on studies not previously evaluated/considered by FSANZ (2017b) and have been further evaluated in **Sections 6.0** to **10.0**.

Legend:

Not stated
Not a health-based value
Thyroid effects in animal studies
Reproductive/developmental effects in animal studies
Immune changes in animal studies
Immune changes in human studies
Effects on the liver in animal studies
Effects on the liver in human studies
Cancer data from animal studies
Cancer data from human studies
Changes in cholesterol/blood chemistry in animal studies
Changes in cholesterol/blood chemistry in human studies
No relevant TRV available (or found in document identified in the literature search)

Table 5-2 Summary of existing drinking water guideline values for the five PFAS included in this report (ng/L)

PFAS	Year	WHO	EC / EU	BfR/ TWK	DOH / NHMRC	Health Canada	RIVM	ATSDR	MDH	WSDH	MPART	NJDEP	OEHHA	Maine DHHS	Alaska DEC / DHHS	Mass. DPH / DEP	CDPH (Conn-ecticut)	US EPA
PFOS	2006			300														
	2008																	
	2011					300									200 (2015)			
	2014																	
	2016					600									70 (2015)		70	70
	2017			100	70 (b)													
	2018					600		14 (d)			70				70 (h)	70		
	2019					600				15	16	10	7 (c)		70 (i)		70	
	2020	100 (a)	100 (LOR)						15		16							
	2021												7 (c)	20				
	2022								15	15						0.02		**0.02** or 4 (LOR)
	2023								4	4 (e)	16		**2 (c)**			4	10	
PFOA	2006			300														
	2008																	400
	2011					300												
	2014																	
	2016					200	87.5										70	70
	2017			100	560													
	2018					200		21 (d)			70				70 (h)	70		
	2019					200				10	8	10	**2 (c)**		70 (i)		70	
	2020	100 (a)	100 (LOR)								8							
	2021												**2 (c)**	20				
	2022								35	10						0.004		**0.004** or 4 (LOR)
	2023								4	4 (e)	8		**3 (c)**			4	16	
PFHxS	2011					300												
	2016					600												
	2017				70 (b)													
	2018							140 (d)							70 (h)	70		
	2019					600				70	51			20				
	2020		100 (LOR)						47		51							
	2021																	9
	2022								47	65	51		2					
	2023									9 (f)							49	
PFBS	2011					15,000												
	2016					15,000												
	2018			6,000 (2017)											2,000	2,000 (other agency)		
	2019					15,000				860 (or 1,300)	420							
	2020		100 (LOR)								420							
	2021												500			2,000		
	2022								100	345								2,000

PFAS	Year	WHO	EC / EU	BfR/ TWK	DOH / NHMRC	Health Canada	RIVM	ATSDR	MDH	WSDH	MPART	NJDEP	OEHHA	Maine DHHS	Alaska DEC / DHHS	Mass. DPH / DEP	CDPH (Conn-ecticut)	US EPA
	2023									2000 (f)	420						760	
GenX Chem-icals	2018																	
	2019a										370							
	2019b																	
	2020										370							
	2021																	10
	2022									10						10		10
	2023									10 (f)	370	20				10		

WHO = World Health Organization. EC/EU = European Commission/European Union. BfR/TWK = Bundesinstituts für Risikobewertung (German Federal Institute for Risk Assessment)/Trinkwasserkommission (Drinking Water Commission). DoH/NHMRC = Department of Health/National Health and Medical Research Council (Australia). RIVM = Rijksinstituut voor Volksgezondheid en Milieu (Dutch National Institute for Public Health and the Environment). ATSDR = Agency for Toxic Substances and Disease Registry. MDH = Minnesota Department of Health. WSDH = Washington State Department of Health. MPART = Michigan PFAS Action Response Team. NJDEP = New Jersey Department of Environmental Protection. OEHHA = California Office of Environmental Health and Hazard Assessment. Maine DHHS = Maine Department of Health and Human Services. Alaska DEC/DHHS = Alaska Department of Environmental Conservation/Department of Health and Human Services. Mass. DPH/DEP = Massachusetts Department of Public Health/Department of Environmental Protection. CDPH = Connecticut Department of Public Health. US EPA = United States Environmental Protection Agency.

(a) A DWG was derived based on pragmatism. A value of 500ng/L is applicable to Total PFAS.
(b) The DWG is applied as a sum: PFOS+PFHxS.
(c) Reference Levels (RLs) and health protective concentration (HPC) for non-cancer effects shown. The RLs and Public Health Goal (PHG) for cancer effects are not shown. In 2019, State Water Resources Control Board (SWRCB) set the Notification Levels (NLs) at the lowest levels at which PFOA and PFOS can be reliably detected in drinking water (OEHHA 2019a).
(d) Drinking water guideline shown is for a child (which is lower than the value derived for an adult).
(e) “Any change to a [State Action Level] SAL or adopting a state [Maximum Contaminant Level] MCL requires rulemaking. WSDH will continue to implement SALs until rulemaking permits use of this value (a MCL from USEPA)"
(f) Health-based water concentration (HBWC) are the “acceptable” values used to create a ratio of observed/acceptable for each of 4 PFAS (PFNA, PFHxS, PFBS and GenX). If the ratios add up to more than 1.0, action must be taken to lower PFAS in the drinking water.
(g) Interim State drinking water standard for the combined sum of six different PFAS: PFOA, PFOS, PFHpA, PFNA, PFDA, and PFHxS.
(h) A 0.07 µg/L action level was set for the sum of the following five (5) PFAS chemicals: PFOS, PFOA, PFNA, PFHxS, and PFHpA.
(i) In order to align state actions to the recently announced EPA plans, Alaska DEC will use the US EPA Lifetime Public Health Advisory (LHA) (PFOS+PFOA above 0.07 µg/L) as the Action Level.

Not stated
Not a health-based value
Thyroid effects in animal studies
Reproductive/developmental effects in animal studies
Immune changes in animal studies
Immune changes in human studies
Effects on the liver in animal studies
Effects on the liver in human studies
Cancer data from animal studies
Cancer data from human studies
Changes in cholesterol/blood chemistry in animal studies
Changes in cholesterol/blood chemistry in human studies
No relevant TRV available (or found in document identified in the literature search)

6.0 Discussion for PFOS

This section provides a discussion of the strengths and limitations of the identified guidance values for PFOS for possible adoption/adaptation into the Guidelines. Critical evaluation was focused on those guidance values derived using underpinning studies not previously considered / evaluated by FSANZ (2017b). FSANZ (2021) also published a review of immunomodulation effects, in which the jurisdiction reviewed a number of studies, findings of which have been used to support subsequent discussions.

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

Candidate guidance values for PFOS described in **Section 4.1** for possible adoption/adaptation in Australia have been evaluated using the Assessment Tool provided in Appendix D in the Technical Report. 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.

Figure 6-1 presents the percentage of criteria (combined technical and administrative criteria) met by each jurisdiction. It is evident from the figure that several publications met similar percentages of criteria, with ATSDR (2021a), EFSA (2020a), FSANZ (2017b), NJDEP (2019b), OEHHA (2023a), and US EPA (2022c, e; 2021b) all meeting relatively high (i.e. ~>60%) proportions of 'must-have' and 'should-have' criteria.

Other jurisdictions (HC 2018a, MDH 2020a, MPART 2019a, OEHHA 2019a) met lower proportions of criteria, indicating these guidance documents potentially do not conform with modern methods of undertaking systematic reviews.

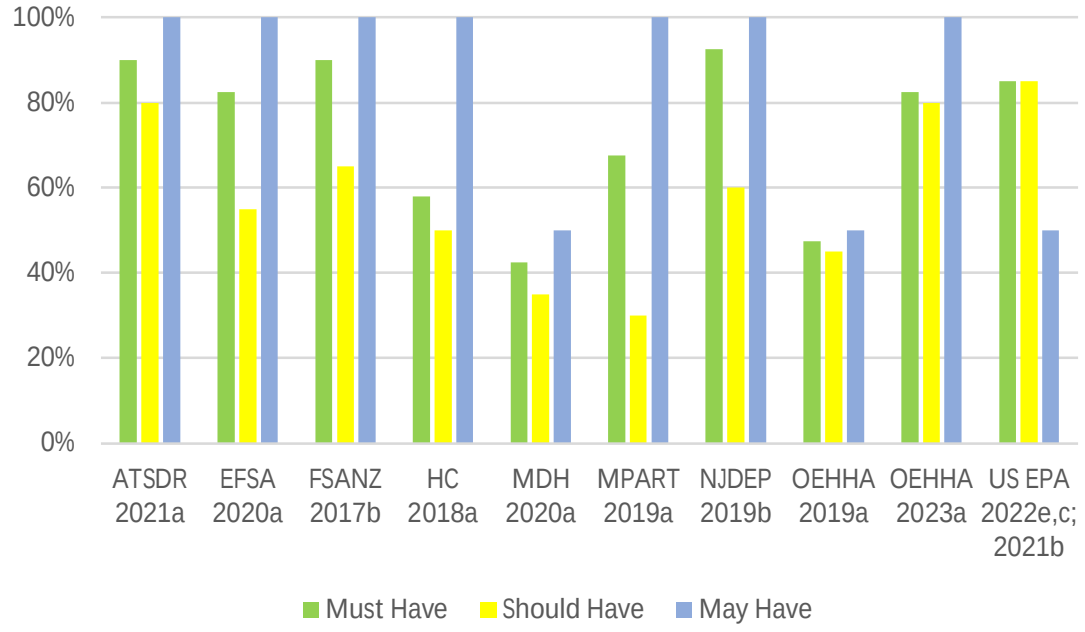


Figure 6-1 Overall proportion of 'must-have', 'should-have' and 'may-have' technical/administrative criteria as per the Assessment Tool met by jurisdictions who have derived candidate guidance values for PFOS for possible adoption/adaptation in Australia

6.2 Critical evaluation of PFOS guidance values based on underpinning studies not previously considered by FSANZ (2017b)

For PFOS, the only guidance values identified in the literature review that based their derivations on underpinning studies not previously considered / cited in the comprehensive review undertaken by FSANZ (2017b) are the following. The discussion in this section therefore focuses on the relevant studies underpinning these guidance values.

- EFSA (2020a) who derived a guidance value for ∑PFOA, PFNA, PFHxS and PFOS of 0.63 ng/kg bw/day (TWI = 4.4 ng/kg bw per week). The critical study this was based on is Abraham et al. (2020), which is a cross-sectional study of 101 healthy 1-year old children which found statistically significant inverse associations between serum levels of PFOA, but not of PFOS, and adjusted levels of vaccine antibodies against *Haemophilus influenza* type b (r = -0.32), diphtheria (r = -0.23) and tetanus (IgG1 only) (r = -0.25). When subjects were stratified according to PFOA concentration, comparison of the highest and lowest quintiles showed that PFOA was associated with antibody levels, on a logarithmic scale, that were 86% lower for *Haemophilus influenza* type b, 53% lower for diphtheria and 54% lower for tetanus. This effect is a marker of immune response. The EFSA CONTAM Panel decided to combine its assessment on the sum of four PFAS, i.e. PFOA, PFNA, PFHxS and PFOS as these four PFAS contribute most to the levels observed in human serum, share toxicokinetic properties in humans and show similar accumulation and long half-lives (EFSA 2020a).
- US EPA (2022c, e; 2021b) who derived a DRAFT guidance value of 0.0079 ng/kg/day for PFOS based on decreased antibody titre following diphtheria vaccination in 1-year old children – also a marker of immune response - in studies

by Grandjean et al. (2012) and Budtz-Jørgensen and Grandjean (2018). It is noted the former study (Grandjean et al. 2012) was previously considered by FSANZ (2017b) but was not selected for derivation of a guidance value.

6.2.1 Abraham et al. (2020) – used by EFSA (2020a)

FSANZ (2021) recently undertook an updated review of PFAS and immunomodulation, in which they provided a critique of the Abraham et al. (2020) study. Strengths of the study, according to FSANZ (2021) included the following.

- *"Children are very close in age.*
- *The investigations of immune parameters were relatively thorough.*
- *Some other persistent organic pollutants were considered.*
- *Differences between breastfed and formula-fed children were considered.*
- *Because the samples were collected in the 1990s, higher PFAS levels were present than in more recent studies."*

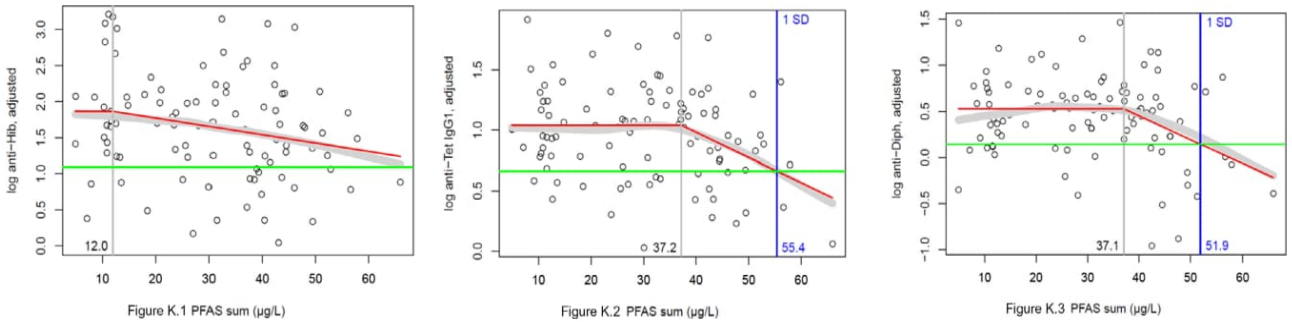
Limitations of the study, according to FSANZ (2021), included the following.

- "*The cohort size was very small, only 101 children overall.*
- *There is substantial interindividual variability in response.*
- *There is a lack of information on whether the decreases in antibody concentrations are clinically relevant. That is, PFOA may cause antibody titres to fall below effective levels sooner than they naturally would have, but if the recommended vaccine schedule is followed, antibody titres might remain sufficient to protect against disease, particularly in formula-fed infants.*
- *The question of the stability of antibodies in samples stored for decades is not addressed."*

EFSA (2020a) themselves noted similar limitations associated with use of the endpoint identified in the Abraham et al. (2020) study for guidance value development. Other submitters' comments on the draft EFSA (2020a) document, according to FSANZ (2021) included the following.

- "*The associations in the studies considered pivotal by the EFSA CONTAM Panel are weak, and cross-sectional studies cannot demonstrate causation.*
- *Vaccination response in humans is known to be highly variable, and the decline in antibodies after vaccination is not well defined but cannot be assumed to be linear.*
- *The mechanism/s by which PFAS affect the immune system are poorly understood.*
- *It is not appropriate to apply Physiologically Based Pharmacokinetic (PBPK) models validated for adults to data obtained from breastfed infants or small children.*
- *It is not appropriate to derive a TRV for adults from data from breastfed infants.*
- *Other authoritative bodies have identified different critical effects for the individual PFAS.*
- *It is clear from the available data that the potencies of the four PFAS included in the guidance value from EFSA (2020a) differ*."

In addition to the limitations identified by FSANZ (2021) and the submitters' comments on the draft EFSA (2020a) document, it becomes clear from the scatter plots of the data for combined PFAS as shown in Appendix K of the EFSA (2020a) report and reproduced in **Figure 6-2** below that there is wide spread in the data and any suggestive inverse association (as shown on the graphs with the red lines) appears to be markedly influenced by the few data points in the 50-60 µg/L serum PFAS range. Thus, the association may partially be an artefact of not having enough data in the highest quintile.



Note: Broad grey band = moving average; red line = fitted 'knee' function; horizontal green line = mean minus one standard deviation of the antibody levels below the 'knee'; vertical grey line = PFAS sum level of the 'knee'; vertical blue line = PFAS sum level of the 'knee' function with antibody levels averagely diminished by one standard deviation.

Figure 6-2 Scatter plot of levels of vaccine antibodies (K.1 *Haemophilus influenza* type b, K.2 Tetanus, K.3 Diphtheria) in relation to the sum of PFAS (PFOA, PFNA, PFHxS, and PFOS) in serum (reproduced from Appendix K in EFSA 2020a)

In the toxicological profile for PFAS from ATSDR (2021a), the agency remarks that there are sufficient epidemiological data to identify possible sensitive targets for many of the PFAS; however, there are two major limitations to establishing dose-response relationships for these effects and using the epidemiological studies to derive TRVs: i) accurate identification of environmental exposure levels producing increased risk for adverse effects (exposure estimates and routes of exposure) and ii) likely co-exposure to mixtures of PFAS. Other limitations include the cross-sectional design of the majority of epidemiological studies and the potential that reverse causality contributes to the observed associations. Although the epidemiological databases for several PFAS provide valuable information on hazard identification, ATSDR (2021a) considered the uncertainties regarding doses associated with adverse effects and possible interactions between compounds preclude use of these data to derive TRVs.

FSANZ (2021) concluded that consistent with earlier observations, the associations between PFAS blood levels and antibody titres in the Abraham et al. (2020) study as well as other studies included in the review were generally weak and partially inconsistent findings have been observed for PFOS, PFOA and other PFAS for the same antigen. It was concluded by FSANZ (2021) while these studies provide limited evidence of statistical associations, "*a causal relationship between increased PFAS blood levels and impaired vaccine response cannot be established with reasonable confidence. The evidence for an association between increasing PFAS blood levels and impaired vaccine response is insufficient for quantitative risk assessment for a number of reasons which include the following*.

- *The small number of studies and participants, and mostly cross-sectional design of studies such that conclusions around causality should be drawn with caution.*
- *Limited dose-response information with most studies investigating a narrow range of blood levels associated with background levels of PFAS exposure.*
- *Inconsistency in antibody response to vaccines between different PFAS congeners which cannot explained by study design.*
- *Potential for confounding by other known environmental immunotoxicants such as PCBs for which inverse associations with blood serum antibody concentrations against tetanus and diphtheria have previously been reported living in the Faroe islands.*

- *Uncertainty about the clinical relevance, if any, of the observed statistical associations to susceptibility to infectious disease."*

This lines up with the information in the Australian Immunisation Handbook (DHAC 2018) 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 titre response, whilst a potential marker of immune response, does not appear to be readily correlated with an adverse response *per se.*

HC (2018a) also commented on the clinical importance in humans of the endpoint under discussion. They cited a study by Grandjean et al. (2012), which, according to HC (2018a) demonstrated that despite decreased vaccine-specific immunoglobulin response in PFOS-exposed children, the number of children with immunoglobulin levels below the clinically protective level was low. They also stated that in humans, evidence of immunosuppression is inconsistent, and the influence of PFOS exposure on clinical immunosuppression (i.e. incidence of illnesses) appears to be more tenuous. Therefore, 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.

6.2.2 Budtz-Jørgensen and Grandjean (2018) – used by US EPA (2022c, e; 2021b)

The authors of the Budtz-Jørgensen and Grandjean (2018) study undertook a benchmark dose analysis on a prospective birth Faroe Islands cohort from previous studies by the same research group (Grandjean et al. 2012, 2017) on associations of serum PFAS with vaccine antibody concentrations. Grandjean et al. (2012) was previously considered / reviewed by FSANZ (2017b) in derivation of the TRVs for PFOS and PFOA. The Grandjean et al. (2017) study was also included in the FSANZ (2021) review when the agency cited a systematic literature review by Kirk et al. (2018) which was conducted as part of the PFAS Health Study in Australia.[6] The main study findings from the Kirk et al. (2018) systematic literature review with respect to immunomodulatory effects of PFAS were as follows.

- For diphtheria vaccine, there was limited evidence for an association between PFOA, PFOS, PFHxS and PFDA, noting that three of the four papers reviewed by Kirk et al. (2018) were on the same cohort in the Faroe Islands.

[6] The PFAS Health Study was commissioned by the Australian Government and was undertaken by the National Centre for Epidemiology and Population Health at the Australian National University (ANU) (https://nceph.anu.edu.au/research/projects/pfas-health-study). The study investigated the exposure levels and potential health effects of PFAS in areas of known contamination in the communities of Williamtown in New South Wales, Oakey in Queensland, and Katherine in the Northern Territory, Australia. Areas in these places have been contaminated with PFAS due to firefighting activities on nearby Defence Force bases. The study found that blood levels of PFOS and PFHxS were elevated in the exposed communities compared to comparison communities. It also found that there were higher levels of psychological distress among people in exposed communities. Higher PFAS levels in blood were associated with higher blood cholesterol in people from Williamtown, and higher uric acid levels in people from Williamtown and Katherine. The effects are small and unlikely to lead to poor health outcomes (ANU 2022). The study found no association with the other health outcomes investigated. The PFAS Health study also found that main risk factors for elevated blood concentrations of PFAS were the length of residence in an exposed community, at least weekly consumption of bore water or certain locally grown foods, and occupational exposure to firefighting foams.

- For response to rubella vaccine, the evidence for an association was limited for PFOA and PFOS, and inadequate for PFHxS and PFNA.
- For all other vaccines (tetanus, measles, mumps and influenza), the evidence for an association was inadequate.
- With regard to associations between PFAS exposure and adverse health outcomes, the evidence for all health outcomes considered (hospitalisations due to infection, middle ear infection, gastroenteritis and colds/influenza) was inadequate.
- The evidence for adverse effects of PFAS on all allergy and hypersensitivity endpoints, including asthma, allergies (including food allergies), plant sensitivity, shrimp allergy, cockroach sensitivity, mould sensitivity, allergic rhinoconjunctivitis, wheezing and eczema, was inadequate.

Many of the same comments made in **Section 6.2.1** also apply to the use of this study for guidance value derivation.

6.3 Summary and Conclusion for PFOS

Although ten health-based guidance values for potential adoption/adaptation were sourced from international jurisdictions reviewed for this report, only two of these used data in the derivation that had previously not been considered / evaluated by FSANZ (2017b).

These were the EFSA (2020a) and US EPA (2022c, e; 2021b) guidance values for PFOS, which used two studies to underpin the derivation that had not been previously considered / evaluated by FSANZ (2017b), i.e. Abraham et al. (2020) and Budtz-Jørgensen and Grandjean (2018).

Based on a brief critical evaluation of the two studies, consistent with the conclusions made by FSANZ (2021), it is concluded that a causal relationship between increased PFAS serum levels and impaired vaccine response cannot be established with reasonable confidence from the available human epidemiological information. The evidence for an association between increasing PFAS serum levels and impaired vaccine response is insufficient for the endpoint to be used for derivation of PFOS TRVs.

It is therefore concluded the current Australian guidance value for PFOS of 20 ng/kg/day and DWG value for PFOS + PFHxS of 70 ng/L are still appropriate. The derivation of these values is briefly shown in **Table 6-1** below.

Table 6-1 Derivation of current Australian drinking water guideline value (ng/L) for PFOS (NHMRC and NRMMC 2011; FSANZ 2017b; DOH 2017)

Parameter	FSANZ 2017b, NHMRC and NRMMC 2011, DOH 2017
Critical study	Luebker et al. 2005b
Study population	Rats
Form of PFOS studied	Potassium PFOS
Exposure route	Oral (gavage)
Study timeframe	Two-generation study (i.e. female F0 rats dosed 42 days prior mating, throughout mating, and day 9 of presumed gestation for rats assigned to caesarean-sectioning, or lactation day 20 for rats assigned to natural delivery; upon weaning, daily dosing of F1 pups began on lactation day 22 for subsequent mating).

Parameter		FSANZ 2017b, NHMRC and NRMMC 2011, DOH 2017
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).
Dose Point of Departure (mg/kg/day)		0.1
Point of Departure human equivalent dose (HED) (mg/kg/day)		0.00051
Uncertainty factors	UF_A	3
	UF_H	10
	$UF_{timeframe}$	1
	$UF_{database}$	1
	$UF_{composite}$	30
Health-based guidance value (ng/kg/day)		20 (rounded up from 17)
Relative source contribution (RSC) to drinking water		0.1
Resulting Health-based DWG(1) (ng/L)		70

DWG = Drinking Water Guideline; HED = Human Equivalent Dose; UF_A = Uncertainty factor for extrapolation from animals to humans; UF_H = Uncertainty factor for human variability; $UF_{timeframe}$ = Uncertainty factor for use of a short-term study; $UF_{composite}$ = Composite (i.e. total) uncertainty factor; $UF_{database}$ = Uncertainty factor to account for the limited database of toxicological studies.

(1) NHMRC and NRMMC (2011) followed the default assumptions for derivation of DWGs in Australia using the following equation:

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

Concentrations of PFOS in uncontaminated distributed drinking water in Australia can range up to 6 ng/L in Queensland (QAEHS 2018a, 2018b)[7] and Sydney (Sydney Water 2023) but up to 16 ng/L in Australia according to WHO (2022). PFOS+PFHxS concentration was found to be at 90% of the Australian DWG (i.e. ~60 ng/L) in one bore in a drinking water borefield supplying Esperance, Western Australia (WCWA 2019, 2020). Once this apparent PFOS/PFHxS contamination was identified, this bore was no longer used (WCWA 2023). Thus, PFOS is unlikely to present a human health risk from uncontaminated 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 existing Australian DWG (and therefore also the candidate DWG suggested in this report) in these cases.

7.0 Discussion for PFHxS

This section provides a discussion of the strengths and limitations of the identified guidance values for PFHxS for possible adoption/adaptation into the Guidelines. Critical evaluation

[7] Note the Queensland data is for raw water catchments.

was focused on those guidance values derived using underpinning studies not previously considered / evaluated by FSANZ (2017b).

7.1 Potential suitability of health-based guidance values for possible adoption/adaptation

Candidate guidance values for PFHxS described in **Section 4.1** for possible adoption/adaptation in Australia have also been evaluated using the Assessment Tool provided in Appendix D in the Technical Report and already described in **Section 6.1** for PFOS.

Figure 7-1 presents the percentage of criteria (combined technical and administrative criteria) met by each jurisdiction. It is evident from the figure that several publications met similar percentages of criteria, with ATSDR (2021a), EFSA (2020a), FSANZ (2017b), OEHHA (2022a), and US EPA (2023) all meeting relatively high (i.e. ~>60%) proportions of 'must-have' and 'should-have' criteria.

Other jurisdictions (MDH 2020b, MPART 2019a) met lower proportions of criteria, indicating these guidance documents potentially do not conform with modern methods of undertaking systematic reviews.

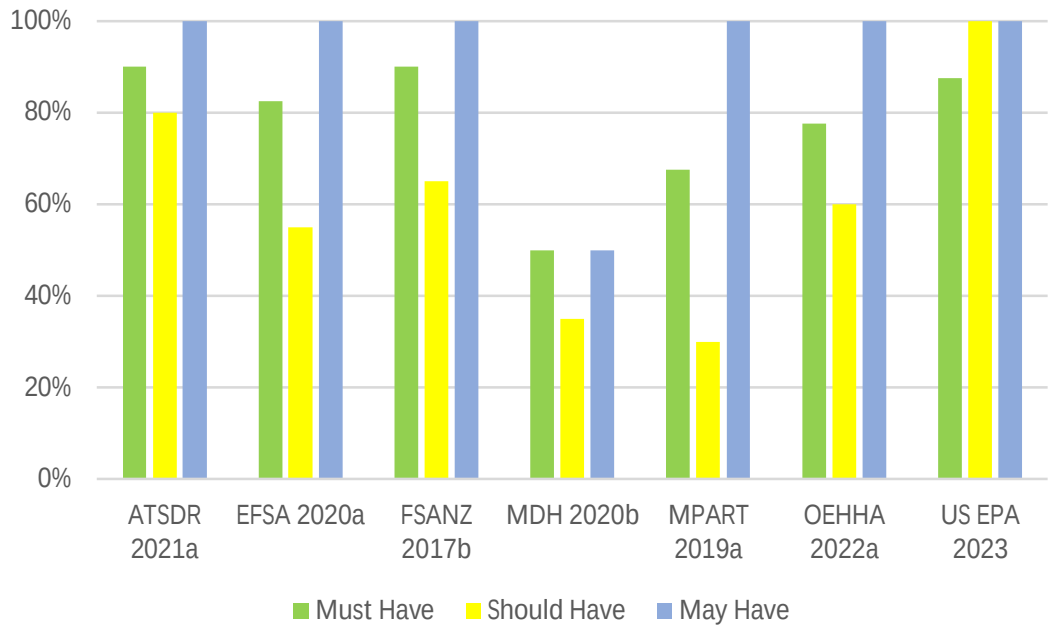


Figure 7-1 Overall proportion of 'must-have', 'should-have' and 'may-have' technical/administrative criteria as per the Assessment Tool met by jurisdictions who have derived candidate guidance values for PFHxS for possible adoption/adaptation in Australia

7.2 Critical evaluation of PFHxS guidance values based on underpinning studies not previously considered by FSANZ (2017b)

For PFHxS, the only guidance values identified in the literature review that based their derivations on underpinning studies not previously considered / cited in the comprehensive

review undertaken by FSANZ (2017b) are the following. The discussion in this section therefore focuses on the relevant studies underpinning these guidance values.

- EFSA (2020a) who derived a guidance value for ∑PFOA, PFNA, PFHxS and PFOS of 0.63 ng/kg bw/day (TWI = 4.4 ng/kg bw per week) for decreased antibody titre to specific vaccines in children. The critical study underpinning this guidance value has already been critically evaluated in **Section 6.2.1**.
- US EPA (2023) who derived a DRAFT guidance value of 0.0004 ng/kg/day for PFHxS based on decreased antibody titre following diphtheria vaccination in 1-year old children. The critical studies underpinning this guidance value have already been critically evaluated either by FSANZ (2017b) (in the case of Grandjean et al. 2012) or in **Section 6.2.2** (in the case of Budtz-Jørgensen and Grandjean 2018).
- Three US State jurisdictions (MDH 2020b, MPART 2019a, OEHHA 2022a) all derived a TRV of 9.7 ng/kg/day for PFHxS based on decreased thyroxine (T4) in rats. The critical study underpinning this derivation is NTP (2022)[8] which was not previously available to FSANZ (2017b) and therefore was not previously considered.

7.2.1 NTP (2022) – used by MDH (2020b), MPART (2019a), OEHHA (2022a)

NTP (2022) conducted 28-day toxicity studies in male and female Sprague Dawley rats (n=10/dose; five doses per chemical) to compare the toxicities of seven PFAS [PFBS, PFHxS potassium salt (PFHxSK), PFOS, and four carboxylates] via gavage in deionised water with 2% Tween® 80. NTP (2022) describes the results for PFBS, PFOS and PFHxSK; a companion report describes the results for the PFAS carboxylates.

Doses for the PFHxSK (>98% purity) treated animals were 0, 0.625, 1.25, 2.5, 5 and 10 mg/kg/day for males and 0, 3.12, 6.25, 12.5, 25 and 50 mg/kg/day for females administered 7 days/week for 28 days. A PPARα agonist (Wyeth-14,643) was used for qualitative comparison to the PFAS evaluated (doses 0 to 25 mg/kg/day). The studies evaluated clinical pathology, thyroid hormones, liver expression of PPARα- and constitutive androstane receptor (CAR)-related genes, liver acyl-coenzyme A oxidase enzyme activity (males only), plasma and liver (males only) PFHxS concentrations and histopathology.

All rats administered PFHxSK survived to scheduled euthanasia and there were no significant treatment-related clinical observations or effects on body weight in males or females. There were no effects on reproductive parameter indications (e.g. sperm count and motility, cyclicity, testis and epididymis weights and histopathology). Plasma concentrations of PFHxS increased with increasing dose in males and females. Although females were administered doses five times higher than those administered to males, the female plasma concentrations were about half of the male concentrations.

In PFHxSK exposed males, the following effects were observed.

- All doses: Significant decrease in free T4, total T4 and total T3 concentrations.
- ≥ 1.25 mg/kg/day: dose-related and significant increases in absolute and relative liver weights. Decreased reticulocyte counts and decreased cholesterol.
- ≥2.5 mg/kg/day: Incidence of hepatocyte hypertrophy (mild to marked) was significantly increased. Relative right adrenal gland weight in 2.5 mg/kg/day group

[8] MPART (2019a) cites this study as NTP (2018) because it was referencing study tables that preceded release of the report, but MDH (2020b) and OEHHA (2022a) cite it as NTP (2019). The 2019 NTP report has since been revised and updated in 2022 (NTP 2022). Minor revisions were made in NTP (2022) from the 2019 report version, all of which are marked up and identified in Appendix F of the NTP (2022) report.

and absolute and relative weights in 5 and 10 mg/kg/day groups were significantly lower. Biological significance of the adrenal gland weight increases are not clear. Decreased triglycerides.

- 10 mg/kg/day: Increased relative right kidney weight. Decreased globulin, resulting in an increase in albumin:globulin ratio.

In females, the following effects were observed.

- All doses: dose-related and significant increases in absolute and relative liver weights.
- ≥6.25 mg/kg/day: Decreased total T4.
- ≥12.5 mg/kg/day: Absolute right adrenal gland weights increased (and relative increased at 50 mg/kg/day). Biological significance of the adrenal gland weight increases are not clear. Decreased free T4.
- 50 mg/kg/day: Incidences of olfactory epithelium degeneration and olfactory epithelium hyperplasia significantly increased. There was also an increase in the incidence of olfactory epithelium inflammation suppurative in this group. These changes were primarily minimal to mild in severity.

In general, the effects in male and female rats administered PFHxSK were of lower magnitude (e.g. liver or clinical pathology findings) or not apparent compared to the effects in rats exposed to PFBS and PFOS. This corresponded, to some degree, with limited to no increases in liver *Acox1* and *Cyp* gene expression changes. Several of the effects observed in the liver were also observed in rats administered Wyeth-14,643, but effects observed outside the liver by the PFAS were not observed with Wyeth-14,643. This indicates that the liver effects are potentially not relevant to humans but relevance of effects in other organ systems cannot be discounted.

Mean plasma concentrations of PFHxS in treated male rats ranged from 66,760 to 198,300 ng/mL, whereas in females they ranged from 37,030 to 95,510 ng/mL.

Changes in thyroid hormone concentrations were observed across three PFAS (PFHxSK, PFBS and PFOS). Total T4, free T4 and total T3 largely decreased in a dose-dependent manner. The magnitude of the effect was stronger in PFBS and PFOS rats compared to PFHxSK rats. Thyroid stimulating hormone (TSH) concentrations were not consistently increased across the three chemicals or sexes in response to the lower thyroid hormone levels, nor were there any histopathological changes in the thyroid gland (e.g. hyperplasia or hypertrophy). 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 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. This explanation is plausible for studies in monkeys; however it is less plausible for studies in rodents given that these species have low levels of plasma thyroid shepherding proteins such as thyroid binding globulin.

Nevertheless, 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 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. This explanation is plausible in rodents. However, it is not plausible in primates where plasma clearance of T4 and T3 is primarily via diodinases and not thyroxine-UDP glucuronosyltransferase. 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 in PFHxSK dosed rats. It could therefore be argued that the decreased T4 and T3 observed in rats administered PFHxSK in the NTP (2022) study may not be relevant to humans.

However, in a reproductive/developmental toxicity study with PFHxS in rats (Butenhoff et al. 2009), effects on thyroid histopathology were indeed observed but these effects occurred at 4-5 times higher serum PFHxS concentrations than found to have resulted in decreased T4 and T3 levels in the NTP (2022) study. In addition, no chronic toxicity study has been conducted with PFHxS which could be used to determine whether the effects observed on thyroid hormone levels in the 28-day study are likely repeatable and relevant to humans, e.g. whether in a chronic study, the effects are repeatable and would be accompanied by changes in TSH or histopathological findings on the thyroid gland or pituitary. It is also noted that associations between PFAS exposure and thyroid hormone status have been observed in some human epidemiological studies, although the associations are not always consistent (e.g. Ballesteros et al. 2017, Boesen et al. 2020, Coperchini et al. 2021). Thus, it is concluded that potential human relevancy of the thyroid hormone changes observed in the 28-day NTP (2022) study with PFHxS cannot be discounted based on currently available information.

Because the NTP (2022) study was conducted in accordance with relevant standardised testing guidelines, evaluated a large number of endpoints, and provided serum PFHxS concentrations, it is concluded to be appropriate new information to potentially adopt/adapt for derivation of candidate guidance/guideline values for PFHxS. The candidate guidance/guideline values are summarised in **Section 7.3**.

7.3 Candidate guidance/guideline values for PFHxS

As indicated in **Section 7.2.1**, the NTP (2022) study represents new suitable information that was not previously available to FSANZ (2017b) when considering derivation of a guidance value for PFHxS, noting the uncertainty with respect to human relevancy of the effect based on currently available information and the potential conservatism in any resulting guidance value. The study has been used by three jurisdictions (MDH 2020b, MPART 2019a, OEHHA 2022a) to derive a guidance value for PFHxS, one of which (OEHHA 2022a) also met a high proportion of technical/administrative criteria for potential adoption/adaptation into the Guidelines (**Section 7.1**).

The three jurisdictions who derived a guidance value for PFHxS using the NTP (2022) study either used a POD of 32,400 ng/mL (i.e. 32.4 mg/L) which represents a lower benchmark dose for 20% reduced T4 in male rats (i.e. $BMDL_{20}$) (MDH 2020b, MPART 2019a) or 28,600 ng/mL (i.e. 28.6 mg/L) which represents a lower benchmark dose for one standard deviation difference from controls ($BMDL_{1SD}$) for the same effect (OEHHA 2022a). There is very little difference between these two PODs.

To derive a human POD from the animal POD, the three jurisdictions derived a similar human clearance value / toxicokinetic adjustment factor[9] (i.e. 0.000085-0.00009 L/kg-day). This resulted in very similar human equivalent dose (HED) PODs of 0.00243 to 0.00292 mg/kg/day. The jurisdictions then applied different uncertainty factors (300 or 1,000) to their HED POD (see **Table 7-1**). The difference is primarily due to OEHHA (2022a) deciding to apply an additional uncertainty factor of 10 for the use of a sub-chronic study.

However, it is noted that a reproductive/developmental toxicity study with PFHxS in rats (Butenhoff et al. 2009) was summarised by FSANZ (2017b), in which the NOAEL (for paternal toxicity) in male rats was stated to be 3 mg/kg/day (for offspring toxicity, it was higher at 10 mg/kg/day). The serum concentration for paternal males at day 42 of the study was 128,670 ng/mL, with some effects on thyroid histopathology (hypertrophy and hyperplasia of the follicular cells) noted in males at this serum concentration. The serum concentration in males at the lower dose of 1 mg/kg/day on day 42 was 89,120 ng/mL. This indicates that histopathological effects in the thyroid are only likely to manifest at higher serum concentrations, i.e. 4-5 times higher, than decreased T4 in male rats. The half-life of PFHxS in male rats (i.e. 3.6-15.9 days or ~10 days, Benskin et al. 2009) suggests that serum PFHxS in these rats was likely at steady state. Thus, the use of an uncertainty factor for use of subchronic study (in addition to the database uncertainty factor) is unlikely to be warranted. The database uncertainty factor is likely to already account for use of a subchronic study, since the former is applied for lack of chronic toxicity studies. It is therefore suggested an overall composite uncertainty factor of 300 rather than 1,000 is likely sufficient and still provides a conservative guidance value.

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 all jurisdictions which have derived DWGs in the literature consulted applied an RSC of 0.2 (i.e. 20%) (e.g. MDH 2020b, OEHHA 2022a) but do not provide the rationale for this. Thus, the default factor of 0.1 has been retained in calculating the potential resulting DWGs for PFHxS using these guidance values in **Table 7-1**, noting that it yields a lower guideline value than use of an RSC of 0.2. Also presented in **Table 7-1** is the derivation of the current Australian DWG for PFOS + PFHxS of 70 ng/L, which is based on a toxicology study for PFOS.

Table 7-1 Potential drinking water guideline values (ng/L) resulting from adaptation of PFHxS guidance values from different jurisdictions based on NTP (2022) critical study as well as current Australian drinking water guideline for PFOS + PFHxS

Parameter	NHMRC and NRMMC 2011, FSANZ 2017b, DOH 2017	MDH 2022b	MPART 2019a	OEHHA 2022a
Critical study	Luebker et al. 2005b	NTP 2022		

[9] i) MDH (2020b) derived the toxicokinetic adjustment factor as follows: Clearance rate = Volume of Distribution (L/kg) x (Ln2/Half-life, days); Clearance rate = 0.25 L/kg x (0.693/1935 days); Clearance rate = 0.00009 L/kg-day
ii) MPART (2019a) used the same toxicokinetic adjustment factor as MDH (2020b).
iii) OEHHA (2022) derived a very similar clearance factor of 0.000085 L/kg-day.

Parameter		NHMRC and NRMMC 2011, FSANZ 2017b, DOH 2017	MDH 2022b	MPART 2019a	OEHHA 2022a
Study population		Rats	Rats		
Form of PFHxS studied		Potassium PFHxS	PFHxSK		
Exposure route		Oral (gavage)	Oral (gavage)		
Study timeframe		2-generation study	28 days		
Critical Effect		↓ BW gain & food consumption in F0 (parental); ↓ pup weight & weight gain during lactation (offspring).	Decreased T4 in male rats		
Serum Point of Departure (mg/L)		0.1 mg/kg/day (dose POD)	$BMDL_{20}$ = 32.4	$BMDL_{20}$ = 32.4	$BMDL_{1SD}$ = 28.6
Clearance Factor (L/kg-day)		-	0.00009	0.00009	0.000085
Point of Departure HED (mg/kg/day)		0.00051	0.00292	0.00292	0.00243
Uncertainty factors	UF_A	3	3	3	√10
	UF_H	10	10	10	10
	$UF_{timeframe}$	1	1	1	10
	$UF_{database}$	1	10	10	√10
	$UF_{composite}$	30	300	300	1,000
Health-based guidance value (ng/kg/day)		20 (rounded up from 17)	9.7	9.7	2.4
Relative source contribution (RSC) to drinking water		0.1	0.1	0.1	0.1
Resulting adaptation to a Health-based DWG(1) (ng/L)		70 (sum of PFOS +PFHxS)	34	34	8.5

DWG = Drinking Water Guideline; ↓ = Decreased; BW = Body weight; F0 = Parental generation; POD = Point of Departure; BMDL = Lower Benchmark Dose; HED = Human Equivalent Dose; UF_A = Uncertainty factor for extrapolation from animals to humans; UF_H = Uncertainty factor for human variability; $UF_{timeframe}$ = Uncertainty factor for use of a short-term study; $UF_{composite}$ = Composite (i.e. total) uncertainty factor; $UF_{database}$ = Uncertainty factor to account for the limited database of toxicological studies (e.g. no two-generation or immunotoxicity studies).

Parameter	NHMRC and NRMMC 2011, FSANZ 2017b, DOH 2017	MDH 2022b	MPART 2019a	OEHHA 2022a
(1) Adaptation 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				

The candidate PFHxS DWGs derived by adapting existing guidance values for this PFAS are 8.5 ng/L using the uncertainty factors from OEHHA (2022a) or 34 ng/L using the uncertainty factors from MDH (2020b) and MPART (2019a); as discussed in the text preceding the table, the difference between the two values is the application of an additional uncertainty factor. The value of 34 ng/L is considered to be more appropriate based on the reasons cited above the table, noting the uncertainty and likely conservatism with respect to human relevancy of the selected endpoint based on currently available information.

Assuming the recommendation in **Section 6.3** for PFOS is accepted, it is noted that, in accordance with enHealth (2016) guidance and current practice in Australia, it is considered reasonable to retain the existing guideline value of 70 ng/L as the sum of PFOS+PFHxS when evaluating concentrations in drinking water in addition to comparison of PFHxS concentrations on their own with the suggested candidate guideline value of 34 ng/L.

In Australian distributed drinking waters, PFHxS concentrations generally may range from <2 to 5 ng/L in Queensland (QAEHS 2018a, 2018b)[10], Sydney (Sydney Water 2023) and Western Australia (WCWA 2023) which are below both candidate DWGs. However, PFOS + PFHxS concentration was measured at 90% of the current Australian DWG (i.e. ~ 60 ng/L) in one bore in a drinking water borefield supplying Esperance, Western Australia (WCWA 2019, 2020). Once this apparent PFOS/PFHxS contamination was identified, this bore was no longer used (WCWA, 2023). This indicates that compliance with the candidate DWGs may present an issue in certain circumstances. Nevertheless, due to the large uncertainty factors, likely conservatism of the selected endpoint with respect to PFHxS, and small RSC incorporated into the derivation of the candidate DWGs, PFHxS 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), PFHxS may be present at concentrations above the candidate DWG and the existing Australian DWG in these cases.

8.0 Discussion for PFBS

This section provides a discussion of the strengths and limitations of the identified guidance values for PFBS for possible adoption/adaptation into the Guidelines.

8.1 Potential suitability of health-based guidance values for possible adoption/adaptation

Candidate guidance values for PFBS described in **Section 4.1** for possible adoption/adaptation in Australia have also been evaluated using the Assessment Tool provided in Appendix D in the Technical Report and already described in **Section 6.1** for PFOS.

[10] Note the Queensland data are for raw water catchments.

Figure 8-1 presents the percentage of criteria (combined technical and administrative criteria) met by each jurisdiction. It is evident from the figure that the highest percentage of 'must-have' and 'should-have' criteria were met by US EPA (2021c), followed by OEHHA (2021c), MPART (2019a) and then MDH (2022g).

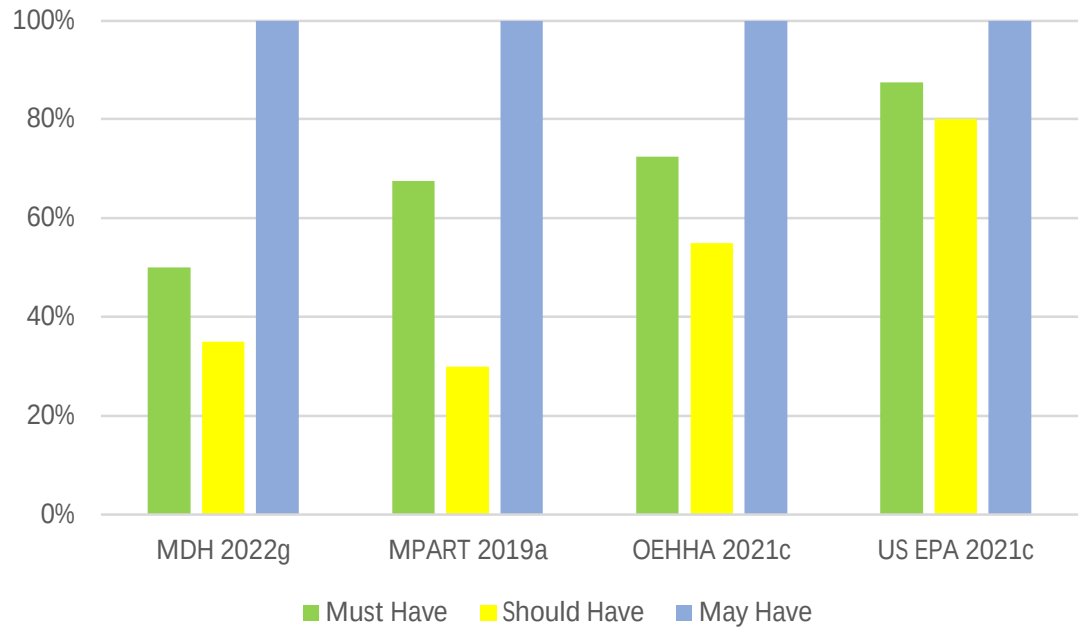


Figure 8-1 Overall proportion of 'must-have', 'should-have' and 'may-have' technical/administrative criteria as per the Assessment Tool met by jurisdictions who have derived candidate guidance values for PFBS for possible adoption/adaptation in Australia

8.2 Critical evaluation of PFBS guidance values

As PFBS was not part of the comprehensive review undertaken by FSANZ (2017b), all guidance values sourced in the literature search for which the derivation was described were evaluated in this section. These include the following.

- 84 ng/kg/day (MDH 2022e, g) (decreased total thyroxine (T4) in rats; critical study: NTP 2022).
- 300 ng/kg/day (MPART 2019a, US EPA 2022c, k; 2021c; WSDH 2019a, 2023a, 2022b) (decreased total thyroxine (T4) in mice; critical study: Feng et al. 2017).
- 600 ng/kg/day (OEHHA 2021d) (decreased total thyroxine (T4) in mice; critical study: Feng et al. 2017).

All jurisdictions have agreed that the most sensitive health endpoint is decreased total thyroxine (T4) in rats or mice. The critical studies underpinning the derivations of the three different guidance values are NTP (2022)[11] and Feng et al. (2017).

[11] MDH (2022g) cites this study as NTP (2019). The 2019 NTP report has since been revised and updated in 2022 (NTP 2022). Minor revisions were made in NTP (2022) from the 2019 report version, all of which are marked up and identified in Appendix F of the NTP (2022) report.

8.2.1 NTP (2022) – used by MDH (2022e, g)

As described in **Section 7.2.1** for PFHxS, NTP (2022) conducted 28-day toxicity studies in male and female Sprague Dawley rats (n=10/dose; five doses per chemical) to compare the toxicities of seven PFAS [PFBS, PFHxSK, PFOS, and four carboxylates] via gavage in deionised water with 2% Tween® 80. NTP (2022) describe the results for PFBS, PFOS and PFHxSK; a companion report describes the results for the PFAS carboxylates.

Doses for the PFBS (>97% purity) treated animals were 0, 62.6, 125, 250, 500 and 1,000 mg/kg/day for both males and females administered 7 days/week for 28 days. A PPARα agonist (Wyeth-14,643) was used for qualitative comparison to the PFAS evaluated doses (0, 6.25, 12.5, or 25 mg/kg/day). The studies evaluated clinical pathology, thyroid hormones, liver expression of PPARα- and constitutive androstane receptor (CAR)-related genes, liver acyl-Coenzyme A oxidase enzyme activity (males only), plasma and liver (males only) PFHxS concentrations and histopathology.

NTP (2022) cites other studies which have shown the half-lives of PFBS after oral administration of 30 mg PFBS/kg in Sprague Dawley rats to be 4.7 and 7.4 hours in males and females, respectively; in humans, a geometric mean half-life of 25.8 days has been estimated.

In PFBS exposed males, the following effects were observed.

- All dose groups: Decreased total protein, due to decreases in globulin, which resulted in increases in albumin/globulin ratio. Decreased cholesterol. Decreased total T4, free T4 and total triiodothyronine (T3) concentrations. TSH levels were unchanged.
- ≥ 62.6 mg/kg/day: Dose-related and significant increases in relative liver weights.
- ≥125 mg/kg/day: Dose-related and significant increases in absolute and relative liver weights (except in 1,000 mg/kg/day group). Mild significant decreases in male rat erythron, characterised by decreased haematocrit, haemoglobin and erythrocyte and reticulocyte counts. Increased incidence of hepatocyte hypertrophy.
- 250 mg/kg/day: Mild significant increases in blood urea nitrogen (BUN) concentrations at this dose and 500 mg/kg/day dose, consistent with decreased water intake (i.e. dehydration). Significantly increased incidence of olfactory epithelium degeneration and olfactory epithelium hyperplasia.
- 500 mg/kg/day: Decreased absolute and relative heart and thymus weight. Increased absolute and relative kidney weights. The biological significance of these changes is not clear. Decreased triglycerides at this dose. Increased ALT, alkaline phosphatase (ALP), and aspartate aminotransferase (AST) activity. Increased sorbitol dehydrogenase (SDH) activity. Increased total bile acid concentrations. Significantly increased hepatocyte cytoplasmic alteration. Significantly increased incidence of olfactory epithelium degeneration and olfactory epithelium hyperplasia.
- 1,000 mg/kg/day: Nine of the ten rats in this group died from day 15 to day 25 and one due to a dosing accident on day 6. Seizure recorded in one male. Body weight was 17% and 19% reduced from controls at 15 and 22 days, respectively. Significantly increased hepatocyte cytoplasmic alteration. One male had hepatocyte necrosis. Increased incidence of mild to marked bone marrow hypocellularity. Significantly increased incidence of olfactory epithelium degeneration and olfactory epithelium hyperplasia. Significantly increased incidence of minimal to mild epithelium hyperplasia in forestomach. Significantly increased incidence of mild to marked thymus atrophy. One male had kidney papilla necrosis.

In females, the following effects were observed.

- All doses: Dose-related and significant increases in relative right kidney weights. Decreased total T4, free T4 and total T3 concentrations. TSH levels were unchanged.
- ≥125 mg/kg/day: Dose-related and significant increases in relative liver weight. Decreased reticulocyte counts. Significantly increased incidence of olfactory epithelium degeneration and olfactory epithelium hyperplasia (the latter from 250 mg/kg/d).
- ≥250 mg/kg/day: One rat died on day 25. Seizures recorded in one female at this dose. Dose-related and significant increases in relative and absolute liver weight. Increased total bile acid concentrations.
- 500 mg/kg/day: One rat died on day 21. Seizures recorded in two females at this dose. Decreased absolute spleen, heart and thymus weights. The biological significance of the latter changes is not clear. Decreased cholesterol at this dose. Increased ALT, ALP, and AST activity. Increased incidence of hepatocyte hypertrophy. Significantly increased hepatocyte cytoplasmic alteration.
- 1,000 mg/kg/day: Eight rats died from day 16 to 27. Seizures recorded in one female at this dose. One rat was lethargic, two had ruffled fur and two were thin. Mean body weight reduced by 14% compared to controls. Increased incidence of hepatocyte hypertrophy. Significantly increased hepatocyte cytoplasmic alteration. Increased minimal hepatocyte necrosis. Increased incidence of mild to marked bone marrow hypocellularity. Significantly increased incidence of mild to marked thymus atrophy. Significantly increased incidence of minimal to mild kidney papilla necrosis.

Except for the deaths related to the dosing accident, all other deaths were considered treatment-related but the cause undetermined. The cause of the seizures was unknown, and they were not repetitive. There was no clinical pathology interpretation in the groups administered the highest dose tested due to the high mortality in these groups.

Plasma concentrations of PFBS increased with increasing dose in both males and females, with males generally having higher (5- to 18-fold) plasma concentrations compared with females across all dose groups.

Male and female rats administered PFBS exhibited a significant increase in expression of *Acox1, Cyp4a1, Cyp2b1,* and *Cyp2b2* compared to controls, indicating significant increased PPARα and CAR activity. Males displayed a greater fold increase in PPARα-related gene expression compared to controls than females, whereas expression of CAR-related genes were more prominent in female rats.

The testicular spermatid count in the 250 mg/kg/day males was lower (10%) than the vehicle control group. When normalised to total testicular weight, counts in the 250 and 500 mg/kg/day groups were 12% and 10% lower, respectively, than the vehicle control group. These differences did not attain statistical significance, but the trend was significant. Left testis and left epididymis weights were not affected by PFBS administration. The histopathologic finding of germinal epithelium degeneration in the testis was noted in one male in the 1,000 mg/kg/day group (sperm assessments were not made in this group due to early mortality). Serum testosterone levels assessed at necropsy in dosed males were similar to the vehicle control group level. Females administered 250 or 500 mg/kg/day PFBS displayed alteration in the oestrous cycle (extended diestrus in the 250 mg/kg/day females, irregular or not cycling in the 500 mg/kg/day females).

Several of the effects observed in the liver were also observed in rats administered Wyeth-14,643, but effects observed outside the liver with PFAS administration were not observed with Wyeth-14,643. This indicates that the liver effects are potentially not relevant to humans but relevance of effects in other organ systems cannot be discounted.

Mean plasma concentrations of PFBS in treated male rats ranged from 2,222 to 43,160 ng/mL (the latter in the 500 mg/kg/day dose group), whereas in females they ranged from 154 to 24,455 ng/mL. However, it is unclear from the study at which time-point post-administration of the final dose these plasma concentrations were measured. This is important for PFBS; due to the relatively short half-life of PFBS in rats (i.e. 4.7-7.4 hours), depending on when samples were collected for analysis, plasma concentrations shortly after administration may have been 2-3 times higher than reported in the study.

MDH (2022e, g) considered the decreased total T4 observed at all doses in female rats to be the critical adverse endpoint and derived a POD as a $BMDL_{1SD}$ of 6.97 mg/kg/day for this effect. They derived a chemical-specific toxicokinetic adjustment factor of 0.0012 representing the difference in half-lives between female rats (1.3 hours) and humans (1050 hours), i.e. 1.3 h ÷ 1050 h = 0.0012. MDH (2022e, g) then derived a HED by multiplying the POD by the toxicokinetic adjustment factor (6.97 mg/kg/day x 0.0012 = 0.0084 mg/kg/day) and dividing this dose by an uncertainty factor of 100 (3x for interspecies differences in toxicodynamics; 10x for intraspecies variability; 3x for database uncertainty due to lack of immunotoxicity, developmental neurotoxicity studies, or 2-generation toxicity study) resulting in a TRV of 84 ng/kg/day. It is noted that if the half-lives cited in the NTP (2022) study (7.4 h in female rats, 619 h in humans) were used instead, the toxicokinetic adjustment factor would change to 0.012 (an order of magnitude difference), and if all other considerations remained the same, this would change the TRV to 833 ng/kg/day (one order of magnitude higher). This highlights the sensitivity of the value of the TRV to the appropriate half-life information.

Similar to the discussion for PFHxS in **Section 7.2.1** with respect to this study, the decreased T4 and T3 observed in the NTP (2022) study in rats administered PFBS was not accompanied by increased TSH or thyroid histopathological findings. This indicates there is uncertainty with respect to the human relevancy of the effect based on currently available information. Nevertheless, it is noted that a developmental/reproductive toxicity study in mice by Feng et al. (2017), described in **Section 8.2.2** below, also found decreased T3 and T4 levels at postnatal day 30 which were accompanied by slight but statistically increased serum TSH. In addition, the human epidemiological literature has found associations between PFAS exposure and thyroid hormone changes (see **Section 7.2.1**), albeit these associations were not always consistent. As there is a lack of chronic toxicity studies with PFBS (similar to PFHxS), and the Feng et al. (2017) study found increased TSH accompanied the decreased T3 and T4 levels, it is concluded that the potential human relevancy of this effect for PFBS cannot be discounted based on currently available information.

It is noted that OEHHA (2021d) did not use the NTP (2022) study in rats for their POD when deriving a guidance value because of the large toxicokinetic differences between female rats and humans, and the uncertainty around the utility of the rat model for effects in humans of maternal thyroid hormone disruption of foetal development.

Because the NTP (2022) study was conducted in accordance with relevant standardised testing guidelines and evaluated a large number of endpoints, it is concluded to be appropriate information to potentially adopt/adapt for derivation of candidate guidance/guideline values for PFBS. The candidate guidance/guideline values are summarised in **Section 8.3**.

8.2.2 Feng et al. (2017) – used by MPART (2019a), US EPA (2022c, k; 2021c), WSDH (2019a, 2023a, 2022b), OEHHA (2021d)

Feng et al. (2017) investigated the influence of gavage exposure to K^+ PFBS (98% purity) (0, 50, 200 or 500 mg/kg/day) in 0.1% carboxymethylcellulose during gestation days 1 to 20 on perinatal growth and development, pubertal onset, and reproductive and thyroid function in

female ICR mice. On postnatal day (PND) 21, all offspring were weaned. Female offspring were transferred to other cages (2-4 per cage). Thirty dams in each dose group were randomly assigned to one of the following three experimental groups: i) group 1, in which perinatal survival and growth, pubertal onset, and ovarian and uterine development were sequentially examined in the same cohorts (50 offspring/10 dams); ii) group 2, in which hypothalamic–pituitary–gonadal hormone and hypothalamic–pituitary–thyroid hormone levels were measured in PND1 offspring (n = 30), PND30 offspring (n = 10), and PND60 offspring (n = 10) obtained from 10 dams; and iii) group 3, in which the levels of serum PFBS were measured (n = 10 dams).

The weight gain of the dams was not different between the different treatment groups. Dams did not exhibit foetal loss or abnormal behaviour during the administration of PFBS.

Number of neonatal PFBS-offspring were not significantly different from that in the control group. All offspring appeared to be active and survived until adulthood. The potentially treatment-related findings in the study were as follows.

- ≥ 200 mg/kg/day:
    - Body weights of PND1 female PFBS-offspring were significantly lower compared to controls. These offspring remained underweight throughout weaning, pubertal and adult periods.
    - Slight but statistically significant delay (approximately 1.5-2 days) in eye opening, delay in vaginal opening, and delay in first oestrous (of up to 5 days) observed in treated offspring compared with control offspring ($p<0.01$). Size of the ovaries of PND60 treated offspring were smaller than those of controls and relative weights were lower ($p<0.05$). PFBS treated offspring at these doses exhibited fewer primordial follicles, primary follicles, secondary follicles, early antral follicles, antral follicles and pre-ovulatory follicles, as well as fewer corpora lutea ($p<0.05$) than controls at diestrus. PND40-60 offspring in these dose groups exhibited a prolongation of diestrus compared with controls ($p<0.05$) with reduced serum E2 levels ($p<0.05$) in PND30 and PND60 offspring, a slight increase in luteinising hormone level in the PND30 offspring only, but no difference in hypothalamic gonadotropin-releasing hormone compared to controls.
    - PND1, PND30 and PND60 offspring in these groups exhibited significantly reduced serum total T3 and T4 levels compared with controls, with the reduction in total T4 lower at PND60 (23%) compared with PND30 (42%). In addition, PND30 offspring in these dose groups showed slight but statistically significant elevations in serum thyroid stimulating hormone (TSH) ($p<0.05$).
    - PFBS treated dams in these dose groups exhibited statistically significantly reduced total T4 ($p<0.05$), total T3 ($p<0.05$) and free T4 ($p<0.05$), as well as increased TSH ($p<0.05$).

The information summarised above indicates a PFBS dose of 50 mg/kg/day was the NOAEL in this study.

Serum PFBS in treated pregnant mice (collected 12 hours after the last administered dose) were 1.73 ± 0.65 ng/mL, 74.01 ± 22.52 ng/mL, 332.41 ± 53.04 ng/mL and 720.86 ± 98.4 ng/mL in the 0, 50, 200, and 500 mg/kg/day groups, respectively. Half-lives of PFBS in male and female CD-1 mice have been reported at 5.8 h in males and 4.5 h in females (Lau et al. 2020) and in humans 619 h (as per NTP 2022). Since serum collection in the Feng et al. (2017) study occurred 12 hours after the last administered dose, serum concentrations in dams are likely to have been 2.7x higher (i.e. ~200 ng/mL at NOAEL dose of 50 mg/kg/day; ~900 ng/mL at LOAEL dose of 200 mg/kg/day) directly after administration of the last dose.

The data from the Feng et al. (2017) study was used to derive TRVs by various jurisdictions as follows.

- MPART (2019a) considered the critical effect to be decreased serum total T4 in PND1 mice and derived a $BMDL_{20}$ of 28.19 mg/kg/day for this effect. They divided this $BMDL_{20}$ by a toxicokinetic adjustment factor of 316 (i.e. human serum half-life of 665 h ÷ female mouse serum half-life of 2.1 h) to derive a HED POD of 0.0892 mg/kg/day. This was divided by an uncertainty factor of 300 (10x for human variability; 3x for interspecies variability in toxicodynamics, 10x for database deficiencies due to lack of neurodevelopmental, immunotoxicological and chronic studies) to derive a TRV of 300 ng/kg/day. WSDH (2019a, 2023a) derived the same TRV in the same manner as MPART (2019a).
- OEHHA (2021d) considered both the NTP (2022) and Feng et al. (2017) studies for deriving a TRV but decided against using the NTP (2022) study because of the large toxicokinetic differences between female rats and humans, and uncertainty around the utility of the rat model for effects in humans of maternal thyroid hormone disruption on foetal development. They derived a similar POD to MPART (2019a) but expressed it as a $BMDL_{1SD}$ of 22.2 mg/kg/day. They adjusted this by a clearance factor of 345 [Ratio of animal to human clearance = (0.056 L/kg/h x 1000 mL/L x 24 h/day) ÷ 3.9 mL/kg/day = 345] to derive a HED POD of 0.064 mg/kg/day. They applied an uncertainty factor of 100 (√10 for interspecies differences in toxicodynamics; 10x for human variability; √10 for database deficiencies) to derive a TRV of 600 ng/kg/day (rounded).
- US EPA (2021c, 2022c, 2022k) also agreed with the critical endpoint of decreased total T4 in newborn mice in the Feng et al. (2017) study. They derived a POD as a lower benchmark dose for half a standard deviation difference from controls ($BMDL_{0.5SD}$) of ~22.1 mg/kg/day[12] and applied a toxicokinetic adjustment factor of 0.0043 (i.e. 4.5 h in female mice ÷ 1050 h in humans) to derive a HED POD of 0.095 mg/kg/day. Note if the human half-life cited in NTP (2022) of 619 h was used, this factor would be 0.0073 (~1.7x difference). This was divided by an uncertainty factor of 300 (3x for interspecies toxicodynamics; 10x for human variability; 10x for database uncertainties) to derive a TRV of 320 ng/kg/day.

The Feng et al. (2017) study was peer reviewed, appears to have been conducted appropriately and evaluated relatively sensitive endpoints of interest (female reproductive performance and developmental effects); it is concluded to be appropriate information to potentially adopt/adapt for derivation of candidate guidance/guideline values for PFBS. The candidate guidance/guideline values are summarised in **Section 8.3**.

8.3 Candidate guidance/guideline values for PFBS

As indicated in **Section 8.2.1** and **8.2.2**, both the NTP (2022) and the Feng et al. (2017) studies represent suitable information for potential guidance value derivation for PFBS, noting the uncertainty with respect to human relevancy of the effect based on currently available information and the potential conservatism in any resulting guidance value. The studies have been used by five jurisdictions (MDH 2022e, g; MPART 2019a; OEHHA 2021d;

[12] Note SLR has estimated this $BMDL_{0.5SD}$ from the information in the US EPA (2021c) report by back-calculating from the POD HED cited in the report (0.095 mg/kg/day) and dividing by the dosimetric adjustment factor derived for female mice compared with humans (see Table 8 in US EPA 2021c). The adjustment factor was 0.0043 (i.e. 4.5 h in female mice ÷ 1050 h in humans). Note if the human half-life cited in NTP (2022) of 619 h was used, this factor would be 0.0073 (~1.7x difference).

US EPA 2021c, 2022c, 2022k; WSDH 2019a, 2023a) to derive a guidance value for PFBS, two of which (OEHHA 2021d; US EPA 2021c, 2002c, 2022k) also met a relatively high proportion of technical/administrative criteria for potential adoption/adaptation into the Guidelines (**Section 8.1**).

The four jurisdictions who derived a guidance value for PFBS using the Feng et al. (2017) study used very similar PODs ranging from 22.1 to 28.19 mg/kg/day for the same critical effect. The jurisdiction that derived a guidance value using the NTP (2022) study derived a different POD of 6.97 mg/kg/day.

To derive a human POD from the animal POD, the various jurisdictions derived human toxicokinetic adjustment factors for the difference between human half-lives and rat or mouse half-lives, depending on the study species; the factors (as the ratio of human to animal half-life) ranged from 233 to 345 for mice and 808 for the rat study. This resulted in similar HED PODs of 0.064 to 0.095 mg/kg/day for the mouse and 0.0084 mg/kg/day for the rat study; although it is noted if the half-lives cited in NTP (2022) were used the latter HED POD would be an order of magnitude higher (0.084 mg/kg/day) and fall within the range of the mouse HED PODs. The jurisdictions then applied different uncertainty factors (100 or 300) to their HED POD (see **Table 8-1**). The difference is due to application of an uncertainty factor of 3 or 10 for database uncertainties.

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 all jurisdictions which have derived DWGs in the literature consulted applied an RSC of 0.2 (i.e. 20%) (e.g. MPART 2019a, OEHHA 2021d, US EPA 2022c, k) but do not provide the rationale for this. Thus, the default factor of 0.1 has been retained in calculating the potential resulting DWGs for PFBS using these guidance values in **Table 8-1**, noting that it yields a lower guideline value than use of an RSC of 0.2.

Table 8-1 Potential drinking water guideline values (ng/L) resulting from adaptation of PFBS guidance values from different jurisdictions based on two critical studies

Parameter	MDH 2022e,g	MPART 2019a, WSDH 2019a, 2022b, 2023a	OEHHA 2021d	US EPA 2022c, k
Critical study	NTP 2022	Feng et al. 2017		
Study population	Rats	Mice		
Form of PFBS studied	PFBS	K^+ PFBS		
Exposure route	Oral (gavage)	Oral (gavage)		
Study timeframe	28 days	Dosing of pregnant animals on GD1 to 20, monitoring of offspring development until PND60		
Critical Effect	Decreased T4 in female rats	Decreased total T4 in female rat offspring on PND1		
Point of Departure (mg/kg/day)	$BMDL_{1SD}$ = 6.97	$BMDL_{20}$ = 28.19	$BMDL_{1SD}$ = 22.2	$BMDL_{0.5SD}$ = ~22.1
Toxicokinetic Adjustment Factor (human half-life ÷ animal half-life)	~808 (83) (2)	316	345	233

Parameter		MDH 2022e,g	MPART 2019a, WSDH 2019a, 2022b, 2023a	OEHHA 2021d	US EPA 2022c, k
Point of Departure HED (mg/kg/day)		0.0086 (0.084) (2)	0.0892	0.064	0.095
Uncertainty factors	UF_A	3	3	√10	3
	UF_H	10	10	10	10
	$UF_{timeframe}$	1	1	1	1
	$UF_{database}$	3	10	√10	10
	$UF_{composite}$	100	300	100	300
Health-based guidance value (ng/kg/day)		86 (840) (2)	297	643	316
Relative source contribution (RSC) to drinking water		0.1	0.1	0.1	0.1
Resulting adaptation to a Health-based DWG(1) (ng/L)		302 (2,939) (2)	1,041	2,252	1,107

DWG = Drinking Water Guideline; BMDL = Lower Benchmark Dose; HED = Human Equivalent Dose; GD = Gestation Day; PND = Postnatal Day; UF_A = Uncertainty factor for extrapolation from animals to humans; UF_H = Uncertainty factor for human variability; $UF_{timeframe}$ = Uncertainty factor for use of a short-term study; $UF_{composite}$ = Composite (i.e. total) uncertainty factor; $UF_{database}$ = Uncertainty factor to account for the limited database of toxicological studies (e.g. no two-generation or immunotoxicity studies).

(1) Adaptation 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

(2) As highlighted in the text in **Section 8.2.1**, the toxicokinetic adjustment factor is very sensitive to the input half-lives assumed for female rats and humans. If the half-lives cited by NTP (2022) of 7.4 h in female rats and 619 h in humans are used instead, the adjustment factor would decrease by a factor of 10, thereby increasing the POD HED and resulting TRV by a factor of 10. The values that would result from using the half-lives cited by NTP (2022) are provided in brackets.

The candidate PFBS DWGs derived by adapting existing guidance values for this PFAS are 302 (or 2,939) ng/L using the rat toxicology study (NTP 2022) or range from 1,041 to 2,252 ng/L using the mouse toxicology study by Feng et al. (2017). The guideline values resulting from adapting the TRV from the rat study (using the half-lives cited in NTP 2022) and the TRVs from the mouse toxicology study are within a factor of three (ranging from 1,041 to 2,939 ng/L) and are considered most applicable within the Australian context. It is reiterated that the endpoint on which these guidance values are based is of uncertain human relevance based on currently available information and therefore the resulting candidate guideline values are conservative.

In Queensland raw water catchments, PFBS concentrations have been recorded up to 2.2 ng/L (QAEHS 2018a, 2018b). There are few PFBS data in drinking water elsewhere in Australia. Based on the limited data available, it appears that PFBS concentrations in distributed drinking water in Australia are markedly lower than any of the candidate DWGs, suggesting PFBS is unlikely to present a human health risk from distributed drinking water in 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), PFBS may be present at concentrations above the candidate DWGs in these cases.

9.0 Discussion for PFOA

This section provides a discussion of the strengths and limitations of the identified guidance values for PFOA for possible adoption/adaptation into the Guidelines. Critical evaluation was focused on those guidance values derived using underpinning studies not previously considered / evaluated by FSANZ (2017b).

9.1 Potential suitability of health-based guidance values for possible adoption/adaptation

Candidate guidance values for PFOA described in **Section 4.1** for possible adoption/adaptation in Australia have also been evaluated using the Assessment Tool provided in Appendix D in the Technical Report and already described in **Section 6.1** for PFOS.

Figure 9-1 presents the percentage of criteria (combined technical and administrative criteria) met by each jurisdiction. It is evident from the figure that several publications met similar percentages of criteria, with ATSDR (2021a), EFSA (2020a), FSANZ (2017b), NJDEP (2019a), OEHHA (2023a), and US EPA (2022c, d; 2021a) all meeting relatively high (i.e. ~>60%) proportions of 'must-have' and 'should-have' criteria.

Other jurisdictions (HC 2018b, MDH 2022f, OEHHA 2019a, MPART 2019a) met lower proportions of criteria, indicating these guidance documents potentially do not conform with modern methods of undertaking systematic reviews.

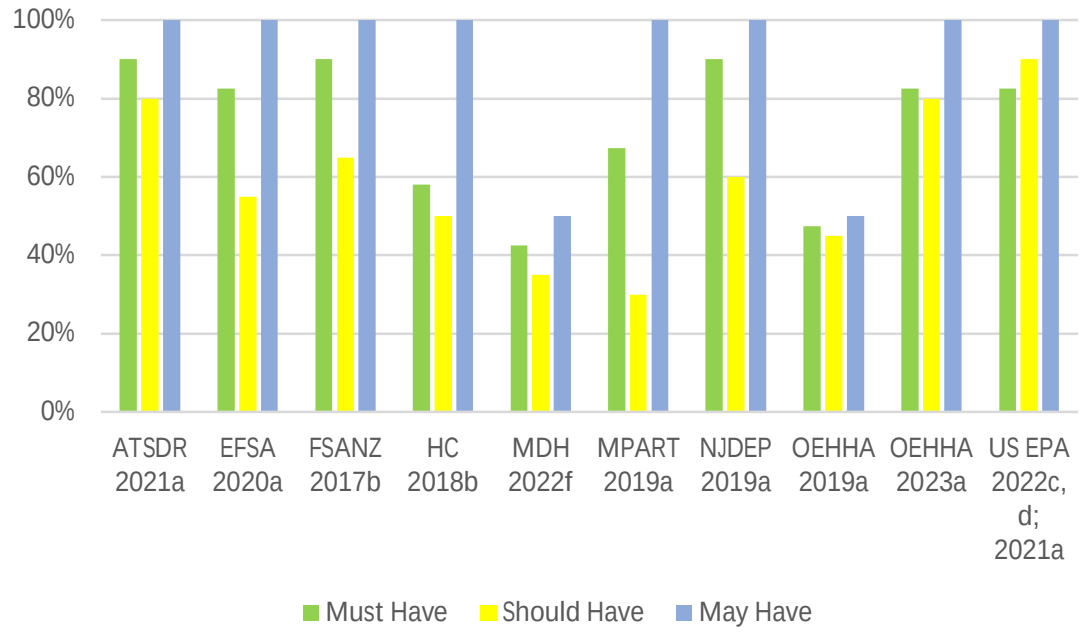


Figure 9-1 Overall proportion of 'must-have', 'should-have' and 'may-have' technical/administrative criteria as per the Assessment Tool met by jurisdictions who have derived candidate guidance values for PFOA for possible adoption/adaptation in Australia

9.2 Critical evaluation of PFOA guidance values

For PFOA, the guidance values identified in the literature review that based their derivations on underpinning studies not previously considered / cited in the comprehensive review

undertaken by FSANZ (2017b) are the following. The discussion in this section therefore focuses on the relevant studies underpinning these guidance values.

- EFSA (2020a) who derived a guidance value for ∑PFOA, PFNA, PFHxS and PFOS of 0.63 ng/kg bw/day (TWI = 4.4 ng/kg bw per week) for decreased antibody titre for specific vaccines. The critical study underpinning this guidance value (Abraham et al. 2020) has already been critically evaluated in **Section 6.2.1**. This study was not available to FSANZ at the time of their 2017 review but was reviewed later by FSANZ (2021).
- US EPA (2022c, d; 2021a) who derived a DRAFT guidance value of 0.0015 ng/kg/day for PFOA based on decreased antibody titre following tetanus vaccination in 7-year old children. The critical studies underpinning this guidance value have already been critically evaluated either by FSANZ (2017b) (in the case of Grandjean et al. 2012), FSANZ (2021), or in **Section 6.2.2** (in the case of Budtz-Jørgensen and Grandjean 2018).
- OEHHA (2019a) derived a non-cancer[13] guidance value of 0.45 ng/kg/day for liver toxicity (and oxidative DNA damage, changes in mitochondrial membrane potential) in female mice. The critical study underpinning this guidance value is Li et al. (2017) and was not previously available to FSANZ (2017b). Therefore, this study has been critically evaluated in **Section 9.2.1**.
- In a later document, OEHHA (2023a) derived a non-cancer guidance value of 0.87 ng/kg/day for increased risk of elevated alanine aminotransferase (ALT) in humans. The critical study underpinning this guidance value is Gallo et al. (2012) which does not appear to have been evaluated by FSANZ (2017b), as assumed by a lack of its citation in the review. Therefore, this study has been critically evaluated in **Section 9.2.2**.
- NJDEP (2019a) derived a guidance value of 2 ng/kg/day for increased liver weight in male mice. The critical study underpinning this guidance value is Loveless et al. (2006), which was also not cited in the FSANZ (2017b) review. Therefore, this study has been critically evaluated in **Section 9.2.3**.
- ATSDR (2021a; adopted by WSDH 2019a, 2022b, 2023a) derived a guidance value of 3 ng/kg/day for skeletal alterations in adult mouse offspring. The critical study underpinning this guidance value is Koskela et al. (2016) which does not appear to have been previously evaluated by FSANZ (2017b). Therefore, this study has been critically evaluated in **Section 9.2.4**.
- MPART (2019a) derived a guidance value of 3.9 ng/kg/day for developmental delays (decreased number of inactive periods, altered novelty induced activity and skeletal alteration such as bone morphology and bone cell differentiation in the femurs and tibias) of mice. The critical studies underpinning this guidance value are Koskela et al. (2016) and Onishchenko et al. (2011), neither of which appear to

[13] As indicated in the tables of the Technical Report and tables in **Section 4.1** of this report, the cancer-based guidance values derived by some jurisdictions were not considered to be applicable to an Australian context, as they use low-dose linear extrapolation as a policy decision in their derivations; Australia's science policy is to only undertake low-dose linear extrapolation for carcinogens acting through a mutagenic mode of action. As there is agreement in the various jurisdictional reviews sourced for this investigation that PFAS are not regarded as being directly mutagenic (see data extraction tables in Technical Report), the guidance values derived for cancer endpoints by low-dose linear extrapolation are not considered applicable to the Australian context and have not been reviewed / critiqued further.

have been previously evaluated by FSANZ (2017b). Therefore, these studies have been critically evaluated in **Section 9.2.4** and **9.2.5**.

In addition, due to there being several 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 9.2.1** to **9.2.5**). 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.

At the request of NHMRC and the Committee, the critical study underpinning the existing Australian guidance / guideline value for PFOA (i.e. Lau et al. 2006) was also assessed for its overall confidence (see **Section 9.2.6**).

9.2.1 Li et al. (2017) – used by OEHHA (2019a)

Li et al. (2017) divided 6-week old Balb/c mice into groups (30/sex/group) and administered each mouse PFOA (98% pure) orally via gavage at doses of 0, 0.05, 0.5, or 2.5 mg/kg/day in corn oil for 28 days. After 28-days of exposure, mice were sacrificed to collect liver and blood samples. Liver and serum samples of 10 mice from each treatment were pooled and homogenised and analysed for PFOA. Liver samples were examined for histology and proteomic change using isobaric tags for relative and absolute quantitation (iTRAQ) and Western Blotting.

In PFOA exposed males, the following effects were observed.

- ≥ 0.5 mg/kg/day: Significantly increased liver weight. Increased incidence of hepatocellular hypertrophy.
- 2.5 mg/kg/day: Decreased body weight gain at 21 days compared to controls. Signs of apoptosis of liver cells.

In females, the following effects were observed.

- All doses: Increased oxidative DNA damage, changes in mitochondrial membrane potential, and increased biomarkers of apoptosis in the liver.
- ≥ 0.5 mg/kg/day: Significantly increased liver weight. Increased incidence of hepatocellular hypertrophy.
- 2.5 mg/kg/day: Signs of apoptosis of liver cells.

Proteomic profiling revealed that reactive oxygen species (ROS) hyper-generation induced by suppression of Complex I was the major pathway to induce apoptosis in female mice at 0.05 mg/kg/day, while PPARα-activation (a mechanism considered not to be relevant to humans) was the mechanism for male mice. A recent review (Corton et al. 2018) indicates that there are a number of modulating factors, such as increased oxidative stress, that potentially alter the ability of PPARα activators to increase rodent liver effects and cancer while not being key events themselves. This indicates the potential that the effects on apoptosis observed in male and female mice by Li et al. (2017) may not be relevant to humans. FSANZ (2017b) concluded in their review that PFOA is known to cause peroxisome proliferation, leading to hepatocellular hypertrophy and increased liver weight, particularly in rodents. Although some liver pathology was seen in some animal studies with PFOA, and there is some evidence of effects of PFOA on the liver that are not mediated by PPARα receptors, it is difficult to separate the effects of PPARα activation from direct effects of PFOA on the liver (FSANZ 2017b). OEHHA (2019a, 2023a) identified a LOAEL of 0.05 mg/kg/day for changes in mitochondrial membrane potential (indicative of mitochondrial

dysfunction), increases in biomarkers of apoptosis (caspase-9 and p53) and increased oxidative DNA damage. It is arguable whether these effects, on their own, can be considered adverse therefore the lowest dose could also be regarded as a NOAEL.

PFOA concentrations in liver and serum increased with PFOA dose, with PFOA concentrations generally higher in liver than serum. The mean serum PFOA concentrations in mice in the 0.05, 0.5 and 2.5 mg/kg/day dose groups were in females / males, respectively: 970 / 1,200 ng/mL; 2,700 / 5,900 ng/mL; 9,500 / 13,400 ng/mL (Li et al. 2017, OEHHA 2023a).

OEHHA (2019a) derived a guidance value using what they considered to be a serum LOAEL of 970 ng/mL. They applied an uncertainty factor of 300 (3x for interspecies extrapolation of toxicodynamics, 10x for human variability, 3x for use of a LOAEL, 3x for database uncertainties due to potential for developmental toxicity at the POD)[14] to derive a target human serum level of 3.2 ng/mL. This was converted to a HED of 0.45 ng/kg/day [0.0032 mg/L x 1.4 x 10^{-4} L/kg/day x 10^{6} ng/mg]. It is noted no such database uncertainty factor was considered to be required by FSANZ (2017b) when deriving a guidance value for PFOA, thus this uncertainty factor would not be considered relevant in the Australian context. In addition, if the POD were considered a NOAEL instead of a LOAEL (as the data suggest), the TRV (without the LOAEL and database uncertainty factors) would be 4.5 ng/kg/day. As indicated above, it is also arguable whether the effects observed on the liver in this study are relevant to humans, particularly as humans are potentially refractory to these types of effects.

Li et al. (2017) was a study focusing on molecular mechanisms of PFOA-induced hepatocyte apoptosis in mice, therefore it did not follow standardised protocols for toxicity experiments. Nevertheless, it provided serum PFOA concentrations, and examined effects on the liver and therefore could be used in a weight of evidence approach for derivation of candidate guidance/guideline values for PFOA. However, it is arguable whether the effects observed at the lowest dose (0.05 mg/kg/day) in female mice can be considered adverse and whether humans may be refractory to liver effects due to PFOA exposure, thus relatively low confidence is assigned to the candidate guidance/guideline value derived using the Li et al. (2017) study. The candidate guidance/guideline values are summarised in **Section 9.3**.

9.2.2 Gallo et al. (2012) – used by OEHHA (2023a)

In a cross-sectional study, Gallo et al. (2012) analysed data for 46,452 adults[15] from the C8 Health Project.[16] 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).

[14] It appears OEHHA (2019a) have rounded up the uncertainty factor of 270 to 300.

[15] 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.

[16] 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. Gallo et al. (2012) used these data to examine the cross-sectional association between serum PFOA and PFOS concentrations and markers of liver function in adults.

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% confidence interval (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 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).

Fitted values of GGT by deciles of PFOA showed an apparent positive association although it was less clear than that for ALT. The suggested association was not confirmed in the logistic regression model, in which no trend across deciles was observed (p=0.213) or for the linear ln-units of PFOA values (OR = 1.01; 95% CI 0.99, 1.04).

For direct bilirubin, there was a suggestion of an inverse U-shaped relationship with PFOA, with increasing levels of bilirubin per increasing levels of PFOA at low PFOA levels and decreasing bilirubin levels for concentrations of PFOA above about 40 ng/mL. The linear regression relationship failed to show any association in the adjusted model.

Multilevel analysis was restricted to subjects living in water districts supplied by contaminated water (n=26,777) and excluding those with private wells. There was a significant difference between the between- and within- district components ALT and direct bilirubin; however, each outcome showed different patterns. The between- water- district regression coefficient from linear regression of ln-PFOA and ALT (0.010; 95% CI: –0.001, 0.020) was lower than the within- water- district coefficient (0.027; 95% CI: 0.022, 0.031). However, both coefficients were significant or borderline significant, in the same direction, and consistent with a positive association between ALT and PFOA levels.

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)[17], 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 association observed for PFOA and ALT appears 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 PFOA with a biomarker of a potential effect should be used as the basis of deriving a health-based guidance value.

[17] 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.

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).

Based on the above evaluation, it is concluded the OEHHA (2023a) guidance value based on the Gallo et al. (2012) study is not suitable for adoption/adaptation in the Australian context and it has not been included in the candidate guidance/guideline value derivation for PFOA in **Section 9.3**.

9.2.3 Loveless et al. (2006) – used by NJDEP (2019a)

Loveless et al. (2006) compared the toxicity of linear ammonium perfluorooctanoate (APFO) with that of 80% linear 20% branched chain APFO (97.99% pure), and a 100% branched form in both rats and mice. The description of the study focuses on the results in mice, as these were used by NJDEP (2019a) for derivation of a guidance value for PFOA. Male Crl:CD-1(ICR)BR mice (10/group) were gavage dosed in NANOpure® water with 0, 0.3, 1, 3, 10, or 30 mg/kg/day of the different APFO form for 14 days. The study was conducted in compliance with US EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards. The study monitored for body weight, clinical signs, mortality, food consumption, clinical pathology (serum lipid parameters), liver and kidney weight, and hepatic ß-oxidation analysis (a measure of peroxisome proliferation).

There were no adverse clinical signs of toxicity observed in any treated mice. One mouse dosed with 30 mg/kg/day linear APFO died during the study with cause of death being undetermined. Mean body weights were significantly lower compared to controls following 7 and 13 days of dosing with 30 mg/kg/day of linear/branched APFO or linear APFO. Mean body weights of control mice increased 1-2 g over the course of the study, whereas in groups treated with ≥ 10 mg/kg/day linear/branched APFO or linear APFO body weights decreased between 1-6g. Treatment-related increases in liver weights decreased the apparent magnitude of body weight effects.

All three forms decreased total and high-density lipoprotein (HDL) cholesterol but triglycerides (Tg) were increased at lower doses. The LOEL was 0.3 mg/kg/day for all of the APFO forms, based on increased relative liver weight, peroxisomal ß-oxidation activity (and increased Tg for linear/branched material). Absolute liver weight was also significantly increased with ≥ 3 mg/kg/day. Serum PFOA (collected approximately 24 hours after the last dose) at the LOEL of 0.3 mg/kg/day ranged from 10,000-14,000 ng/L.

It is noted that an increase in liver weight, in the absence of histopathological findings, may be indicative of an adaptive response (ATSDR 2021a). Nevertheless, the effect has been noted to occur in other animal studies with PFOA; as it was accompanied by peroxisome proliferation, humans may be less susceptible to the effect although NJDEP (2019a) notes that similar increases in liver weight were observed in a 90-day study in cynomolgus monkeys at comparable serum levels to those observed in mice. NJDEP (2019a) also notes that increases in liver weight and other types of hepatic toxicity occur through both PPARα dependent and independent modes of action and are considered relevant to humans. ATSDR (2021a) did not consider the liver effects (increase in liver weight, hepatocellular hypertrophy, alterations in serum lipids in the absence of other degenerative changes) to be appropriate endpoints for deriving a TRV. It is also noted that, although FSANZ (2017b) did not cite the Loveless et al. (2006) study explicitly in their review, they considered increases in absolute and/or relative liver weight in rodents, in the absence of hepatocellular degeneration or necrosis, to not be an adverse effect for the purpose of identifying a NOAEL or LOAEL. Similarly, FSANZ (2017b) has not interpreted increased absolute liver weight in monkeys as an adverse effect because there was no significant effect on relative liver weight, and no histological evidence of hepatocellular hypertrophy or liver lesions.

NJDEP (2019a), on the other hand, considered the effect appropriate for determining a guidance value and conducted BMD modelling of the serum PFOA data for the branched/linear APFO from the Loveless et al. (2006) study to derive a PFOA serum $BMDL_{10}$ in mice for increased relative liver weight of 4,350 ng/mL. They applied an uncertainty factor of 300 (3x for interspecies extrapolation of toxicodynamic differences, 10x for human variability, 10x for database uncertainties for potential adverse effects on mammary gland development occurring at lower doses than increased relative liver weight) to the POD to derive a target human serum level of 14.5 ng/mL. It is noted the latter database uncertainty factor was not applied in the FSANZ (2017b) derivation of a TRV and therefore would be unlikely applied if adapting the value to the Australian context. ATSDR (2021a) also noted that the mammary gland effect did not result in an adverse effect on lactational support at maternal doses of PFOA as high as 1 mg/kg/day, based on normal growth and survival in F2 pups. Given that milk production was adequate to support growth, ATSDR (2021a) considered the biological significance of the delayed development of the mammary gland observed at very low doses is uncertain.

NJDEP (2019a) then converted the target human serum level to a dose by applying a clearance factor (1.4×10^{-4} L/kg/day) sourced from US EPA (2016a, as cited in NJDEP 2019a). This resulted in a TRV of 2 ng/kg/day (i.e. 14.5 ng/mL x 1.4×10^{-4} L/kg/day x 10^3 mL/L). It is noted this TRV would likely be 10-fold higher (i.e. 20 ng/kg/day) in the Australian context if the additional database uncertainty factor was not applied (see also **Section 9.3**). In line with the conclusions in the FSANZ (2017b) review, there is uncertainty with respect to the human relevance of the liver effects observed in the Loveless et al. (2006) study due to the dearth of mode of action information for these effects and suggested human refractoriness for some of these effects. Thus, the candidate guideline value resulting from adaptation of the NJDEP (2019a) guidance value is considered to be of low confidence (see **Section 9.3**).

9.2.4 Koskela et al. (2016) – used by ATSDR (2021a)

Koskela et al. (2016)[18] exposed pregnant C57BL/6/Bk1 mice orally in the diet to 0 (n=10) or 0.3 (n=6) mg PFOA/kg/day (96% purity) throughout pregnancy from GD1 to presumably 21, and female offspring (groups of five) were studied at age 13 or 17 months. Body weight was measured as well as morphometrical and biomechanical properties of femurs and tibias with micro-computed tomography and 3-point bending, and bone PFOA concentrations were determined by mass spectrometry. The effects of PFOA on bone cell differentiation were studied in osteoclasts from C57BL/6/Bk1 mice and in the MC3T3 pre-osteoblast cell line.

Litter mates of the offspring in the Koskela et al. (2016) study were examined for neurobehavioral effects in a study conducted by Onishchenko et al. (2011). As reported in the latter study, there were no differences in dam weight gain, litter size or sex ratio or pup body weight or brain weight at birth in the treated group compared to controls. Offspring body weight was significantly higher in comparison with controls at 13 and 17 months of age (9.9 and 7.8%, respectively), which Koskela et al. (2016) speculate may be due to an increased amount of adipose tissue.

In 17-month-old offspring, there was a 6.8% increase in periosteal area of the femoral cortical bone ($p<0.05$) and increases in the peri- and endosteal perimeters (3.2%, $p<0.05$ and 5.2%, $p<0.01$, respectively) and the marrow area (10.0%) ($p<0.05$); an increase in medullary area was also observed. There were no differences in femoral cortical bone area or femoral mineral density. In the tibia, the total area inside the periosteal envelope and the periosteal perimeter were increased (4.9 and 3.5%, respectively) ($p<0.05$). Although the

[18] The Onishchenko et al. (2011) study (discussed in **Section 9.2.5**) and Koskela et al. (2016) study are reports of different endpoints examined in the same study.

study authors noted in the text that tibial medullary areas were "*essentially the same between groups*," data in Figure 2 of the paper show a statistically significant increase at 17 months (but absolute values were indeed similar). Significant decreases in tibial mineral density were observed at 13 and 17 months. There were no significant differences in the tibial medullary area or the endosteal perimeter. There were no significant effects on any other measured biochemical parameter in the femur or tibia (stiffness, maximum energy, absorption).

According to ATSDR (2021a), the "*Koskela et al. (2016) study has a number of strengths including examination of several measures of bone status tested at different ages, measurement of bone PFOA levels, and tests to evaluate potential mechanisms of action. To evaluate whether developmental exposure resulted in bone damage in mature animals, the study evaluated bone morphology (periosteal, cortical, and medullary areas and bone mineral density) and bone biomechanical properties (stiffness, maximum force, and maximum energy); all tests were conducted on femur and tibia bone. Measurement at two ages (13 and 17 months) allowed for an evaluation of whether the effect of PFOA on bone changed as the animals aged. The companion in vitro study of osteoclasts and osteoblasts provided mechanistic support for the in vivo findings. Additionally, the in vitro study evaluated four PFOA concentrations and found concentration-related differences."*

There are several study limitations that affect the interpretation of the study results; these include the small number of animals tested, use of only one PFOA dose level, inadequate reporting of dietary PFOA levels, and lack of measured serum PFOA levels. Tests of potential alterations in bone mineral density and bone biomechanical properties were only evaluated in 5–6 female offspring per group; however, support for the finding comes from the consistency of the findings at 13 and 17 months of age. The use of only one PFOA dose level does not allow for the establishment of dose-response relationships. This study limitation is mitigated by the extensive intermediate-duration oral exposure database, which allows for an overall assessment of dose-response. The dams were exposed to PFOA dissolved in alcohol and sprayed onto the food pellets. The study did not measure the amount of residual alcohol or the actual amount of PFOA on the food pellets. Koskela et al. (2016) measured PFOA levels in the tibias and femurs but did not measure serum PFOA levels. ATSDR estimated the TWA serum PFOA concentrations using the Wambaugh et al. (2013) model. The lack of measured serum PFOA levels did not allow for validation of whether the model accurately predicted serum levels; the model was validated using data from other intermediate-duration PFOA studies in rats and mice (ATSDR 2021a)".

The ATSDR (2021a) estimated mouse serum PFOA concentration at the administered dose of 0.3 mg/kg/day was 8,290 ng/mL. This serum concentration was converted by ATSDR (2021a) to a HED POD of 0.000821 mg/kg/day [(Css x Ke x Vd) ÷ AF = (8.29 mg/L) x 0.693/1,400 d x 0.2 L/kg ÷ 1] and an uncertainty factor of 300 was applied (10x for use of a LOAEL, 3x for interspecies extrapolation of toxicodynamic differences, 10x for human variability).

Despite the limitations outlined by ATSDR (2021a) for the Koskela et al. (2016) study, the outcome does appear to be compelling and, if relevant to humans, could potentially increase the risk of bone fractures later in life. This study was included in the candidate guidance/guideline values summary in **Section 9.3**. However, due to the small animal numbers in the study (n=6 in treated group), the fact there was only a single treatment group, the study not following standardised testing guidelines, and the uncertainty with respect to the clinical relevance of the findings, the confidence in the resulting adapted guideline value is considered to be very low.

9.2.5 Onishchenko et al. (2011) – used by MPART (2019a)

As described in the previous section (Koskela et al. 2016 reporting the same study), Onishchenko et al. (2011) exposed pregnant C57BL/6/Bk1 mice (n=6/group, n=10 for controls) to PFOA (96% purity) or PFOS (as heptadecafluorooctanesulfonic acid potassium salt, purity ≥ 98%) at 0 or 0.3 mg/kg/day via the diet (dissolved in ethanol and applied to food, then evaporated for 2 hours) from GD1 throughout pregnancy (presumed GD21). Tissue samples (whole brain and liver) were collected from pups at birth and concentrations of PFOS and PFOA analysed. Tests for locomotor and circadian activity were performed on offspring at age 5-8 weeks. Afterward, animals were tested for emotion-related behaviour in elevated plus maze and forced swim tests. Tests for muscle strength and motor coordination were performed in animals 3- to 4-month old.

Dams exposed to PFOS or PFOA gained weight normally during pregnancy and did not differ from control females at any gestational age. Litter size and sex ratio were unaffected by treatment and there were no differences in offspring body or brain weights between groups at birth. Liver weights were normal in PFOS-exposed pups, but significantly increased in PFOA-exposed mice (77 ± 2 mg vs. 58 ± 1 mg in control, $p<0.001$).

PFOS-exposed males walked significantly less than controls when exploring a new environment, while females did not differ significantly from controls. PFOA exposure did not have a significant effect on locomotor activity. PFOS-exposed males also showed decreased activity in social groups using the TraffiCage system during the first two hours. A similar trend was observed in PFOS-exposed females, but the difference during the second hour of the test did not reach statistical significance. PFOA-exposed males were more active ($p = 0.013$), while PFOA-exposed females showed a decreased activity ($p = 0.036$) than the controls. However, these alterations were observed when animals were tested in social groups, while individual testing did not reveal any differences. After habituation to the new home cage, animal activity declined to a low, diurnal level. All groups of animals had a normal circadian pattern with higher levels of activity during the dark phase and early morning hours, followed by lower activity levels during the light phase.

In the elevated plus maze test, PFOS-exposed male mice travelled equally long distance exploring the closed arms as controls, but the exposed animals spent significantly more time being inactive than controls. The preference for exploration of open (potentially dangerous) versus closed (safe) areas did not seem to be altered in the exposed animals. PFOS-exposed females as well as all PFOA-exposed groups did not differ from their respective controls in any behavioural parameter tested in the elevated plus maze.

There was no effect on immobility time in the forced swimming test. However, in the hanging wire test, PFOS-exposed male mice had significantly shorter fall latency than controls ($p =0.04$) but females and PFOA-exposed mice were unaffected.

Overall, the behavioural changes found in this study were of a small magnitude and study groups were also relatively small. Serum PFOS and PFOA concentrations were not measured in this study, but ATSDR (2021a) estimated the mouse serum PFOA concentration at the administered dose of 0.3 mg/kg/day was 8,290 ng/mL. The jurisdiction did not use the results of the Onishchenko et al. (2011) study for derivation of a TRV for PFOA, since circadian activity was assessed using a TrafficCage system in which all animals in the group were placed in a single cage and activity was measured; thus, activity was only measured on a group basis and it is possible that one animal could skew the results. It is noted ATSDR (2021a) did not calculate the serum PFOS concentration in this study.

It is noted another study with a similar study design but including more than one dose (0, 0.1, 0.3, 1.0 mg/kg/day) via gavage found no changes to motor-related behaviours at PFOA doses below 1 mg/kg/day (Goulding et al. 2016).

MPART (2019a) used both the Onishchenko et al. (2011) and Koskela et al. (2016) studies (which are reports of different endpoints examined in the same study) and considered the dose of 0.3 mg/kg/day as a LOAEL. They used the ATSDR (2021a) estimated serum concentration of 8,290 ng/mL to calculate a HED LOAEL of 0.001163 mg/kg/day [TWA serum x ke x Vd = 8.29 mg/L x 8.2 x 10^{-4} x 0.17 L/kg]. It is noted that the parameters used to convert the serum concentration to a HED differ from those used by ATSDR (2021a).[19] Using the parameters from ATSDR (2021a) results in a slightly different HED LOAEL of 0.000821 mg/kg/day. MPART (2019a) then applied the same rounded uncertainty factor of 300 (3 for use of a LOAEL since a NOAEL for immune effects was similar to the selected LOAEL and the selected LOAEL represented less severe effects, 10x for human variability, 3x for interspecies differences in toxicodynamics, 3x for database deficiencies as the mammary gland effects were considered to signal a concern for other low dose endocrine effects) to the HED LOAEL to derive a TRV of 3.9 ng/kg/day.

The study was included in the candidate guidance/guideline values summary in **Section 9.3**. However, due to the marked limitations with the study identified by ATSDR (2021a), the fact it was not conducted in accordance with standardised testing guidelines, and the apparent small absolute differences between the treated and control groups, the confidence in the resulting adapted guideline value is considered to be very low.

9.2.6 Lau et al. (2006) – used by NHMRC and NRMMC (2011), FSANZ (2017b), DOH (2017)

Lau et al. (2006) is a developmental toxicity study conducted with PFOA (ammonium salt, >98% pure) in which timed-pregnant CD-1 mice were administered 0, 1, 3, 5, 10, 20, or 40 mg PFOA/kg bw/day by oral gavage from gestational day (GD) 1 to 17 inclusive. Some mice were sacrificed on GD18 for teratological evaluation, while others were dosed on GD18 and allowed to proceed to spontaneous parturition. In the control group, 45 mice were terminated pregnant and 23 proceeded to spontaneous parturition, whereas for the treated groups, the corresponding numbers were 17/8, 17/8, 27/19, 26/21, 42/7 and 40/0, respectively.

All dams in the 40 mg/kg/day group resorbed their litters. Weight gain in dams that carried pregnancy to term was decreased in the 20 mg/kg/day group. Increased liver weight was observed in dams sacrificed at GD18 at all doses. Percentage of live foetuses at birth was lower only in the 20 mg/kg/day group, and foetal weight was also decreased. No significant increases in malformation were noted in any treatment group. Growth was delayed in all PFOA-treated litters except the 1 mg/kg/day group. Ossification (i.e. number of sites) of the forelimb proximal phalanges was significantly decreased at all doses except 5 mg/kg/day.

Reduced ossification of the calvaria and enlarged fontanel was observed at 1, 3, and 20 mg/kg/day and at ≥ 10 mg/kg/day in the supraoccipital bone. Postnatal survival was also significantly decreased at ≥ 5 mg/kg/day. According to the study authors, accelerated sexual maturation was observed in male offspring (i.e. time to preputial separation was decreased in male pups), but not in females, at all doses of PFOA. However, FSANZ (2017b) noted in their assessment of the study that the data presented in the paper do not support this conclusion.[20]

[19] MPART (2019a) considered the PFOA serum half-life of 840 days (2.3 years) more relevant for exposure to the general population than the ATSDR (2021a) assumed half-life of 1,400 days.

[20] Age at preputial separation was similar in the high dose group (31.7 ± 1.1 days) to that in controls (30.5 ± 0.2 days), therefore there was no clear dose response for this effect.

Average serum PFOA concentrations in pregnant mice at term were 21.9, 40.5, 71.9, 116, 181, and 271 µg/mL in the 1, 3, 5, 10, 20, and 40 mg/kg/day groups, respectively (FSANZ 2017b).

The maternal NOAEL was 10 mg/kg/day (i.e. 116 µg/mL), based on decreased body weight gain at ≥ 20 mg/kg/day (FSANZ 2017b). The NOAEL for foetal toxicity was 1 mg/kg/day (i.e. maternal serum of 21.9 µg/mL), based on decreased body weight gain at doses of ≥ 3 mg/kg/day (i.e. maternal serum of 40.5 µg/mL) (FSANZ 2017b). Lau et al. (2006) derived lower benchmark doses for a 5% effect ($BMDL_5$s) for various effects observed in the study. The $BMDL_5$ for decreased pup weight at weaning was 0.86 mg/kg/day (serum $BMDL_5$ not reported), which is similar to the NOAEL nominated by FSANZ (2017b). The lowest $BMDL_5$ derived by Lau et al. (2006) was 0.17 mg/kg/day for increased maternal liver weight at term; however as noted previously, these effects, in the absence of concomitant histopathological findings, are unlikely to be relevant to humans.

FSANZ (2017b) considered the Lau et al. (2006) study suitable for derivation of a health-based guidance value. FSANZ (2017b) used pharmacokinetic modelling to predict average serum concentrations from predicted areas-under-the-curve over the duration of dosing using parameters also used by the US EPA. The average PFOA serum concentration from the modelling at the NOAEL of 1 mg/kg/day was determined to be 35.1 µg/mL; this was converted to a human equivalent dose (HED) of 0.0049 mg/kg/day using a clearance factor of 0.00014 L/kg/day (the same factor also used by several other jurisdictions). FSANZ (2017b) then applied an uncertainty factor of 30 (3x for interspecies differences in toxicodynamics, 10x for human variability) to derive a tolerable daily intake of 160 ng/kg/day.

The study and the resulting FSANZ (2017b) guidance value were included in the guidance / guideline values summary in **Section 9.3**. The Lau et al. (2006) study appears to have been conducted using a protocol similar to OECD TG 414 (prenatal developmental toxicity study) and examined a large number of standard endpoints[21] in a sufficiently large number of treatment groups and treated animals. Thus, the confidence in the resulting guideline value is considered to be high.

9.3 Candidate guidance/guideline values for PFOA

As indicated in preceding sections, a number of additional studies (summarised in **Sections 9.2.1** to **9.2.5**) that had not been previously explicitly considered / evaluated in the FSANZ (2017b) review of PFOA were used by various jurisdictions as critical studies for derivation of PFOA guidance values. Of those studies, all except the cross-sectional one by Gallo et al. (2012) were considered potentially suitable for adoption/adaptation for candidate DWG derivation in the Australian context.

The potentially suitable studies were used by four jurisdictions (Loveless et al. 2006 by NJDEP 2019a; Koskela et al. 2016 by ATSDR 2021a; Onishchenko et al. 2011 and Koskela et al. 2016 by MPART 2019a; and Li et al. 2017 by OEHHA 2019a) to derive a guidance value for PFOA, two of which (NJDEP 2019a and ATSDR 2021a) also met a high proportion of technical/administrative criteria for potential adoption/adaptation into the Guidelines (**Section 9.1**). However, it is noted that, due to various considerations, the confidence in the resulting adapted candidate guideline values ranges from very low to low.

The jurisdictions have all chosen different endpoints for derivation of guidance values, at times have used slightly different toxicokinetic adjustment factors for converting an animal

[21] Endocrine disruptor relevant parameters (i.e. anogenital distance in foetuses and thyroid hormones in dams) were only added to the OECD TG in 2018. These endpoints were not included in the Lau et al. (2006) study, since the OECD TG update superseded the conduct and publication of the Lau et al. (2006) study.

serum concentration to a human dose, and the choices of uncertainty factors also differ between jurisdictions (see **Table 9-1**).

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 all jurisdictions which have derived DWGs in the literature consulted applied an RSC of 0.2 (i.e. 20%) (e.g. OEHHA 2019a, 2023a; US EPA 2021a, 2022d) but do not provide the rationale for this. Thus, the default factor of 0.1 has been retained in calculating the potential resulting DWGs for PFOA using the guidance values in **Table 9-1**, noting that it yields a lower guideline value than use of an RSC of 0.2.

Also presented in **Table 9-1** 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 to have high confidence in **Section 9.2.6**.

Table 9-1 Potential drinking water guideline values (ng/L) resulting from adaptation of PFOA guidance values from different jurisdictions [(1)]

Parameter	NHMRC and NRMMC 2011, FSANZ 2017b, DOH 2017	NJDEP 2019a	ATSDR 2021a	MPART 2019a	OEHHA 2019a
Critical study	Lau et al. 2006	Loveless et al. 2006	Koskela et al. 2016	Onishchenko et al. 2011, Koskela et al. 2016	Li et al. 2017
Study population	Mice	Mice	Pregnant mice		Mice
Form of PFOA studied	PFOA Ammonium salt (98.9% linear / 1.1% branched)	Branched/ linear PFOA (as APFO)	PFOA		PFOA
Exposure route	Oral (gavage)	Oral (gavage)	Oral (diet)		Oral (gavage)
Study timeframe	Throughout pregnancy (GD1-17)	14 days	Throughout pregnancy (presumably GD1-21)		28 days
Critical Effect	↓ pre-weaning growth rate in pups	↑ relative liver weight in male mice	Skeletal alterations (i.e. altered femur and tibial bone morphology, ↓ tibial mineral density) in adult offspring	Skeletal alterations (see cell to the left) and altered exploratory behaviour in adult offspring (↑ in males, ↓ in females)	↑ oxidative DNA damage, changes in mitochondrial membrane potential, and ↑ biomarkers of apoptosis in liver of female mice[(5)]
Serum Point of Departure (mg/L)	NOAEL = 35.1	$BMDL_{10}$ = 4.35	LOAEL = 8.29 *(estimated)*	LOAEL = 8.29 *(estimated)*	LOAEL = 0.97 (NOAEL = 0.97)[(5)]
Clearance Factor (L/kg-day)	0.00014 (*back-calculated from POD HED*)	0.00014 (from US EPA)	0.000099	0.00014	0.00014
Point of Departure HED (mg/kg/day)	0.0049	0.000609	0.000821	0.001161	0.000136

Parameter		NHMRC and NRMMC 2011, FSANZ 2017b, DOH 2017	NJDEP 2019a	ATSDR 2021a	MPART 2019a	OEHHA 2019a
Uncertainty factors	UF_A	3	3	3	3	3
	UF_H	10	10	10	10	10
	UF_{LOAEL}	1	1	10	3 (4)	3 (1) (5)
	$UF_{database}$	1	10 (1) (2)	1	3 (1) (2)	3 (1) (6)
	$UF_{composite}$	30	300 (30) (2)	300	300 (90) (2)	300 (30) (5,6)
Health-based guidance value (ng/kg/day)		160 (11)	2 (20) (2)	2.7	3.9 (12.9) (2)	0.45 (4.5) (5,6)
Relative source contribution (RSC) to drinking water		0.1	0.1	0.1	0.1	0.1
Resulting adaptation to a Health-based DWG (3) (ng/L)		560	7 (71) (2)	9.6	13.5 (45) (2)	1.6 (16) (5,6)
Confidence in candidate guideline value		High(7)	Low (8)	Very low (9)	Very low (9, 10)	Low (5)

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; $UF_{composite}$ = 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 PFOS, there are various reasons why the epidemiological information for associations of PFAS serum concentrations with decreased antibody titre for specific vaccines (i.e. Abraham et al. 2020, Budtz-Jørgensen and Grandjean 2018) is not considered suitable in the Australian context for derivation of guidance values for PFAS. Similarly, the cross-sectional study by Gallo et al. (2012) for increased ALT associated with increased PFOA concentrations in serum was not considered suitable for adoption/adaptation in the Australian context for PFOA health-based guidance value development (see **Section 9.2.2**). For this reason, these studies have not been included in this table.

(2) The additional uncertainty factor of 3x (for MPART 2019a) or 10x (for NJDEP 2019a) was applied for potential adverse effects on mammary gland development occurring at lower doses than the endpoint selected. As discussed in **Section 9.2.3**, this additional database uncertainty factor is unlikely to be required. The values that would result from not applying this uncertainty factor are provided in brackets.

(3) Adaptation 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

Parameter	NHMRC and NRMMC 2011, FSANZ 2017b, DOH 2017	NJDEP 2019a	ATSDR 2021a	MPART 2019a	OEHHA 2019a
(4) Since a NOAEL for immune effects was similar to the selected LOAEL and the selected LOAEL represented less severe effects, MPART (2019a) used a reduced uncertainty factor of 3x for use of a LOAEL. (5) As discussed in **Section 9.2.1**, it is arguable whether the effects observed at the lowest dose in this study (0.05 mg/kg/day) in female mice can be considered adverse. If the lowest dose in the study (0.05 mg/kg/day) is considered to be a NOAEL instead of a LOAEL, the alternative values that would result are provided in brackets. In addition, FSANZ (2017b) indicates that humans may be refractory to the liver effects observed in rodents as a result of PFOA exposure, thus there is low confidence in the relevance of this candidate guideline value. (6) As discussed in **Section 9.2.1**, the use of the additional database uncertainty factor is unlikely to be required. The values that would result from not applying this uncertainty factor are provided in brackets. (7) The Lau et al. (2006) study appears to have been conducted using a protocol similar to OECD TG 414 (prenatal developmental toxicity study) and examined a large number of standard endpoints in a sufficiently large number of treatment groups and treated animals (see **Section 9.2.6**). Thus, the confidence in the resulting guideline value is considered to be high. (8) Considered to be of low confidence, since increases in absolute and/or relative liver weight in rodents, in the absence of hepatocellular degeneration or necrosis, was not considered by FSANZ (2017b) to be an adverse effect for the purpose of identifying a NOAEL or LOAEL. Humans may also be more refractory to these effects than rodents (**Section 9.2.3**). (9) Due to the small animal numbers in the Koskela et al. (2016) study (n=6 in treated group), the fact there was only a single treatment group, and the uncertainty with respect to the clinical relevance of the findings, the confidence in the resulting adapted guideline value is considered to be very low (**Section 9.2.4**). (10) Due to the marked limitations with the Onishchenko et al. (2011) study identified by ATSDR (2021a), the fact it was not conducted in accordance with standardised testing guidelines, and the apparent small absolute differences between the treated and control groups, the confidence in the resulting adapted guideline value is considered to be very low (**Section 9.2.5**). (11) An international collaboration of scientists (Burgoon et al. 2023) recently derived a guidance value of 70 ng/kg/day for PFOA using the same study by Lau et al. (2006). The group used a NOAEL of 23 µg/mL (i.e. 23 mg/L) and applied uncertainty factors of 2.5 for potential toxicodynamic differences between mice and humans, 3 for toxicodynamic differences between humans, and 8.4 for toxicokinetic differences between humans [23 mg/L ÷ 63 = 0.3 mg/L]. This was converted to a guidance value by multiplying the guidance serum concentration by the geometric mean for clearance of PFOA in humans from a study by Zhang et al. (2013) assuming steady state [0.3 mg/L x 0.00023 L/day/kg = 0.00007 mg/kg/day or 70 ng/kg/day].					

The candidate PFOA DWGs derived by adapting existing guidance values for this PFAS range from 1.6 to 71 ng/L depending on the endpoint selected and uncertainty factors used, with the existing DWG at 560 ng/L. However, when excluding the values from the candidate DWGs likely not applicable to the Australian context due to differences in application of uncertainty factors or differences in endpoint selection (see **Table 9-1**), the range is 9.6 to 71 ng/L. These values all incorporate at least an uncertainty factor of 30x 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. However, it is noted that, due to various reasons outlined in **Sections 9.2.1** to **9.2.5**, the confidence in the candidate guideline values is considered very low to low, whereas the confidence in the existing Australian guideline value is considered to be high (**Section 9.2.6**) .

It is also noted that a recently published paper by Burgoon et al. (2023) which became available at the time of writing this report describes the outcome of an international collaboration of three teams consisting of a total of 24 scientists from eight countries tasked with reviewing relevant information and independently developing ranges for estimated PFOA safe doses (i.e. guidance values). All three teams determined that the available epidemiological information could not form a reliable basis for a PFOA safe dose assessment in the absence of mechanistic data that are relevant for humans at serum concentrations seen in the general population. This conclusion is in line with the conclusions made in the current report with respect to the available epidemiological data. The international collaboration estimated PFOA guidance values ranging from 10 to 70 ng/kg/day based instead on dose-response data from five studies of PFOA-exposed laboratory animals (including the study underpinning the existing Australian guideline value, i.e. Lau et al. 2006). The collaboration considered all of these values to be protective of human health (Burgoon et al. 2023). This range of guidance values is not dissimilar from the range of PFOA guidance values shown in **Table 9-1** adapted for the Australian context from international jurisdictions (i.e. 2.7 to 20 ng/kg/day), with the top end of the range given by Burgoon et al. (2023) (i.e. 70 ng/kg/day) being approximately two times lower than the guidance value derived by FSANZ (2017b) (i.e. 160 ng/kg/day). The difference in the latter two values is due to:

i) selection by Burgoon et al. (2023) of a slightly lower serum NOAEL (23 mg/L)[22] than FSANZ (2017b) (35.1 mg/L) from the Lau et al. (2006) study;

ii) use of a slightly larger uncertainty factor by Burgoon et al. (2023) (63 vs. 30);[23] and

[22] It is unclear to SLR how the serum POD corresponding to the NOAEL of 23 mg/L was derived by Burgoon et al. (2023). Data summarised by FSANZ (2017b) indicates the measured serum concentration at the NOAEL dose was 21.9 mg/L, whereas FSANZ (2017b) adjusted this serum concentration to 35.1 mg/L as this was the estimated average area-under-the-curve for the duration of dosing.

[23] FSANZ (2017b) used a composite uncertainty factor of 30 consisting of 3x for interspecies toxicodynamic differences and 10x for human variability. Burgoon et al. (2023) used a composite uncertainty factor of 63 composed of:

1) 2.5x for interspecies toxicodynamic differences (instead of 3x used by FSANZ),
2) 25.2x for human variability (instead of the default factor of 10x used by FSANZ) consisting of 3x for intra-human differences in toxicodynamics and 8.4x for intra-human differences in toxicokinetics [i.e. 0.79 mL/day/kg arithmetic mean clearance of average group from a study by Zhang et al. (2013) divided by 0.094 mL/day/kg arithmetic 95% lower bound clearance for a sensitive group from the same study = 8.4].

iii) a slightly different human clearance value (0.00023 L/kg/day vs. 0.00014 L/kg/day).[24] Collectively, these differences result in approximately a factor of 2x difference in the resulting guidance values.

Since the candidate guideline values for PFOA summarised in **Table 9-1** (9.6 to 71 ng/L) are based on data from studies considered to be of very low to low confidence for guideline derivation, it is suggested the information is not of high enough quality to warrant revision of the existing Australian guideline value for PFOA (560 ng/L) at this time, which is based on data for which there is high confidence.

In Australian distributed drinking waters or raw water catchments, PFOA concentrations generally may range up to 10 ng/L in various locations (QAEHS 2018a, 2018b, Sydney Water 2023, WHO 2022, WCWA 2023). This maximum concentration is at or below the candidate DWGs of 9.6 to 71 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.

10.0 Discussion for GenX Chemicals

This section provides a discussion of the strengths and limitations of the identified guidance values for GenX Chemicals for possible adoption/adaptation into the Guidelines.

10.1 Potential suitability of health-based guidance values for possible adoption/adaptation

Candidate guidance values for GenX Chemicals described in **Section 4.1** for possible adoption/adaptation in Australia have also been evaluated using the Assessment Tool provided in Appendix D in the Technical Report and already described in **Section 6.1** for PFOS.

Figure 10-1 presents the percentage of criteria (combined technical and administrative criteria) met by each jurisdiction. It is evident from the figure that the higher percentage of ‘must-have’ and ‘should-have’ criteria were met by US EPA (2021e), followed by MPART (2019a).

[24] FSANZ (2017b) converted the serum POD obtained from the Lau et al. (2006) study to a HED by multiplying by the clearance rate for PFOA in humans (i.e. 0.00014 L/kg/day) prior to applying the composite uncertainty factor. Burgoon et al. (2023) applied the composite uncertainty factor of 63 to the serum POD [i.e. 23 mg/L ÷ 63 = 0.3 mg/L (rounded)] and then applied a clearance rate for PFOA in humans of 0.00023 L/kg/day [the geometric mean clearance rate from Zhang et al. (2013)] to derive the guidance value of 0.00007 mg/kg/day (i.e. 70 ng/kg/day).

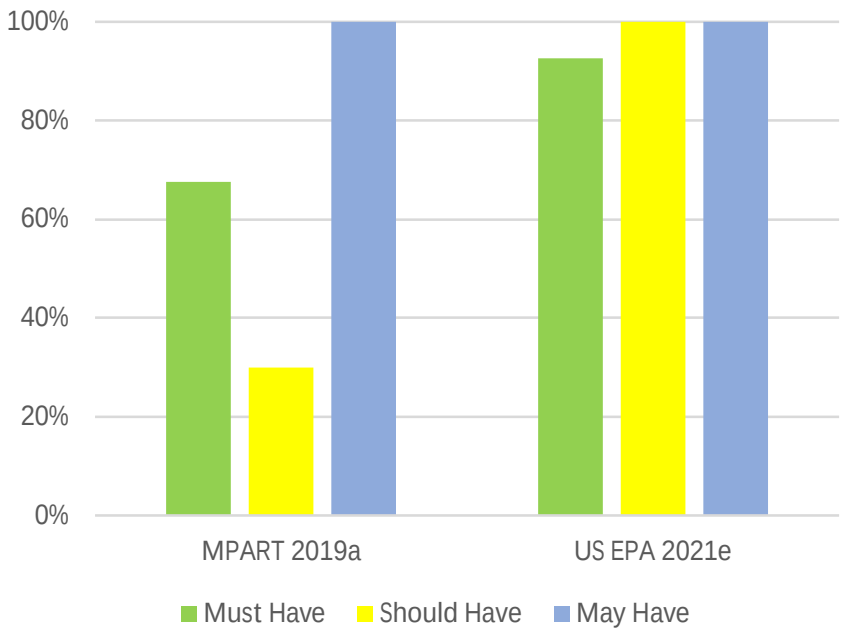


Figure 10-1 Overall proportion of 'must-have', 'should-have' and 'may-have' technical/administrative criteria as per the Assessment Tool met by jurisdictions who have derived candidate guidance values for GenX chemicals for possible adoption/adaptation in Australia

10.2 Critical evaluation of GenX Chemicals guidance values

As GenX Chemicals were not part of the comprehensive review undertaken by FSANZ (2017b), all guidance values sourced in the literature search for which the derivation was described were evaluated in this section. These include the following.

- 3 ng/kg/day (US EPA 2021e, 2022c, j; also adopted by WSDH 2022, 2023a and NJDEP 2023a) (liver effects in mice; critical study: DuPont 2010 unpublished study).
- 77 ng/kg/day (MPART 2019a) (liver effects in mice; critical study: DuPont 2010 unpublished study).

Both jurisdictions have agreed that the most sensitive health endpoint is liver effects (increased absolute and relative weight and histopathologic findings, i.e. liver single cell necrosis in parental mice) from an unpublished Reproduction/ Developmental Toxicity Study in Mice [conducted according to OECD TG 421; modified according to the Consent Order, DuPont-18405-1037 (2010)]. As the original study is not available to SLR, descriptions in the next section rely on the descriptions provided in the reviews by MPART (2019a) and US EPA (2021e).

10.2.1 DuPont (2010) – used by MPART (2019a) and US EPA (2021e)

DuPont (2010) conducted a combined oral gavage reproductive/developmental toxicity study in mice with HFPO dimer acid ammonium salt (GenX), administering the chemical (purity 84%) to Crl:CD1(ICR) mice (25/sex/group) in deionised water at doses of 0, 0.1, 0.5, or 5 mg/kg/day. Parental males were dosed for 70 days prior to mating and throughout mating through one day prior to scheduled termination, for a total of 84-85 doses. Parental females were dosed for two weeks prior to pairing and through lactation day (LD) 20 for a total of 53-65 doses. F1 females (offspring) were dosed daily beginning on PND21 through PND40.

US EPA evaluated the methods and data submitted as part of the DuPont (2010) unpublished study and deemed the study acceptable; they also requested an independent review of the study by the National Toxicology Program. The study was conducted according to OECD Test Guideline 421 and followed Good Laboratory Practices (GLP). This study was accompanied by additional testing also considered by the US EPA; the additional testing included repeated dose metabolism and pharmacokinetic studies in mice and rats, 90-day oral gavage toxicity study in mice and rats, and a combined chronic toxicity / carcinogenicity study in rats.

In GenX exposed males, the following effects were observed.

- ≥ 0.5 mg/kg/day: Increased absolute and relative liver weight and histopathological findings (increases in hepatocellular hypertrophy, single-cell necrosis, mitotic figures and lipofuscin pigment). Mild increases in tubular cell hypertrophy in kidneys of males.
- 5 mg/kg/day: F1 pups exhibited lower mean body weights at PND 4, 7, 14, 21, 28, 35, and 40. Delay in balanopreputial separation was also observed but was considered by US EPA to be of equivocal biological significance. Final body weight was significantly increased from controls by 9%.

In females, the following effects were observed.

- ≥ 0.5 mg/kg/day: Increased absolute and relative liver weight and histopathological findings (increases in hepatocellular hypertrophy, single-cell necrosis, mitotic figures and lipofuscin pigment).
- 5 mg/kg/day: F1 pups exhibited lower mean body weights at PND 4, 7, 14, 21, and 28. Delay in vaginal patency was also observed but was considered by US EPA to be of equivocal biological significance. Final body weight was significantly increased from controls by 14%. Increased relative kidney weight (by 6.5%) compared to controls in parental females.

Three males (one in each dose group) and six females (one in control, three in low dose, one each in mid- and high- dose groups) did not survive until scheduled sacrifice; the cause of death was undetermined in all cases except the male in the mid-dose group, which appeared to have ulcerative dermatitis. Due to the lack of dose response, the study authors concluded that these deaths were not treatment related.

No treatment-related effects were identified for reproductive parameters (mating, fertility and copulation indices; mean days between pairing and coitus). No treatment-related effects were observed for mean gestation length, mean numbers of implantation sites, mean numbers of pups born, live litter size, percentage of males at birth, postnatal survival, or general condition of pups. The NOAEL was 0.1 mg/kg/day. No plasma/serum concentration measurements were reported in the study descriptions by MPART (2019a) and US EPA (2021e).

From the study descriptions provided in MPART (2019a) and US EPA (2021e), and the independent review of the study findings by NTP, the unpublished DuPont (2010) study was conducted in accordance with relevant standardised testing guidelines and evaluated a range of endpoints. Therefore, it is concluded to be appropriate information to potentially adopt/adapt for derivation of candidate guidance/guideline values for GenX Chemicals. The candidate guidance/guideline values are summarised in **Section 10.3**.

10.3 Candidate guidance/guideline values for GenX Chemicals

As indicated in **Section 10.2.1**, the DuPont (2010) study likely represents suitable information for potential guidance value derivation for GenX Chemicals. The study was used

by two jurisdictions (MPART 2019a; US EPA 2021e; the latter also adopted by WSDH 2022, 2023a and NJDEP 2023a 2023a) to derive a guidance value for GenX Chemicals, of which US EPA (2021e) met a higher proportion of technical/administrative criteria for potential adoption/adaptation into the Guidelines (**Section 10.1**).

The two jurisdictions who derived a guidance value for GenX Chemicals using the DuPont (2010) study used similar PODs; MPART (2019a) used a $BMDL_{10}$ of 0.15 mg/kg/day for liver single cell necrosis, whereas US EPA (2021e) used a $BMDL_{10}$ of 0.09 mg/kg/day for the constellation of liver lesions in parental females. Both jurisdictions used an allometric scaling approach to translate the POD to a HED POD[25] by applying a factor of 0.15 to the POD. This gave HED PODs of 0.01 mg/kg/day (US EPA 2021e) or 0.0225 mg/kg/day (MPART 2019a).

The jurisdictions then applied different uncertainty factors (300 or 3,000) to their HED POD (see **Table 10-1**). The difference is due to application of an additional uncertainty factor of 10 by US EPA (2021e) for database uncertainties. However, as discussed for PFHxS in **Section 7.3**, it is not considered warranted to apply full uncertainty factors of 10x each for both the use of a subchronic study and database uncertainties.

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 that both jurisdictions which have derived DWGs in the literature consulted applied an RSC of 0.2 (i.e. 20%) (e.g. MPART 2019a, US EPA 2022j) but do not provide the rationale for this. Thus, the default factor of 0.1 has been retained in calculating the potential resulting DWGs for GenX Chemicals using these guidance values in **Table 10-1**, noting that it yields a lower guideline value than use of an RSC of 0.2.

Table 10-1 Potential drinking water guideline values (ng/L) resulting from adaptation of GenX Chemicals guidance values from different jurisdictions based on DuPont (2010)

Parameter	MPART 2019a	US EPA 2021e, 2022c, j; also adopted by WSDH 2022, 2023a and NJDEP 2023a
Critical study	DuPont 2010	
Study population	Mice	
Form of GenX studied	HFPO dimer acid ammonium salt	
Exposure route	Oral (gavage)	
Study timeframe	Combined reproductive/developmental toxicity (Parental males = 70 days prior to mating and throughout mating through one day prior to scheduled termination for a total of 84-85 doses. Parental females = two weeks prior to pairing and through LD 20 for a total of 53-65 doses. F1 females (offspring) = daily beginning on PND21 through PND40).	
Critical Effect	Liver single cell necrosis in parental males	Constellation of liver lesions in parental females
Point of Departure (mg/kg/day)	$BMDL_{10}$ = 0.15	$BMDL_{10}$ = 0.09

[25] The approach involves $BW^{3/4}$ scaling, i.e. (body weight in animal^{¼} ÷ body weight in human^{¼}) = [(0.0372 kg in male mouse)$^{1/4}$ ÷ (80 kg)$^{1/4}$] = 0.15. If the convention of 70 kg used in the Guidelines were used in this equation, the factor of 0.15 would remain unchanged, so this has no influence on the POD.

Parameter		MPART 2019a	US EPA 2021e, 2022c, j; also adopted by WSDH 2022, 2023a and NJDEP 2023a
Allometric dosing conversion factor (2)		0.15	0.15
Point of Departure HED (mg/kg/day)		0.0225	0.01
Uncertainty factors	UF_A	3	3
	UF_H	10	10
	$UF_{timeframe}$	3	10
	$UF_{database}$	3	10
	$UF_{composite}$	300	3,000
Health-based guidance value (ng/kg/day)		75	3.3
Relative source contribution (RSC) to drinking water		0.1	0.1
Resulting adaptation to a Health-based DWG(1) (ng/L)		263	12

DWG = Drinking Water Guideline; BMDL = Lower Benchmark Dose; HED = Human Equivalent Dose; LD = Lactation Day; PND = Postnatal Day; UF_A = Uncertainty factor for extrapolation from animals to humans; UF_H = Uncertainty factor for human variability; $UF_{timeframe}$ = Uncertainty factor for use of a short-term study; $UF_{composite}$ = Composite (i.e. total) uncertainty factor; $UF_{database}$ = Uncertainty factor to account for the limited database of toxicological studies (e.g. no two-generation or immunotoxicity studies).

(1) Adaptation 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

(2) The approach involves $BW^{3/4}$ scaling, i.e. (body weight in animal^{¼} ÷ body weight in human^{¼}) = [(0.0372 kg in male mouse)$^{1/4}$ ÷ (80 kg)$^{1/4}$] = 0.15. If the convention of 70 kg used in the Guidelines were used in this equation, the factor of 0.15 would remain unchanged, so this has no influence on the POD.

The candidate GenX Chemicals DWGs derived by adapting existing guidance values for this PFAS are 263 ng/L using the uncertainty factors used by MPART (2019a) or 12 ng/L using the additional uncertainty factor employed by US EPA (2021e). As discussed in the text preceding the table, the main difference between the two values is the application of higher uncertainty factors (10 each for timeframe and database deficiencies by US EPA 2021e, 2022c, j; but only 3 each by MPART 2019a).

However, it is noted that there is only one toxicological study available on which to base a candidate DWG. There is also concern with respect to the reported purity (i.e. 84%) of GenX in the DuPont (2010) study. Therefore, a value of 263 ng/L could be regarded as a concentration of potential concern rather than a DWG *per se*.

Unfortunately, no information regarding GenX Chemical levels in Australian distributed drinking water was identified from the literature retrieved. Therefore, it is unknown whether GenX Chemicals are present at concentrations lower or higher than the concentration of potential concern. It is suggested additional research is needed to determine whether GenX Chemicals are found in any Australian drinking waters, which would also inform whether a health-based DWG is required.

11.0 Conclusions

The targeted screening of existing health-based guidance/guideline values for the five PFAS of interest identified numerous candidate guidance/guideline values for potential adoption/adaptation.

The volume of information found and needing to be assessed was very large. Due to resource constraints and with agreement from NHMRC with advice from the Committee, critical evaluation of studies was prioritised to those studies that had not been previously reviewed and/or considered by an Australian agency for guidance/guideline value development. The latest review by an Australian jurisdiction in which guidance values were derived for three of the PFAS under consideration (PFOS+PFHxS and PFOA) was the FSANZ (2017b) document. This forms the basis of the current TRVs for PFOS/PFHxS and PFOA which have been used by NHMRC to derive the current guideline values in drinking water for these chemicals. FSANZ (2021) also published a review of immunomodulation effects, in which the jurisdiction reviewed a number of studies, findings of which are used to support discussions in this report on relevant PFAS.

A summary of the conclusions and DWG options from potential adoption/adaptation of suitable information for each of the five PFAS is provided in **Table 11-1**. Bolded guideline values in the table below are considered to be most relevant to the Australian context in terms of selection of uncertainty factors and endpoints.

Table 11-1Conclusions and DWG options from potential adoption/adaptation of suitable information for PFOS, PFHxS, PFBS, PFOA, and GenX Chemicals

PFAS	Candidate DWGs (ng/L) (1)	Conclusion
PFOS		Although 10 health-based guidance values for potential adoption/adaptation were sourced from international jurisdictions reviewed for this report, only two of these used data in the derivation that had previously not been considered / evaluated by FSANZ (2017b). These were the EFSA (2020a) and US EPA (2022c, e; 2021b) guidance values for PFOS, which used two studies to underpin the derivation that had not been previously considered / evaluated by FSANZ (2017b), i.e. Abraham et al. (2020) and Budtz-Jørensen and Grandjean (2018). Based on a brief critical evaluation of the two studies, consistent with the conclusions made by FSANZ (2021), it is concluded that a causal relationship between increased PFAS serum levels and impaired vaccine response cannot be established with reasonable confidence from the available human epidemiological information. The evidence for an association between increasing PFAS serum levels and impaired vaccine response is insufficient for the endpoint to be used for derivation of PFOS TRVs. It is therefore concluded the current Australian guidance value for PFOS of 20 ng/kg/day and guideline value of **70 ng/L** are still considered appropriate. It is therefore considered reasonable to retain the existing guideline value of **70 ng/L** as the sum of PFOS+PFHxS, in line with the recommendations from enHealth (2016) to sum these two compounds. Concentrations of PFOS in uncontaminated distributed drinking water or raw water catchments in Australia can range up to 6 ng/L in Queensland (QAEHS 2018a, 2018b) and Sydney (Sydney Water 2023) but up to 16 ng/L in Australia according to WHO (2022). PFOS+PFHxS concentration was found to be at 90% of the Australian DWG (i.e. ~60 ng/L) in one bore in a drinking water borefield supplying Esperance, Western Australia (WCWA 2019, 2020). Once this apparent PFOS/PFHxS contamination was identified, this bore was no longer used (WCWA 2023). Thus, provided drinking water catchments are protected from PFOS contamination and alternative water supplies are available if PFOS contamination is identified, 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

PFAS	Candidate DWGs (ng/L) [1]	Conclusion
	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 exceeding the existing Australian DWG in these cases.	
PFHxS	• 8.5 ng/L using UF from OEHHA (2022a), or • **34 ng/L** using UF from MDH (2020b) and MPART (2019a). Both of these candidate DWGs use the same toxicological study in rats (NTP 2022). It is noted there is uncertainty with respect to human relevancy of the critical endpoint (decreased thyroid hormone levels) from this study based on currently available information and therefore the resulting candidate guidance value is likely conservative. It is also considered reasonable to retain the existing guideline value of **70 ng/L** as the sum of PFOS+PFHxS.	The value of 34 ng/L for PFHxS and the existing 70 ng/L guideline value for PFOS+PFHxS are considered to be appropriate based on currently available information. In practice this means it is considered reasonable to retain the existing guideline value of **70 ng/L** as the sum of PFOS+PFHxS, with PFHxS not exceeding **34 ng/L**. In Australian raw water catchments and distributed drinking waters, PFHxS concentrations generally may range from <2 to 5 ng/L in Queensland (QAEHS 2018a, 2018b), Sydney (Sydney Water 2023) and Western Australia (WCWA 2023) which are below both candidate DWGs. However, PFOS + PFHxS concentration was measured at 90% of the current Australian DWG (i.e. ~ 60 ng/L) in one bore in a drinking water borefield supplying Esperance, Western Australia (WCWA 2019, 2020). Once this apparent PFOS/PFHxS contamination was identified, this bore was no longer used (WCWA 2023). This indicates that compliance with the candidate DWGs for PFHxS may present an issue in certain circumstances. Nevertheless, due to the large uncertainty factors and small RSC of 10% incorporated into the derivation of the candidate DWGs, PFHxS 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), PFHxS may be present at concentrations greater than the candidate DWGs and the existing Australian DWG in these cases.
PFBS	• **2,939**[2] **ng/L** using rat toxicology study (NTP 2022) (used by MDH 2022e,g), or • **1,041** to **2,252 ng/L** using a mouse toxicology study by Feng et al. (2017) (used by MPART 2019a; US EPA 2021c, 2022c, k; OEHHA 2021d; WSDH 2019a, 2022b, 2023a).	Any of the values in the range of **1,041** to **2,939 ng/L** would be appropriate at this time. These values are also likely conservative due to time of serum collection after the last administered dose; due to the short half-life of PFBS, serum concentrations in dams in both studies may have been 2-3x higher directly after administration of the last dose. Using higher serum concentrations to derive guidance values would also result in higher (i.e. less stringent) guideline values.

PFAS	Candidate DWGs (ng/L) [1]	Conclusion
	It is noted there is uncertainty with respect to human relevancy of the critical endpoint (decreased thyroid hormone levels) from these studies based on currently available information and therefore the resulting guidance values are likely conservative.	In Queensland raw water catchments, PFBS concentrations have been recorded up to 2.2 ng/L (QAEHS 2018a, 2018b). There are few available PFBS data in distributed drinking water elsewhere in Australia. Based on the limited data available, provided drinking water catchments are protected from PFBS contamination and alternative water supplies are available if PFBS contamination is identified, it appears that PFBS concentrations in distributed drinking water in Australia are markedly lower than any of the candidate DWGs, suggesting PFBS is unlikely to present a human health risk from distributed drinking water in 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), PFBS may be present at concentrations greater than the candidate DWGs in these cases.
PFOA	• 71[2] ng/L using toxicology study in mice (Loveless et al. 2006) (used by NJDEP 2019a), or • 9.6 ng/L using developmental toxicology study in mice (Koskela et al. 2016) (used by ATSDR 2021a), or • 45[2] ng/L using developmental toxicology study in mice (Koskela et al. 2016, Onishchenko et al. 2011) (used by MPART 2019a), or • 16[2] ng/L using toxicology study in mice (Li et al. 2017) (used by OEHHA 2019a). It is noted that, due to various reasons outlined in **Sections 9.2.1** to **9.2.5**, the confidence in the candidate guideline values is considered very low to low. It is therefore suggested the information is not of high enough quality to warrant revision of the existing Australian guideline value for PFOA (**560 ng/L**).	Any of these values would be conservative as they all incorporate at least an uncertainty factor of 30x 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. However, since the candidate guideline values for PFOA (9.6 to 71 ng/L) are based on data from studies considered to be of very low to low confidence for guideline derivation, it is suggested the information is not of high enough quality to warrant revision of the existing Australian guideline value for PFOA (**560 ng/L**), for which the confidence in the underpinning study is high. In Australian distributed drinking waters and raw water catchments, PFOA concentrations generally may range up to 10 ng/L in various locations (QAEHS 2018a, 2018b, Sydney Water 2023, WHO 2022, WCWA 2023). This maximum measured concentration is at or below the candidate DWGs. Due to the uncertainty factors and small RSC incorporated into the derivation of the candidate DWGs, PFOA is unlikely to present a human health risk from distributed drinking water in uncontaminated regions of Australia. However, this is based on limited data and it would be worthwhile to undertake additional analysis of PFOA in distributed drinking water. There are many sites of PFAS contamination in Australia, and, if water from

PFAS	Candidate DWGs (ng/L) (1)	Conclusion
		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.
GenX Chemicals	• **263 ng/L** using reproductive / developmental toxicology study in mice (DuPont 2010) using uncertainty factors used by MPART (2019a), or • 12 ng/L using the same study but using an additional uncertainty factor employed by US EPA (2021e).	There is currently insufficient evidence to derive a health-based DWG for GenX Chemicals. However, a concentration of potential concern of 263 ng/L could be derived based on the limited toxicity data available. Concentrations of GenX Chemicals in overseas distributed drinking waters (<5 ng/L) are lower than the concentration of potential concern. Unfortunately, no information regarding GenX Chemical levels in Australian distributed drinking water was identified from the literature retrieved. Therefore, it is unknown whether GenX Chemicals are present at concentrations lower or higher than the candidate DWGs in Australia. It is suggested additional research is needed to determine whether GenX Chemicals are found in any Australian drinking waters, which would also inform whether a health-based DWG is required.

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 (see detailed discussions in **Section 6.0** to **10.0** 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 (2017b). However, it is noted that the candidate guideline values for PFOA (9.5 to 70 ng/L) are based on data from studies considered to be of very low to low confidence for guideline derivation.

From the available information gathered on exposure to the five PFAS of interest in Australian distributed drinking waters and the information gathered to inform supporting information in the Fact Sheet, all DWG options would be readily measurable with current commercial analytical techniques. Although existing treatment technologies do not appear to be particularly effective at removing PFAS from water, DWG options are/would be achievable if uncontaminated source waters are utilised. However, the DWG options may not be achievable for local drinking water supplies in contaminated areas without addition of a PFAS-removal treatment step or use of an alternative water supply.

12.0 Review Team

Name	Position	Responsibilities
Ms Tarah Hagen, MSc, DABT, RACTRA	Technical Director – Toxicology & Risk Assessment, SLR	Report author and technical oversight of literature review

Name	Position	Responsibilities
Ms Maria Consuelo Reyes Campos, MSc	Project Consultant – Land Quality & Remediation	Literature searching, preliminary title screen, compilation of Appendix A
Mr Giorgio De Nola, MSc, RACTRA	Principal Consultant – Toxicology & Risk Assessment, SLR	Data extraction, internal peer review

13.0 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 this review as described in the Research Protocol. • Been involved in preparation and/or review of draft and final Technical and Evaluation Reports for previous and/or current consultancies with NH&MRC (evidence evaluations for 11 inorganic chemicals, full reviews for 4 inorganic chemicals). • 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 2017b). This paper is mentioned in the context of the previous review by FSANZ (2017b).
Ms Maria Consuelo Reyes Campos	As part day-to-day consulting activities at SLR Consulting Ms Reyes Campos has: • Been involved in literature searching for a current consultancy with NH&MRC (full evidence evaluation for 4 inorganic chemicals).
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 and/or current consultancies with NH&MRC (evidence evaluations for 11 inorganic chemicals, full reviews for 4 inorganic chemicals). • 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.

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Appendix A List of Critical Studies Underpinning Guidance Value Derivation

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 Report

National Health and Medical Research Council

SLR Project No.: 640.V30693.20000

17 October 2024

The full list of critical studies underpinning each of the guidance values derived by various national and international jurisdictions (for which data extraction is provided in the accompanying Technical Report) is shown in **Table A-1** below, along with an indication of whether or not the critical study had been previously evaluated / considered by FSANZ (2017b, 2021). If they have been previously evaluated, the response to the question in the table 'Previously Evaluated / Considered by FSANZ?' would be 'Yes' and this is denoted with a tick (i.e. '✓'); conversely if the study(ies) have not been previously evaluated by FSANZ (2017b, 2021), the response to the question in the table would be 'No' and this is denoted with a cross (i.e. '×'). Note the guidance values which have been subjected to further critical evaluation are those marked with a cross in the FSANZ (2017b) column (i.e. '×') in the **Table A-1**, i.e. those not previously evaluated / considered by FSANZ (2017b, 2021). If studies are marked with a tick (i.e. '✓') in that column, these critical studies have not been subjected to further evaluation in this report.

Table A-1: List of critical studies underpinning each of the guidance values for the five PFAS covered in this review and indication of whether or not the critical study had been previously evaluated / considered by FSANZ (2017b, 2021)

Jurisdiction Reference	Critical Study(ies) Underpinning Guidance Value	Previously Evaluated / Considered by FSANZ?	
		FSANZ (2017b)	FSANZ (2021)
PFOS			
ATSDR (2021a)	Luebker et al. 2005	✓	×
BfR (2019a)	Steenland et al. 2009	✓	×
	Eriksen et al. 2013	✓	×
	Nelson et al. 2010	✓	×
EFSA (2020)	Abraham et al. 2020	×	✓
FSANZ (2017b)	Luebker et al. 2005	✓	×
	Seacat et al. 2002	✓	×
	Butenhoff et al. 2012b/ Thomford 2002	✓	×
	Thibodeaux et al. 2003 / Lau et al. 2003	✓	×
HC 2018a	Butenhoff et al. 2012b	✓	×
MDH 2020a	Dong et al. 2011	✓	×
MPART 2019a	Dong et al. 2009	✓	×
NJDEP 2019b	Dong et al. 2009	✓	×
	Butenhoff et al. 2012b	✓	×
	Dong et al. 2012	✓	×
OEHHA 2019a	Dong et al. 2009	✓	×
OEHHA 2023a	Steenland et al. 2009	✓	×
RIVM 2021a (same as EFSA)	Abraham et al. 2020	×	✓
	Grandjean et al. 2012	✓	✓

Jurisdiction Reference	Critical Study(ies) Underpinning Guidance Value	Previously Evaluated / Considered by FSANZ?	
		FSANZ (2017b)	FSANZ (2021)
US EPA 2021b, 2022c, e	Budtz-Jørgensen and Grandjean 2018	×	×
WSDH 2019a, 2022b, 2023a	Dong et al. 2011	✓	×
PFHxS			
ATSDR 2021a	Butenhoff et al. 2009a	✓	×
EFSA 2020a	Abraham et al. 2020	×	✓
MDH 2020b	NTP 2018, 2019, 2022	×	×
MPART 2019a	NTP 2018, 2019, 2022	×	×
OEHHA 2022a	NTP 2018, 2019, 2022	×	×
US EPA 2023	Grandjean et al. 2012	✓	✓
	Budtz-Jørgensen and Grandjean 2018	×	×
WSDH 2019a, 2022b, 2023a	NTP 2018, 2019, 2022	×	×
PFBS			
MDH 2022e, g	NTP 2022	×	×
MPART 2019a	Feng et al. 2017	×	×
OEHHA 2021d	NTP 2022	×	×
	Feng et al. 2017	×	×
US EPA 2021c, 2022c, k	Feng et al. 2017	×	×
WSDH 2019a, 2022b, 2023a	Feng et al. 2017	×	×
PFOA			
ATSDR 2021a	Koskela et al. 2016	×	×
BfR 2019a	Steenland et al. 2009	✓	×
	Eriksen et al. 2013	✓	×
	Nelson et al. 2010	✓	×
EFSA (2020)	Abraham et al. 2020	×	✓
FSANZ 2017b	Lau et al. 2006	✓	×
	Butenhoff et al. 2002	✓	×
	Perkins et al. 2004	✓	×
HC 2018b	Perkins et al. 2004	✓	×
MDH 2022d, f	Lau et al. 2006	✓	×
MPART 2019a	Koskela et al. 2016	×	×
	Onishchenko et al. 2011	×	×

Jurisdiction Reference	Critical Study(ies) Underpinning Guidance Value	Previously Evaluated / Considered by FSANZ?	
		FSANZ (2017b)	FSANZ (2021)
NJDEP 2019a	Loveless et al. 2006	×	×
OEHHA 2019a	Li et al. 2017	×	×
OEHHA 2023a	Gallo et al. 2012	×	×
US EPA 2021a, 2022c, d	Grandjean et al. 2012	✓	✓
	Budtz-Jørgensen and Grandjean 2018	×	×
WSDH 2019a, 2022b, 2023a	Koskela et al. 2016	×	×
	Onishchenko et al. 2011	×	×
GenX Chemicals			
MPART 2019a	DuPont 2010	×	×
NJDEP 2023a	DuPont 2010	×	×
US EPA 2021e, 2022c, 2022j	DuPont 2010	×	×

✓ = This study was previously evaluated / considered by FSANZ (2017b) or FSANZ (2021). × = This study has not been previously evaluated / considered by FSANZ (2017b) or FSANZ (2021).

Grey shading indicates the guidance value is based on an underpinning critical study which has not been previously evaluated / considered by FSANZ (2017b), and therefore has been further considered in this Evaluation Report (see also addendum to Research Protocol in **Section 3.4** of Technical Report).

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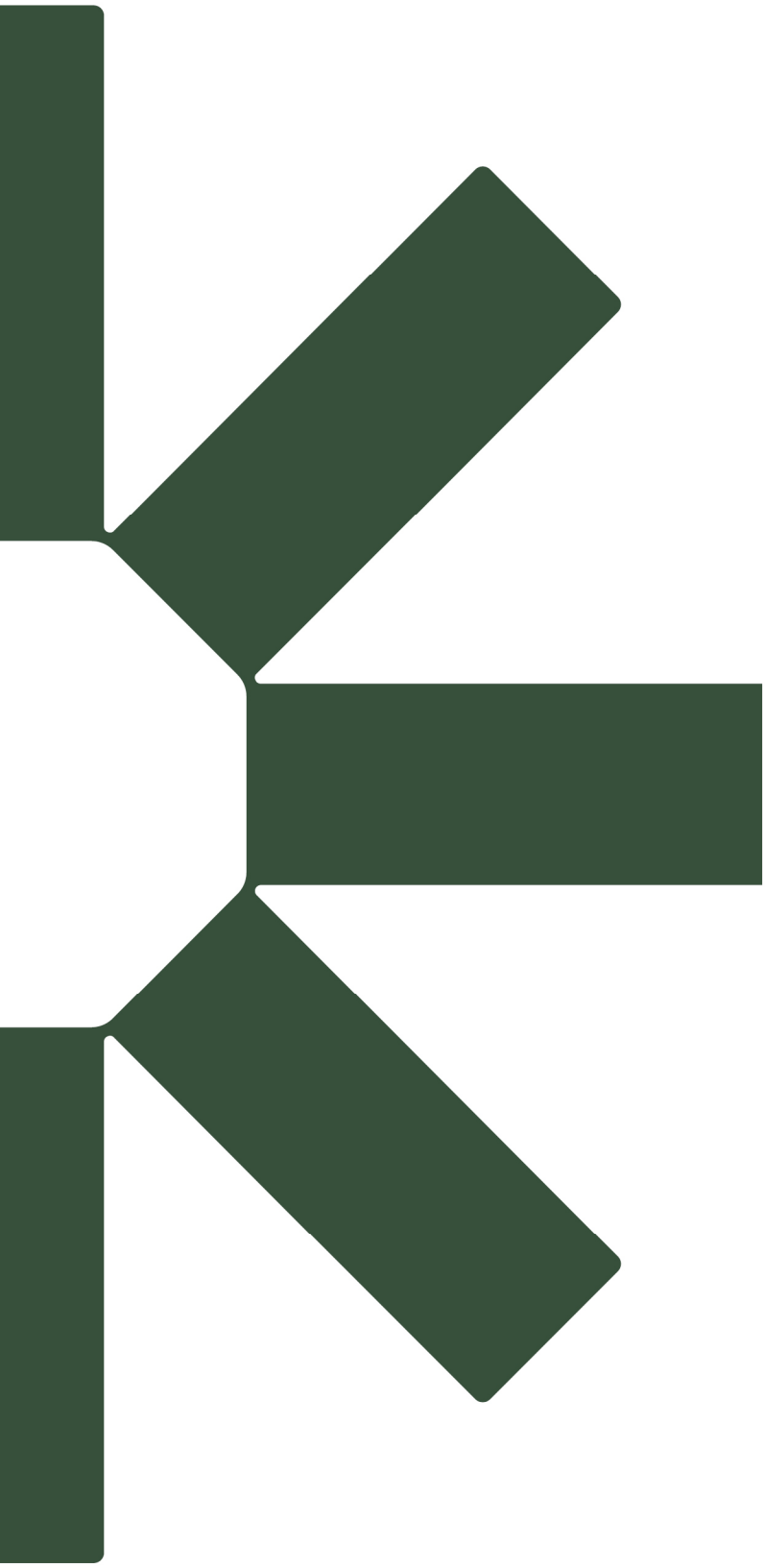

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