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PFOS and PFOA in Drinking-water

Background document for development of
WHO Guidelines for Drinking-water Quality

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Preface

To be completed by WHO Secretariat

Acknowledgements

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Acronyms and abbreviations

BMD	benchmark dose
$BMDL_{10}$	95% lower confidence limit on the benchmark dose for a 10% response
$BMDU_{10}$	95% upper confidence limit on the benchmark dose for a 10% response
bw	body weight
CI	confidence interval
CONTAM Panel	Panel on Contaminants in the Food Chain (European Food Safety Authority)
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
GDWQ	*Guidelines for drinking-water quality*
GV	guideline value
HBGV	health based guidance value
LOAEL	lowest-observed-adverse-effect level
MOE	margin of exposure
NOAEL	no-observed-adverse-effect level
OR	odds ratio
PFAS	per- and polyfluoroalkyl substances
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
USA	United States of America
WHO	World Health Organization

Contents

The scope of this background document includes PFOA and PFOS, with more limited consideration and information provided on other PFAS. The evidence presented is primarily drawn from existing authoritative reviews, including those from the Agency for Toxic Substances and Disease Registry (ATSDR), the European Food Safety Authority (EFSA), Health Canada, and the Environmental Protection Agency (US EPA). Examples of robust studies from the primary literature are also described to provide an overview of health effects in humans and animals. However, this document is not intended as a comprehensive summary of the primary literature and not all studies are cited.

EXECUTIVE SUMMARY

To be written after public review

1. GENERAL DESCRIPTION

Per- and polyfluoroalkyl substances (PFAS) is the collective name for a large group of fluorinated compounds. As of 2018 approximately 4730 substances were identified to be on, or likely to have been on, the global market (OECD, 2018). The perfluoroalkyl moieties of PFOS and PFOA are hydrophobic and lipophilic, whereas their acid groups (sulfonate or carboxylate) are hydrophilic, add polarity and increase acidity. Although the stability of PFOS and PFOA as well as their surfactant properties make them useful in consumer and industrial applications, their persistence can be of concern regarding environmental and human health. PFAS compounds are distributed across the globe and their degradation products occur in biota and environmental media, often at great distances from their original source. Therefore, due to these concerns and the resulting increase in regulatory restrictions globally, the commercial use of these compounds has declined in recent years.

1.1. Identity

The OECD has defined PFAS as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it). In other words, with a few noted exceptions, any chemical with at least one perfluorinated methyl group (–CF3) or one perfluorinated methylene group (–CF2–) is a PFAS. In these cases, both a perfluorinated methyl group and a perfluorinated methylene group are considered saturated and aliphatic (OECD, 2021).

PFAS as a class encompass a wide range of chemical substances, including high molecular weight fluoropolymers, oligomeric substances, surface-active compounds, and low molecular weight volatile substances. Similarly, PFAS are used in a wide range of applications, such as aerosol propellants, solvents, pesticides, antifoaming agents; surface treatments for textiles, leather, masonry and paper and board; leveling agents in paints, coatings and waxes; plastics; lubricants and greases; and fire-fighting foams. An overview of the more than 200 use categories for more than 1400 PFAS have been identified and published (Glüge et al., 2020).

There are a wide range of PFAS that contain a chain of aliphatic carbon atoms that are fully fluorinated and terminated with a perfluorinated methyl group ($–CF_3$). The fluorocarbon moiety is frequently functionalized and used to chemically link the perfluoroalkyl moiety into more complex molecules, such as so-called “side-chain fluorinated polymers” or surface-active chemicals. While these molecules contain the persistent perfluoroalkyl moiety, other portions of the molecule may degrade biotically or abiotically to liberate the fully fluorinated

perfluoroalkyl acids (PFAAs). Complex PFAS that can yield these highly persistent PFAS are referred to as precursor substances. PFAAs can be further delineated into perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) and these have varied chain lengths. PFCAs containing ≥ 7 perfluorinated carbon atoms and PFSAs containing ≥ 6 perfluorinated carbon atoms are classed as long-chain substances (ATSDR, 2021). The most widely studied of these PFAAs are perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) which belong to the category of perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) respectively.

PFOS

Chemical Name: Perfluorooctanesulfonic acid
CAS No: 1763-23-1
Formula: $C_8HF_{17}O_3S$

PFOA

Chemical Name: Perfluorooctanoic acid
CAS No: 335-67-1
Formula: $C_8HF_{15}O_2$

1.2. Physicochemical properties, including speciation

The physicochemical properties of PFOS and PFOA are shown in table 1.1. In general, both compounds are lipophilic and soluble in water to varying degrees, with PFOS being more hydrophobic and less water-soluble than PFOA.

Table 1.1: Physicochemical properties of PFOS and PFOA

Property	PFOS	PFOA
Molecular Weight (grams per mole [g/mol])	500.13	414.09
Colour/Physical State	White powder (potassium salt)	White powder (ammonia salt)
Boiling Point	133 – 249 ºC (experimental) 219 – 244 ºC (predicted)	188 - 199 ºC (experimental) Stable when bound
Melting Point	15.2 – 185 ºC (predicted)	47.5 – 59.5 ºC (experimental) ≥400°C (potassium salt) [#]
Vapor Pressure	2.48 x 10^{-6} millimeter Mercury (mm Hg) at 25 ºC (experimental and predicted)	1.65 x 10^{-2} – 10.0 mm Hg at 25 ºC (experimental) 0.111 – 0.345 mm Hg at 25 °C (predicted)
Henry's Law Constant	1.8 x 10^{-11} atm-m3/mole (predicted)	1.92 x 10^{-10} atm-m3/mole (predicted)
Log K_{ow}: Octanol-Water	4.30 – 7.03 (experimental)	1.92 – 3.6 (experimental)
Organic carbon water partitioning coefficient (K_{oc})	2.57	2.06
Solubility in Water	680 mg/L	9.50 x 10^3 mg/L at 25 ºC (estimated)
Half-life in Water	Stable (41 years) [#]	Stable (92 years) [#]
Half-life in Air	Stable (114 days) [#]	Stable when bound

All data adapted from US EPA (https://comptox.epa.gov/dashboard/DTXSID3031864 (accessed March 20, 2021) except that marked [#] which was sourced from ATSDR 2021.

The C8 perfluoroalkyl moiety of PFOS is hydrophobic and lipophobic while the sulfonic acid (or sulfonate group – its conjugate base) adds polarity. PFOS is exceptionally stable due to the strength of the carbon-fluorine bond which is the strongest covalent bond in organic chemistry. PFOS is a surface-active substance which lowers the surface tension of water more than that of hydrocarbon surfactants. Attention is typically focused on the straight-chain isomer (n-PFOS) which is dominant in commercial mixtures and environmental samples.

PFOA contains a C7 perfluoroalkyl moiety that is hydrophobic and lipophobic while the carboxylic acid (or carboxylate group) is hydrophilic and adds polarity. PFOA is exceptionally stable due to the strength of the carbon-fluorine bond that is the strongest covalent bond in organic chemistry. PFOA is a surface-active substance that lowers the surface tension of water more than that of hydrocarbon surfactants. Attention is typically focused on the straight-chain isomer (n-PFOA) which is dominant in commercial mixtures and environmental samples.

1.3. Organoleptic properties

No information could be identified indicating taste or odour thresholds for PFOS or PFOA.

1.4. Major uses and sources

1.4.1. PFOS

PFOS and its precursors are widely used in a number of applications. PFOS precursors include high molecular weight urethane and acrylate polymers, phosphate esters and low molecular weight substances) (3M, 1999). The principal uses are for water, oil soil and grease repellents used as surface treatments for a variety of substances such as paper and board; leather; masonry; textiles, carpet, fabric and upholstery. Specialised chemical applications include mining and oil well surfactants, hydraulic fluids and use in fire-fighting foams.

The manufacture of PFOS was largely discontinued in 2002 in the USA, when the US manufacturer 3M, ceased production[1]. In January 2009, Canada published regulations adding PFOS and its salts and precursors to its Virtual Elimination List compiled under subsection 65(2) of the Canadian Environmental Protection Act (CEPA, 1999). PFOS and its salts are listed as "persistent organic pollutants" (POPs) under the Stockholm Convention, and in May 2009, PFOS and its salts, and perfluorooctane sulfonyl fluoride were included in Annex B of the Stockholm Convention[2], which restricts its manufacture, import and export. The European Union banned PFOS use in finished and semi-finished products in 2006, and it is also regulated as a POP. However, some uses are still permitted by the EU with certain restrictions, including those relevant to photo-resistant or anti-reflective coatings, photolithography, and photographic coatings (EU, 2019). In substances or preparations, PFOS is allowed at a maximum level of 10 mg/kg, in semi-finished articles or parts it is restricted to <0.1% (by weight), and in coatings a maximum level of 1 µg/m^2 is permitted. Although most industrialised countries have ceased PFOS production, PFOS and PFOS-related chemicals are currently

[1] https://www.3m.com/3M/en_US/pfas-stewardship-us/pfas-history/ (accessed 31 March 22)

[2] Stockholm Convention decision SC-4/17 http://chm.pops.int/Portals/0/download.aspx?d=UNEP-POPS-COP.4-SC-4-17.English.pdf (accessed 5 May 22)

produced in China, which remains a major producer and user (HAES 2021; Li et al. 2015; Lim et al. 2011).

Perfluoroalkyl derivatives that can degrade to PFOS in the environment are still in wide use globally and are considered "legacy chemicals" (ATSDR, 2021; Glüge et al., 2020). Due to the curtailing of the manufacture of PFOS and PFOS-related substances in most jurisdictions and the increasing restrictions placed on PFOS precursors, manufacturers have replaced PFOS precursors with short-chain analogues, which in some cases may not have the same performance, implying that the overall production is expected to be increased. Chief amongst these are substances that can degrade to perfluorobutane sulfonic acid (PFBS) as well as precursors to perfluorohexane sulfonic acid (PFHxA) (Brendel et al., 2018, Poulson et al. 2005).

1.4.2. PFOA

The principal uses of PFOA and its precursors are for water, soil and grease repellents used as surface treatments for a variety of substrates such as paper and board; leather; masonry; textiles, carpets, fabric and upholstery. PFOA is widely used as an industrial surfactant in chemical processes and as a starting material for the manufacture of other PFAS. Specialized chemical applications of PFOA precursors include mining and oil well surfactants; leveling agents in paints, coatings and sealants; and use in fire-fighting foams. Perfluorooctanoate (the conjugate base), usually as the ammonium salt, was long used as a surfactant in the manufacture of fluoropolymers such as polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), and fluoroelastomers via emulsion polymerization. PTFE itself is a polymer used in a wide variety of applications, including non-stick coatings in kitchenware; nonreactive containers for corrosive materials; electrical wire insulation; lubricants, and many other uses.

PFOA is currently manufactured principally in China. It was also previously manufactured in the United States and Europe however major manufacturers voluntarily agreed to phase out production of PFOA by the end of 2015. (ATSDR, 2021). In October 2016, Canada published Regulations Amending the Prohibition of Certain Toxic Substances Regulations, which came into force in December 2016. These amendments prohibit PFOA, its salts and its precursors and products containing them in Canada unless present in manufactured items[3]. PFOA, its salts and PFOA-related compounds were included in Annex A of the Stockholm Convention on POPs in May 2019[4] and was designated to be completely prohibited, for manufacture, import and export; however some exemptions have been permitted with prior registration of these uses. As discussed below, exposure to PFOA remains possible, even where it is no longer manufactured or used due to its legacy uses, degradation of precursors, and extremely high persistence in the environment and the human body (Glüge et al., 2020).

Notably PFOA (along with other PFCAs – both long-chain and short-chain) can be formed by the degradation of fluorotelomer compounds and fluorotelomer derived substances. The most significant products for the fluorotelomer industry in terms of volume, the so-called "side-chain" fluorotelomer-based polymers, have been shown to degrade to form PFOA and related

[3] https://www.canada.ca/en/health-canada/services/chemical-substances/other-chemical-substances-interest/perfluorooctanoic-acid-salts-precursors.html (accessed 23 Sept 21)

[4] Stockholm Convention decision SC-9/12 http://www.pops.int/Portals/0/download.aspx?d=UNEP-POPS-COP.9-SC-9-12.English.pdf (accessed 5 May 22)

PFCA compounds with half-lives of decades, both biotically and by simple abiotic reaction with water (Washington et al., 2015; Washing and Jenkins, 2015). PFOA will be the primary degradation product, biotically and abiotically, of the 8:2 fluorotelomer alcohol $F(CF_2)_8CH_2CH_2OH$, (abbreviated 8:2 FTOH). Fluorotelomer alcohols (FTOHs) have been widely used in the production of polymers and surface coatings for decades with an estimated annual production in 2004 of 11,000 - 13,000 tonnes per year (Lindstrom et al., 2011). Fluorotelomer-based polymers are used widely as water, oil, soil and grease repellents used as surface treatments for a variety of substrates such as paper and board; leather; masonry; textiles, carpet, fabric and upholstery. Other fluorotelomer-based substances are used widely in food packaging applications, as their lipophobicity makes paper resistant to absorbing oils from fatty foods. For example, fluorotelomer coatings are used in microwave popcorn bags, fast food wrappers, and pizza boxes. Other specialized chemical applications of fluorotelomer substances include surfactants and leveling agents in paints, coatings and sealants; and use in fire-fighting foams.

1.5. Environmental fate

The partitioning, transport, and transformation of PFAS occurs across multiple media types. Most literature focuses on the PFAAs, PFOS and PFOA, and this will be the focus here. The resistance of PFOS and PFOA to biotic or abiotic degradation means that physical transport processes are critical for their transport and ultimate environmental fate. However, processes that affect precursor PFAS that can degrade to PFOS or PFOA over time and their transport processes will also be considered.

PFOS and PFOA are acids with low pKa values, which means that, at relevant environmental pH values, PFOS and PFOA are present primarily as their respective conjugate base organic anions. The anions will exhibit low volatility and low sorption coefficients such that, when released to surface water, they will tend to remain in solution; although they have also been demonstrated to associate with the organic carbon fraction that may be present in soil or sediment (Higgins et al., 2016). These anions can be found in soil and sediment due to exposure of impacted media such as the application of biosolids, landfill leachates or direct releases at manufacturing sites. Soils and sediments can then act as secondary sources to groundwater and surface water through leaching and percolation processes. PFOS and PFOA (along with other long-chain PFCAs) are typically the predominant PFAS identified in surface sediments (Rankin et al., 2017). Once in surface water, PFAAs can contaminate groundwater through groundwater recharge or can be transported to the oceans where they are then transported globally by ocean currents (Joerss, 2020). Upon release to water, oceans are likely the ultimate destination for PFOS and PFOA anions as ocean waters have been estimated to contain the majority of PFCAs historically released into the environment (Armitage et al., 2006). However, contamination of surface water and ground water with these substances is prevalent globally at sites proximate to factories, disposal sites and sites where aqueous film-forming foams (AFFFs) have been used in firefighting or training (such as airports and military bases). The estimated half-lives in water for PFOS (as its potassium salt) and PFOA are 41 years and 92 years, respectively (ATSDR, 2021).

Releases of ionic PFAS from factories to air are likely tied to particulate matter that settle to the ground in dry weather and are also scavenged by precipitation (Barton et al., 2006). The ionic forms of PFOA and PFOS, which are characterized by low vapor pressure and high-water solubility, tend to be the dominant species found in airborne particulate matter. PFOS is

generally associated with larger, coarser particles while PFOA is associated with smaller, ultrafine particles in the atmosphere (Furuuchi et al., 2017). Deposition depends on the amount of PFAS emissions, local topography, weather patterns, and release characteristics such as smokestack height, effluent flow rate, and effluent temperature.

Short-range atmospheric transport and deposition may result in PFAS contamination of terrestrial and aquatic systems proximate to sites of significant emissions, thereby contaminating soil, surface water and groundwater, as well as those several miles from these industrial emission sources (Shin et al., 2011). Therefore, while PFOS and PFOA exhibit relatively low volatility, airborne transport has been a relevant migration pathway following industrial releases from stack emissions.

Atmospheric deposition can occur via dry or wet deposition, both of which have been found relevant for PFOA (Barton et al., 2017). When precipitation washes out PFOA-containing aerosols, the process is known as wet deposition. During dry deposition, PFOA is associated with liquid or particle phases in air that can be deposited onto surfaces by sedimentation. Wet and dry deposition are the major mechanisms of removal of PFAS from the atmosphere and can occur from the scavenging of particle-bound PFAS or partitioning of gaseous PFAS to water droplets. Both wet and dry deposition are generally considered removal processes that influence local sources and reduce long-range atmospheric transport.

Both PFOS and PFOA exhibit surfactant properties because they contain a perfluoroalkyl moiety that is hydrophobic and lipophobic as well as an acid group (sulfonate or carboxylate) that is hydrophilic and adds polarity. Their surface-active properties result in films formed at the air-water interface. Experiments suggest that marine aerosols may act as a significant long-range atmospheric transport mechanism for PFOS and PFOA. PFAAs are highly enriched in sea spray aerosols and, as PFOS and PFOA do not environmentally degrade, their presence in marine aerosols can be a continuous source to terrestrial environments and subsequently to surface water and ground water (Johansson et al., 2019).

In addition to short-range transport and deposition, long-range transport processes are responsible for the wide distribution of PFAS across the globe as evidenced by their occurrence in biota and environmental media in remote regions as far as the Arctic and Antarctic (Muir et al., 2019). In contrast to anionic PFAS (PFOS and PFOA), other neutral PFAS such as fluorotelomer alcohols (FTOHs) and fluoroalkylsulfonamides (FOSAs) remain neutral at environmentally relevant pHs, have higher volatility and tend to partition into air. Some PFAS, such as FTOHs and FOSAs, once released to the air are subject to photooxidation during transport, but they can also accumulate to measurable levels in soil and surface water through atmospheric deposition (Maybury et al., 2010). PFOS and PFOA precursors, once deposited terrestrially and in surface waters, can undergo biotic and/or abiotic degradation to the PFAAs and thereby contaminate the environment at great distance from the original source.

Once airborne, neutral precursors to PFOA or PFOS can occur in a gaseous state or be associated with particulate matter or other aerosols suspended in air. Volatile precursor compounds, such as FOSAs and FTOHs have been measured over urban centres (Ahrens et al., 2012), over the open ocean (Zhiyong Xie et al., 2016) and in remote regions (Zhiyon Xie et al., 2015). In these cases, FTOHs are observed to be the dominant neutral PFAS present, almost entirely in the gas phase. Atmospheric deposition of neutral precursor substances can occur via dry or wet deposition and ultimately contribute to the global distribution of PFOS and PFOA.

2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Human exposure to PFAS, including PFOS and PFOA, occurs through multiple media and routes; dietary exposure, dust and drinking-water are key exposure routes for which quantitative exposure data are available (Domingo and Nadal, 2019).

2.1. Water

2.1.1. General water sources

PFOS and PFOA have been measured in samples representing its multiple forms and states and these data have been previously outlined in available literature (for example, see ATSDR, 2021). It is not within the scope of this document to cover these details here, however some specific examples from more recent publications (2010 – present) are outlined below to provide a general awareness of the extent of environmental levels.

United States

- PFOS and PFOA were widely detected in surface water samples collected from various rivers, lakes, and streams in the United States (ATSDR, 2021), with concentrations in surface water samples ranging between <1 ng/L to 1090 ng/L.

- Higher levels of PFOS and PFOA are detected in surface and ground waters near perfluorochemical industrial facilities with concentrations of 144 and 598 µg/L, respectively reported in surface water downstream of the 3M Decatur, Alabama facility (ATSDR, 2021).

Asia Region

- Lu et al. (2015) reported PFOS and PFOA concentrations in samples of surface water (n=29 rivers, 6 lakes and 4 reservoirs) in Shanghai, Jiangsu and Zhejiang Provinces of eastern China during 2011. Concentrations of PFOS ranged from <0.07 to 9.7 ng/L and PFOA <0.7 to 668 ng/L.

- In Japan, the National Survey on the presence of PFOS and PFOA in fiscal year 2019 reported levels of between 0.1 and 1462.8 ng/L for PFOS and 0.2 and 1812 ng/L for PFOA in samples from a number of rivers, groundwaters, lakes and marshes, sea areas and spring waters (M Asami, National Institute of Public Health, Japan, personal communication, May 2021). This compares to PFOS and PFOA levels (maximum) of 2.85 and 8.43 ng/L for source water (n = 8 samples) in the Philippines and 1.3 and 10.7 ng/L (n = 15 samples) in Thailand (Guardian et al., 2020).

- Contamination of river waters with fire-fighting foam in Okinawa, Japan was associated with PFOS levels between 65 and 196 ng/L, and PFOA levels of 0.4–19 ng/L (Yukioka et al., 2020).

Europe

- More than 90% of investigated European rivers were contaminated with PFAS at concentrations between 3 – 1400 ng/L (Loos et al., 2007; Möller et al., 2010; Hölzer et al., 2008; Ericson et al., 2009; Wilhelm et al., 2009). A more recent review of European PFAS occurrence data (WRC, 2020) concluded that concentrations of PFOS and PFOA in surface waters have high variability. PFOS levels ranged from 0.04 ng/L to 2709 ng/L and PFOA levels from 0.21 ng/L to 3640 ng/L. Groundwater levels of PFOS and PFOA were more consistent and, in general, < 100ng/L.

- An extensive monitoring programme by the UK water Industry between 2015 and 2020 called the (Chemicals Investigation Programme phase 2 (or CIP 2)) included monitoring for PFOS and PFOA. Results from several UK river water samples had upper bound mean concentrations of 5.6 ng/L for PFOS and 4.3 ng/L for PFOA.

- Longitudinal monitoring data for PFOS and PFOA in groundwater, including from the Veneto region in Italy during the period 2013 – 2020 are available (L Lucentini, Istituto Superiore di Sanità, F Russo, Veneto Region, personal communication, May 2022). Monitoring was put in place in the Veneto region following the discovery of accidental contamination of both groundwater sources and drinking water through release from a chemical plant producing perfluorinated compounds (closed in 2018). The 50^{th} and 95^{th} percentiles for PFOS were 20 and 84 ng/L respectively in 2013, and ≤LOQ (5 ng/L) and 55 ng/L respectively in 2021. For PFOA, 50^{th} and 95^{th} percentile levels in groundwater in 2013 were 327 and 2050 ng/L respectively, and in 2021 were ≤LOQ (5 ng/L) and 200 ng/L respectively.

2.1.2. Drinking-water

In general, PFAS co-occur in water sources with the specific compounds and concentrations of these present varying from source to source. The widespread presence of PFOS and PFOA at typically low concentrations (i.e., ng/L) in surface and groundwater across many countries indicates that drinking water taken from these sources may contain both substances. Although it is likely that such contamination is a global issue, current data are not adequate to confirm this. Some specific examples from more recent publications are highlighted below.

- Kaboré et al. (2018) evaluated levels of a large number (n=133) of PFAS in bottled and drinking water samples taken worldwide. PFOS was detected in 18% of bottled water samples and 85% of drinking water samples, with maximum detected levels of 0.67 and 4.1 ng/L respectively.

- Domingo and Nadal (2019) summarised data published in the scientific literature (Scopus and PubMed) on the concentrations of PFAS in drinking water published after 2009 (i.e., a 10-year period). The authors noted that the data were predominately related to the European Union, USA and China, with no information available for most countries worldwide. Although a large proportion of the data collated by the authors relate to PFOS and PFOA, data are generally reported by the authors as total PFAS. However, it is difficult to compare data between studies due

to variability in the individual compounds included and analytical methods used for determination as well as in the sampling strategies employed.

Asia region

- PFOA and PFOS were detected in tap water sampled from the household kitchen, from 79 cites (one sample per city) in 31 provincial-level administrative regions throughout China (except for Taiwan, Macao, and Hainan). Median reported PFOS and PFOA levels of 0.25 ng/L (LOQ = 0.01 ng/L) and 0.74 ng/L for PFOS and PFOA (LOQ = 0.3 ng/L), respectively (Li et al., 2019).

- The National Survey on the presence of PFOS and PFOA in fiscal year 2019 in Japan, reported PFOS and PFOA levels in drinking water sampled from 39 water treatment plants sampled between January and March 2020; (Mari Asami, personal communication, National Institute of Environmental Health, Japan, May 2021). PFOS was detected at levels up to 25.1 ng/L (not detected in 22 samples, with minimum level of detection varying between treatment plants) and PFOA at levels up to 44 ng/L (not detected in 11 samples, with minimum level of detection varying between treatment plants).

- Guardian et al. (2020) determined drinking water levels of 0.39 and 3.01 ng/L (maximum) for PFOS and PFOA in the Philippines (n = 7 samples) and of 0.33 and 7.89 ng/L (maximum) respectively in Thailand (n = 16).

Australia

- Evaluation of PFAS in drinking water from 62 samples taken at 34 locations across Australia showed that PFOS and PFOA were more commonly detected than other PFAS (49% and 44% of samples respectively). The highest concentration of PFOS was 16 ng/L and for PFOA, 9.7 ng/L (Thompson et al., 2011).

United States

- Occurrence data for PFOS and PFOA in drinking water has been collected as part of the US EPA Unregulated Contaminant Monitoring Rule (UCMR). The most recent data are available for the period 2013 – 2015 and indicated that the sum of reported PFOS and PFOA concentrations ranged from 0.02 to 7.22 µg/L (US EPA, 2021a). These findings are discussed in more detail in section 2.1.2.1.

- PFOS and PFOA levels were measured in source and treated water of 25 drinking-water treatment plants across the USA, included in a broader study of emerging contaminants. PFOS was quantifiable in 88% of source water samples, with median and maximum concentrations of 2.28 and 48.3 ng/L respectively. In treated drinking water, PFOS was quantifiable in 80% of samples, with median and maximum concentrations of 1.62 and 36.9 ng/L respectively. PFOA was quantifiable in 76% of source and drinking water samples, with median and maximum concentrations of 6.32 and 112, and 4.15 and 104 ng/L respectively. The authors noted that only one drinking-water treatment plant exceeded the current US EPA health advisory level of 70 ng/L (Boone et al., 2019).

Europe

- In an evaluation of human exposure to PFOS and PFOA in the EU, EFSA reported that for the category 'Drinking water', PFOS was found above the limit of detection/limit of quantification (LOD/LOQ) in 12% of samples (56/451), with the mean concentration ranging from 0.1 ng/L (lower bound mean) to 3.0? ng/L (upper bound mean). PFOA was quantified in 22% of samples (99/453) analysed, with mean concentrations ranging from 1.0 ng/L (lower bound mean) to 3.0 ng/L (upper bound mean) (EFSA, 2020).

- In Turkey, drinking water sampled from 33 provinces (n=94 samples) were found to contain both PFOS (13% of samples) and PFOA (11% of samples). Maximum concentrations in drinking water were reported as 2.04 and 2.37 ng/L respectively (Ünlü et al., 2019).

- Drinking water samples were also evaluated in the review of UK and European PFAS occurrence data (WRC 2020). PFOS data was identified for four European countries (the Netherlands, Germany, France and Spain) and for six European countries (UK, Germany, Spain, France, the Netherlands and Greece) for PFOA. A high variability in levels reported was seen for both; PFOS was present with average concentrations ranging from 0.33 ng/l (in Lleida, Spain) to 46 ng/L (unspecified area in Spain). Soil contamination was linked to higher levels, rather than reflecting typical drinking water levels. PFOA was detected at concentrations ranging from 0.63 ng/L (Utrecht, Netherlands) to 519 ng/L in the Rhine, Ruhr and Moehne area, with the higher levels reflecting local soil contamination (WRc, 2020).

- Longitudinal monitoring data for PFOS and PFOA in drinking water samples taken from the Veneto region, Italy, are also available for the period 2013 - 2021 (L Lucentini, Istituto Superiore di Sanità, F Russo, Veneto region, personal communication, May 2022). This is detailed in section 2.1.2.2 below.

2.1.2.1. UCMR 3 monitoring study

The Unregulated Contaminant Monitoring Rule (UCMR) is a monitoring programme that collects data for contaminants suspected to be present in drinking water without health-based standards under the Safe Drinking Water Act (SDWA). It represents a robust, large scale study utilising recommended analytical methodologies. The third round of UCMR (UCMR3) included PFAS (PFOS; PFOA; perfluorononanoic acid (PFNA); PFHxS; perfluoroheptanoic acid (PFHpA); PFBS) in samples taken from 2013 to 2015. All public water systems (PWSs) serving more than 10,000 people (i.e., large systems) and a small portion of small systems (800 representative PWSs serving 10,000 or fewer people) were included. Table 2.1 shows the levels of each PFAS measured in drinking water in 2015 from different water source types following treatment.

Table 2.1: PFAS levels in drinking water as reported by the UCMR 3 monitoring programme

Source water type	Average of Analytical Result Value (µg/L)	Max. of Analytical Result Value (µg/L)	Min. of Analytical Result Value (µg/L)	No. of detects / Total no. samples	% detection
Ground water	0.047	0.156	0.014		
PFHpA	0.029	0.410	0.010	144/22494	0.64%
PFHxS	0.150	1.600	0.030	179/22494	0.80%
PFNA	0.035	0.056	0.022	18/22494	0.08%
PFOA	0.045	0.349	0.020	278/22494	1.24%
PFOS	0.199	7.000	0.040	224/22494	1.00%
Total PFAS	*0.382*				
Ground water under direct influence of surface water	0.069	0.086	0.050		
PFHpA	0.011	0.011	0.011	1/436	0.23%
PFHxS	0.063	0.071	0.048	5/436	1.15%
PFNA	ND	ND	ND	0/436	ND
PFOA	0.026	0.040	0.020	7/436	1.61%
PFOS	0.062	0.086	0.045	9/436	2.06%
Total PFAS	*0.139*				
MX[1]	0.043	0.043	0.043		
PFHpA	0.011	0.013	0.010	4/814	0.49%
PFHxS	0.085	0.180	0.060	6/814	0.74%
PFNA	ND	ND	ND	0/814	ND
PFOA	0.033	0.042	0.020	10/814	1.23%
PFOS	0.047	0.060	0.040	10/814	1.23%
Total PFAS	*0.043*				

Surface water	0.029	0.040	0.022		
PFHpA	0.019	0.060	0.010	87/13228	0.66%
PFHxS	0.065	0.190	0.030	17/13227	0.13%
PFNA	0.054	0.054	0.054	1/13228	0.01%
PFOA	0.031	0.100	0.020	84/13228	0.64%
PFOS	0.084	0.400	0.040	49/13228	0.37%
Total PFAS	*0.066*				

Calculated from UCMR3 monitoring programme data (US EPA, 2015. Occurrence data for the unregulated contaminant monitoring rule. Zip file of UCMR 3 Occurrence Data available at: https://www.epa.gov/dwucmr/occurrence-data-unregulated-contaminant-monitoring-rule [accessed 7 April 2021]).

[1] - MX – any combination of surface water, ground water and ground water under the direct influence of surface water. All PFAS were measured for each source water type, however only those present at quantifiable levels are included.

PFOS and PFOA are the most frequently detected PFAS, both in ground water and surface water. Andrews and Naidenko (2020) evaluated the full US EPA UCMR3 data set in relation to occurrence of PFOS and PFOA. The authors highlight that there is significant variation in PFAS occurrence both within and between states, with occurrences above the US EPA drinking water health advisory level of 70 ng/L (combined PFOS and PFOA). These are linked to contamination from, for example, manufacturing sites and fire training areas. Combined levels of PFOA and PFOS > 70 ng/L were detected in 0.3% of samples. In addition, Andrews and Naidenko (2020) estimated that between 18 and 80 million people in the US receive tap water with levels of PFOS and PFOA (combined) of ≥ 10 ng/L and > 200 million with concentrations ≥ 1 ng/L.

2.1.2.2. Veneto region monitoring study

In spring 2013, groundwater in a large area of the Veneto Region (northeastern Italy) was found to be contaminated by PFAS from a manufacturing plant that had been active since the late 1960s. Residents were exposed to PFAS (particularly PFOA) through drinking water until autumn 2013, as demonstrated by the human biomonitoring studies carried out with sampling in 2015-2016 (Ingelido et al. 2018). A range of PFAS have been monitored in drinking water samples from the Veneto region during the period 2013 – 2020, including: perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonic acid (PFHxS), PFOA and branched isomers (PFOA_TOT), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDeA), PFOS and branched isomers (PFOS_TOT), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluoroheptanesulfonic acid (PFHpS), Gen-X (HFPO-DA), fluorotelomer 4:2 (4:2-FTS), fluorotelomer 6:2 (6:2-FTS), fluorotelomer 8:2 (8:2-FTS), and (cis/trans)-Perfuoro([5-methoxy1,3-dioxolan (cC6O4). These PFAS were selected based on analytical standard availability. Tables 2.2 and 2.3 below show the levels of PFOS and PFOA detected in each year. In addition, levels for a wider range of PFAS from were reported by Pitter et. al (2020) and WHO (2016).

Table 2.2: PFOS levels in drinking water in the Veneto region of Italy 2013 – 2021 (L Lucentini, Istituto Superiore di Sanità, F Russo, Veneto region, personal communication, May 2022)

Drink. water	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020/2021**
PFOS TOT	***ng/L***	***ng/L***	***ng/L***	***ng/L***	***ng/L***	***ng/L***	***ng/L***	***ng/L***
Min	≤LOQ	≤LOQ	≤LOQ	≤LOQ	5,0	≤ <LOQ	≤ LOQ	≤ LOQ
5° prct	≤LOQ	≤LOQ	≤LOQ	≤LOQ	5,0	≤ LOQ	≤ LOQ	≤ LOQ
25° prct	≤LOQ	≤LOQ	≤LOQ	≤LOQ	5,0	≤ LOQ	≤ LOQ	≤ LOQ
50° prct	12,0	≤LOQ	≤LOQ	≤LOQ	5,0	≤ LOQ	≤ LOQ	≤ LOQ
75° prct	21,0	≤LOQ	15,0	10,0	5,0	≤ LOQ	≤ LOQ	≤ LOQ
95° prct	57,0	21,4	22,0	21,0	13.4	≤ LOQ	≤ LOQ	≤ LOQ
Max	117,0	36,0	45,0	34,0	34,0	≤ LOQ	6,0	≤ LOQ

PFOS TOT: Perfluorooctanesulfonic Acid and branched isomers; LOQ: limit of quantification = 5 ng/L

Table 2.3: PFOA levels in drinking water in the Veneto region of Italy 2013 – 2020 (L Lucentini, Istituto Superiore di Sanità, F Russo, Veneto region, personal communication, May 2022.)

Drink. water	2013	2014	2015	2016	2017	2018	2019	2020	2021
PFOA TOT	***ng/L***	***ng/L***	***ng/L***	***ng/L***	***ng/L***	***ng/L***	***ng/L***	***ng/L***	***ng/L***
Min	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ
5° prct	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ
25° prct	183,8	54,3	41,0	33,0	≤LOQ	≤LOQ	≤LOQ	≤LOQ	≤LOQ
50° prct	247,5	127,0	113,0	56,0	44,5	≤LOQ	≤LOQ	≤LOQ	≤LOQ
75° prct	361,8	185,0	144,3	123,5	124,5	≤LOQ	≤LOQ	≤LOQ	≤LOQ
95° prct	866,3	270,4	199,0	211,4	203,0	≤LOQ	≤LOQ	≤LOQ	≤LOQ
Max	1475,0	386,0	290,0	394,0	245,0	21,0	8,0	7,0	10,0

PFOA TOT: Perfluorooctanoic acid and branched isomers; LOQ: limit of quantification = 5 ng/L

A decreasing trend is seen following the introduction of a water safety plan in 2013. Technical improvements in the water treatment plant in 2018 may have increased the rate of decrease for both PFOS and PFOA. The plateau observed for drinking water may be due to the LOQ which was 5 ng/L.

See section 9.4 for further information on PFAS occurrence in drinking-water from a treatment context. This section also includes additional data and treatment details, from the Veneto region of Italy.

2.2. Food

Food is a significant source of exposure to PFOS and PFOA with contamination occurring mainly through bioaccumulation in aquatic and terrestrial food chains. Contamination can also occur through the transfer of PFOS, PFOA and their precursors from contact materials used in food processing and packaging (EFSA, 2020). As for water, these data have been previously outlined in available literature and are not detailed here. Some specific studies are outlined below to provide a general awareness of the extent of environmental levels (EFSA, 2020; ATSDR, 2021).

The occurrence of PFOS and PFOA in foods was recently evaluated by EFSA in a large study where 21,411 food samples from 16 European countries were analysed (n=10,889 for PFOS and n=10,522 for PFOA). The assessment was based on a final number of 20,019 samples (n=10,191 and 9,828 for PFOS and PFOA respectively) that met EFSA's quality criteria, although a relatively large proportion of the samples were below the LOD/LOQ (74% for PFOS and 91% for PFOA respectively). Gas chromatography-based methods gave the highest sensitivity with median LOQs of 0.2 and 0.4 µg/kg for PFOS and PFOA respectively. Liquid chromatography-tandem mass spectrometry-based methods gave a median LOQ of 1.0 µg/kg for both compounds (EFSA, 2018).

EFSA reported that high mean concentrations of PFOS and PFOA were associated with 'meat and meat products', which was affected by high mean concentrations in the liver from game animals. When offal was excluded, the mean lower and upper bound PFOS and PFOA levels in meat and meat products were 0.55 and 0.75 µg/kg for PFOS and 0.10 and 0.34 µg/kg for

PFOA. The 'Fish and seafood' category also contained high levels, with mean lower and upper bound levels of 2.08 and 2.59 µg/kg for PFOS and 0.18 and 0.90 µg/kg for PFOA (EFSA, 2018).

Trudel et al. (2008) reported that comparable levels of PFAS uptake would be expected in North American and Europe from food and water. The authors estimated intakes in the range of 3 – 220 ng/kg bw/day for PFOS and 1 – 130 ng/kg bw/day for PFOA. PFOS and PFOA were also frequently detected in foods sampled as part of the Canadian Total Diet Study (TDS) collected between 1992 and 2004. Levels ranged from 0.5 – 4.5 ng/g food with estimated intakes of 250 ng/day in adults (Tittlemier et al. (2007). Similar levels of dietary intake were reported in a German study with median daily intakes of 1.4 and 2.9 ng/kg bw/day for PFOS and PFOA respectively (Fromme et al., 2007).

2.3. Air

PFAS are present in indoor air due to the release from treated consumer products (e.g. carpets and textiles), as highlighted in select examples below. The reported mean concentrations of perfluoroalkyls measured in four indoor air samples collected from Tromsø, Norway (no further details provided) were < 47.4 pg/m^3 for PFOS and 4.4 mg/m^3 for PFOA (Barber et al., 2007).

Outdoor air emissions of PFOA from the DuPont 3M works, West Virginia, were recorded at levels up to 75,000-900,000 pg/m^3 time during operational periods prior to 2004 (ATSDR, 2021). In urban locations in the USA (Albany, New York) and Japan, and rural locations in Norway and Ireland, mean PFOA levels of between 1.54 and 15.2 pg/m^3 have been reported (ATSDR, 2021).

Laboratory studies demonstrate that PFOA (and other perfluoroalkyl carboxylic acids) are formed by the atmospheric photooxidation of precursor compounds including fluorotelomer alcohols and perfluoroalkyl sulphonamides (Makey et al., 2017; ATSDR, 2021).

2.4. Indoor dust

PFOS and PFOA have also been detected in indoor dust . For example, PFOS and PFOA were reported to be present in dust samples from Canadian homes at mean levels of 443.68 and 19.72 ng/g respectively. In Japan, dust samples collected from vacuum cleaners in homes contained between 11–140 and 69–380 ng/g PFOS and PFOA respectively. Household dust samples collected from the United Kingdom, Australia, Germany, and the United States showed the presence of perfluoroalkyl substances (Kato et al. 2009a). Mean levels of PFOS were reported as 479.6 ng/g (maximum of 18,071 ng/g) and PFOA as 667.7 ng/g (maximum of 9,818 ng/g). Median levels of PFOS in dust samples collected in homes, apartments, day-care centres, offices, and cars in Sweden were 39, 85, 31, 110, and 12 ng/g, respectively (Bjorklund et al. 2009). Median PFOA levels in dust samples from the same study were 54, 93, 41, 70, and 33 ng/g in homes, apartments, day-care centres, offices, and cars, respectively. Strynar and Lindstrom (2008) detected PFOS and PFOA in 74.4% and 64.1% of indoor dust samples collected from homes and day-care centres in North Carolina and Ohio, respectively.

2.5. Relative contribution of drinking water to total exposure levels

In the evaluation carried out by EFSA (2020), the contribution of drinking water to overall PFOS and PFOA intake (as lower bound mean exposure) in the general population was found to be highest in the infant age group, with a maximum of 10% and 60% respectively. Other studies support food as being the major source (>70%) of exposure to PFOS and PFOA in the general population living in areas not characterised by heavy contamination by PFAS. For example, a study in 41 Norwegian women assessed the contribution of drinking water and other sources of PFOS and PFOA to total exposure levels (food, house dust and indoor air). Food contributed between 88 and 99% of the median total intake of PFOS and between 67 and 84% of the median total intake of PFOA. The median relative contribution from drinking water was reported as between 0.57 and 0.68% for PFOS and 9.1 and 11% for PFOA (EFSA, 2020). However, in areas where drinking-water contamination has occurred, a relative contribution for drinking water above 75% has been reported (Emmett et al., 2006; Hölzer et al., 2008; Steenland et al., 2009; Vestergren and Cousins, 2009; Ingelido et al., 2018; Xu et al., 2021) particularly in farmers consuming their own produced foods (Ingelido et al., 2020). Gebbink et al. (2015) assessed contributors of direct and indirect (via precursors) pathways of human exposure to PFOS and PFOA using data published since 2008. Total exposure was highest for direct exposure to PFOS and PFOA, with precursor contributions between 11-33% for PFOS and 13-64% for PFOA. In a review of pathways of human exposure to PFAS (which included those publications discussed here), Sunderland et al. (2019) reported that for PFOS, drinking water contributed between <1 and 22% of total exposure in adults. For PFOA, drinking water contribution ranged between <1 and 37% of total exposure in adults. In 2016, the US EPA applied a relative source contribution of 20 percent for the final drinking water health advisories for PFOA and PFOS, based on their physical properties and available information indicating that significant potential exposure sources other than drinking water ingestion exist (US EPA, 2016a,b). In 2019, the US EPA conducted a broad literature search to evaluate evidence for pathways of human exposure to PFOA and PFOS, and in 2021 released a draft analysis that supports application of a 20 percent relative source contribution for PFOA and PFOS in drinking water (US EPA, 2021b,c). Ingelido et al. (2018, 2020) reported that the relative contribution of PFOS and PFOA to total PFAS exposure in the Italian region of Veneto, varied depending on whether food or water, respectively, was the main source of exposure.

2.6. Bioaccumulation

Bioaccumulation of PFOS and PFOA is possible in aquatic organisms, in land-based food chains (i.e., plants) and mammals, including farm animals, and humans (EFSA, 2020). The partitioning to albumins in blood, liver and eggs is a key bioaccumulation mechanism for PFAS, in contrast to lipid accumulation that is typical of other POPs.

The bioaccumulation factor from water to fish (*Clupea harengus*) increases from 2.5 to 4.7 with increasing chain lengths between 7 and 10 carbon atoms, which indicates a greater potential for accumulation in fish relative to the surrounding environment as chain length increases. Data for the marine food web from the Eastern Canadian Arctic (from 1996 to 2002) indicate that PFOS biomagnifies with a trophic magnification factor of 3.1 (HC, 2018a). Although the bioaccumulation processes for terrestrial food chains is more complex, it is estimated that the trophic magnification factor is lower than that for aquatic food chains (HC, 2018a). PFAS present in soil are taken up by the roots of plants, with longer chain lengths associated with a greater potential to bioaccumulate in the plant (Felizeter et al., 2012). Due to

the evidence of bioaccumulation for several PFAS in humans and other mammals, it is assumed that this will also occur in farm animals (Olsen et al., 2007; Houde et al., 2006, 2011). Ongoing monitoring studies may help to show an effect on biomagnification in animals and humans due to the phasing out of PFOS and PFOA. Similar data will be needed to determine the bioaccumulation potential of the replacement substances (ATSDR 2021).

In addition, it has been reported that the sediment-water distribution coefficient for PFAS increases from 0.3 to 2.5 as chain lengths increase from four to eight carbon atoms (EFSA, 2020).

3. TOXICOKINETICS AND METABOLISM IN HUMANS AND ANIMALS

Studies related to the toxicokinetics and metabolism of PFOS and PFOA in animals and humans have been comprehensively reviewed and summarized elsewhere (ATSDR, 2021; EFSA, 2020; HC, 2018a; HC, 2018b; US EPA, 2016a 2016b; US EPA 2021a 2021b). Although this section highlights several studies related to the toxicokinetics of PFOA and PFOS in animals and humans that are relevant to these endpoints as illustrative examples, it is not intended to be a comprehensive summary of all the data available.

3.1. Absorption

3.1.1. PFOS

Several experimental studies report that in rats, PFOS is rapidly absorbed from the GI tract. For example, in Sprague-Dawley rats, approximately 94% of a relatively high administered single oral dose of 4.2 mg/kg PFOS was recovered in the carcass 48 hours after dosing, whereas only 3.32% of the total dose was found in the digestive tract and 3.24% in the faeces (Chang et al., 2012) indicating that most of the PFOS dose was systemically absorbed. The time to C_{max} (T_{max}) after a single oral dose of 2 mg/kg PFOS in male and female rats was approximately 11 hours (Kim et al., 2016), and 14.3 – 16.4 hours (males) or 12.2 – 13.7 hours (females) after single oral doses of 2 and 20 mg/kg (Huang et al., 2019). Additionally, based on data from Kim et al. (2016) and Huang et al. (2019), the oral bioavailability of PFOS in rats administered oral doses of 2 mg/kg is about 100%.

Based on the physicochemical properties of PFOS (high molecular weight and low volatility), the inhalation and dermal routes are unlikely to be of significance for humans when exposure occurs via drinking water (i.e. exposures from bathing or showering) (HC, 2018a); however, a quantitative evaluation of the contribution from dermal uptake due to showering or bathing has not been conducted.

3.1.2. PFOA

A pharmacokinetic evaluation was conducted as part of a phase 1 clinical trial conducted in 43 adult human patients with cancer, to evaluate the potential for PFOA use in chemotherapy (Elcombe et al., 2013; as summarized by Dourson et al., 2019 and Dourson and Gadagbui, 2021). Patients were given doses of 50 - 1200 mg PFOA per week for up to six weeks (doses ranging from approximately 0.1 to 2.3 mg/kg-day), and plasma concentrations were monitored. Absorption of PFOA was rapid, with time to C_{max} (T_{max}) ranging from approximately 1.5 - 3 hours. After a single dose, C_{max} values ranged from approximately 20 – 30 µM at the lowest

dose (50 mg) to approximately 300 – 700 µM at the highest dose (1200 mg); however, these values rise with repeated dosing, with steady state plasma concentrations being reached after 12-36 weeks (Dourson et al., 2019). It is unclear to what extent these data from patients with cancer (who may be receiving special medications or have disrupted functions relevant to ADME) are relevant to persons without cancer, or to communities with longer term, lower environmental exposure. Additionally, absorption of PFOA was demonstrated in residents exposed to PFOA from contaminated drinking water, in which elevated serum PFOA concentrations were reported (Emmett et al., 2006).

In experimental studies in animals, PFOA is rapidly absorbed from the GI tract. A carbon tracer study in rats found that 92-93% of an initial single oral dose of 5 or 20 mg/kg PFOA was absorbed (Cui et al., 2010). Similar absorption rates were derived for mice, rats, hamsters, and rabbits in relevant studies cited by Health Canada (2018b); uptakes were generally higher for males than females for all species other than rabbits. In rats, absorption was 2-3 times higher under fasting than under non-fasting conditions (HC, 2018b). In rats, data from Kim et al. (2016) suggest large intersex differences in the oral absorption rate of PFOA; the time to C_{max} (T_{max}) after a single oral dose of 2 mg/kg PFOS in male and female rats was 2.07 and 0.06 days, respectively (Kim et al., 2016). Similarly, T_{max} values associated with larger single oral doses ranging from 40 – 160 mg/kg in rats were disparate between males and females although less pronounced, with values ranging from 4.86 – 8.33 hours for males and 2.33 – 3.22 hours for females. The oral bioavailability of PFOA in male rats administered oral doses of 2 or 40 mg/kg is about 100% (Dzierlenga et al., 2019). However, in female rats oral bioavailability was 85% (Kim et al., 2016).

Due to the high molecular weight and low volatility of PFOS and PFOA, Health Canada (2018a; 2018b) suggested that inhalation and dermal routes are not of major significance in cases where the exposure to a contaminate water source occurs during bathing/showering.

3.2. Distribution

3.2.1. PFOS

Volunteer studies to assess the distribution of PFOS in humans are not available for any route of exposure; however, evidence of its distribution can be derived from other sources. Autopsy tissue samples in the US, reflecting exposure from all routes, showed a good correlation of PFOS levels in the liver with those in serum with a mean liver-to-serum ratio of 1.3 (Olsen et al., 2003). Other tissues including cerebrospinal fluid and thyroid were not seen as partitioning sites in humans (ATSDR, 2021). The mechanism of PFOS distribution occurs through binding to serum albumin and, to a lesser extent, to plasma γ-globulin, α-globulin, α-2-macroglobulin, transferrin and β-lipoproteins, although the binding to lipoproteins is limited in humans (≤ 9%) (Butenhoff et al., 2012). PFOS was also shown to competitively bind to the human thyroid hormone transport protein transthyretin (TTR), with less than one-tenth of the T4 affinity (Weiss et al., 2009).

Placental transfer of PFOS in humans has also been reported; for example, in a study conducted in 32 pregnant women in China, PFOS cord blood levels were statistically-significantly ($p < 0.001$) correlated with maternal serum concentrations, with the mean levels in cord blood, placenta, and amniotic fluid reported as 21%, 56%, and 0.14% of the mean levels in the mother's blood, respectively (Zhang et al., 2013). Post-natal transfer of PFOS is also possible

via breastmilk, and breastmilk PFOS concentrations have been reported in several publications. In the United States, the median breast milk PFOS concentration was 30 pg/mL with a range of 6 – 187 pg/mL (Zheng et al., 2021). A comparative study of breastmilk PFAS concentrations sampled between 2010 and 2016 reported higher levels of PFOS in breastmilk samples from the Chinese cities of Shanghai, Jiaxing, and Shaoxing (65 – 119 pg/mL for linear PFOS; 7 – 12 pg/mL for branched PFOS) compared to samples from Stockholm, Sweden (39 pg/mL for linear PFOS; 7 pg/mL for branched PFOS) (Awad et al., 2020). Additionally, a review of milk and maternal serum concentrations estimated maternal PFOS milk:serum ratios ranging from 0.01 – 0.03 (Liu et al., 2011). Lastly, Nyberg et al. (2018) evaluated temporal trends in PFAS breastmilk concentrations in Sweden, reporting declining levels of PFOS over time based on breastmilk samples collected in Stockholm (1972 to 2016) and Gothenburg (2007 to 2015). Similar findings were reported in the Czech Republic based on samples collected from 2006 to 2017 (Černá et al., 2020).

Studies relevant to the distribution of PFOS in animals have also been conducted. Adult cynomolgus monkeys administered PFOS at doses of 0, 0.03, 0.15, or 0.75 mg/kg bw per day orally by intragastric intubation over a 26-week period showed a linear increase in serum PFOS levels with duration up to a dose of 0.15 mg/kg bw per day. A non-linear increase was seen at the highest dose of 0.75 mg/kg bw per day and levels also plateaued after about 100 days at this dose. The proportion of the cumulative dose of PFOS in the liver at the end of the dosing regimen ranged between 4.4% to 8.7% with no difference seen between dose or gender (Seacat et al., 2002).

In rats, distribution of PFOS following exposure via the diet (0, 20, 50 or 100 mg PFOS/kg diet for 4 weeks) was reported as highest in the liver, with much lower distributions to the spleen and heart. No consistent differences were found between sexes for levels in the liver, however for the spleen and heart levels of PFOS tended to be higher in females than males at all doses (Curran et al., 2008). Administration of ^{35}S-PFOS to rats in the diet (0.031 mg/kg/day and 23 mg/kg/day) for between 1 and 5 days also showed the liver to be the main tissue for PFOS deposition (40 – 50% of total dose at the highest dose), with plateauing of levels at the highest dose after 3 days (Bogdanska et al., 2011).

Placental transfer of PFOS was also shown to occur in rats, with foetal serum levels approximately 1–2 times greater than maternal serum levels at GD 20. The resulting levels of PFOS in foetal liver were not elevated when compared to maternal liver; however, the concentrations of PFOS in foetal brain tissue were approximately ten-fold higher than maternal brain tissue (Chang et al., 2009), the latter of which the US EPA (2016a, 2021a) attributed to an immature blood-brain barrier. Also, in pregnant rats, maternal liver-to-serum PFOS ratios of 1.8 to 4.9, and maternal brain-to-serum ratios of 0.04 to 0.09 have been reported (Chang et al., 2009). In pregnant mice administered a single dose of 12.5 mg/kg bw ^{35}S-PFOS (Borg et al., 2010), distribution of PFOS was highest to the liver and lungs (four-fold and two-fold higher, respectively, than serum levels) in the dams and was highest to the liver and kidneys in the foetuses.

3.2.2. PFOA

Little data are available to assess the distribution of PFOA in humans; however, biomonitoring and epidemiology studies (including autopsy tissue samples) suggest that PFOA distributes to the liver, lungs, kidneys, thyroid and bones, with some tissues having very low levels (HC,

2018b). As with PFOS, the distribution of PFOA occurs primarily through binding to serum albumin, with less binding to plasma γ-globulin, α-globulin, α-2-macroglobulin, transferrin and β-lipoproteins. Protein binding can also occur in organs and tissues (HC, 2018b).

Pharmacokinetic data from the Elcombe et al. (2013) clinical study in patients with cancer described previously (see Section 3.1.2), were evaluated by Dourson et al. (2019), and mean volumes of distribution (V_d) were 6.8 L after one week of dosing and 9.3 L after 6 weeks of dosing, suggesting that some of the absorbed dose will be distributed into tissues, and there was no correlation between PFOA V_d and the dose administered. However, as previously noted, this study included patients with cancer who were being treated in a clinical setting and the relevance of the data to drinking-water exposure in the general population is unclear.

Placental transfer of PFOA can also occur in humans. For example, in an analysis of 29 matched maternal and foetal cord blood samples collected in China (Zhang et al., 2013), PFOA cord blood levels were statistically-significantly ($p < 0.001$) correlated with maternal serum concentrations, with median PFOA levels of 2.96 ng/mL and 1.73 ng/mL in maternal serum and foetal cord blood, respectively. Additionally, PFOA levels were detected in the amniotic fluid, but at lower levels than in cord blood and placenta, with mean PFOA levels of 47% (cord blood), 59% (placenta), and 1.3% (amniotic fluid) of the maternal serum PFOA levels (Zhang et al., 2013).

Post-natal transfer of PFOA is also possible via breastmilk, and breastmilk PFOA concentrations have been reported in several publications. In the United States, the median breast milk PFOA concentration was 0.014 ng/mL with a range of less than 0.01 to 0.051 ng/mL (Zheng et al., 2021). A study carried out in three cities in China (Shanghai, Jiaxing, and Shaoxing), sampled between 2010 and 2016, reported levels of PFOA in breastmilk in the 0.094 – 0.226 ng/mL range (Awad et al., 2020). Additionally, a review of milk and maternal PFOA serum concentrations estimated maternal milk:serum ratios ranging from 0.11 – 0.12 (Liu et al., 2011). Nyberg et al. (2018) evaluated temporal trends of PFAS substances in human milk and reported declining levels of PFOA in breastmilk over time based on samples collected in Stockholm, Sweden between 1972 and 2016, and Gothenburg, Sweden between 2007 and 2015).

In animals, experimental distribution studies were carried out in several species including monkeys, rats, and mice. In cynomolgus monkeys, oral administration of PFOA daily for a 6-month period at doses of 0, 3, 10, or 20 mg/kg bw resulted in a plateauing of serum levels within 4–6 weeks and urine levels after 4 weeks, in all dose groups. In the 3 and 10 mg/kg bw dose groups, PFOA concentrations in the liver ranged from 6.29 to 21.9 µg/g, whereas liver concentrations in two monkeys exposed to 20 mg/kg bw were 16.0 and 83.3 µg/g (Butenhoff et al., 2004a). In rats administered PFOA (as ^{14}C-PFOA) at doses of 1, 5, or 25 mg/kg bw by oral gavage, the primary tissues for distribution were reported as the liver, blood, skin, muscle, bone, G.I. tract, and fat (unpublished data cited by US EPA, 2016b). Gender differences in distribution patterns were reported in rats administered PFOA at 10 mg/kg bw by oral gavage for 20 days, after which serum concentrations of 111 µg/mL and 0.69 µg/mL were reported in males and females, respectively (Lau et al., 2006); however, this disparity was not evident in mice, in which serum levels of 181 – 191 µg/mL in males and 171 – 178 µg/mL were reported after gavage administration of 20 mg/kg bw for 7 or 17 days. In mice exposed to single doses of 1 or 10 mg/kg bw PFOA, slightly lower peak serum concentrations and higher final serum concentrations were reported in males in comparison to females, and liver as well as kidney

concentrations also were higher in males than in females (Lou et al., 2009). The US EPA (2016b) suggested that this indicates a longer half-life in males than in females.

Placental transfer of PFOA has also been reported in animal studies. Pregnant rats were administered PFOA at doses of 0, 3, 10, and 30 mg/kg bw per day during gestation days 4–10, 4–15, and 4–21, or from GD 4 to lactational day (LD) 21. In the group exposed from GD 4 to LD 21, foetal plasma PFOA levels at GD 21 were around half of the maternal plasma levels (unpublished data cited by US EPA, 2016b, 2021b). In neonatal pups, PFOA levels in plasma decreased between birth and LD 7, and from LD 7 to LD 21, pup plasma levels were similar to those observed in maternal milk (unpublished data cited by US EPA, 2021b). In pregnant mice orally exposed to PFOA at doses of 0, 0.1, 1, and 5 mg/kg bw on GD 17, serum PFOA in pups was significantly higher than that in maternal serum but decreased between birth and post-natal day (PND) 18 (Fenton et al., 2009). Accumulation of PFOA in the brain (0.7 µg/g) and liver (16.3 µg/g) was reported in pups born to C57BL/6/Bkl mice exposed to PFOA in the diet equivalent to a dose of 0.3 mg/kg bw per day from GD 1 to the end of pregnancy (Onishchenko et al., 2011). In addition, in an NTP (2020) chronic exposure study with combined gestational and lactational exposure (see section 5.3.2), the offspring of Sprague-Dawley rats exposed to 300 ppm PFOA in the diet beginning at GD 6 had mean plasma PFOA concentrations that were 30% and 14% of maternal serum levels when measured at GD 18 and PND 4, respectively.

3.3. Metabolism

All available human and animal data indicate that PFOS and PFOA are not metabolised due to a lack of metabolism for the perfluorinated carbon chain[5] (HC 2018a, 2018b; ATSDR, 2021).

3.4. Elimination

3.4.1. PFOS

PFOS elimination half-lives reported in the literature vary widely. In humans, the longest mean half-life described in the literature is 5.4 years (95% CI = 3.9–6.9 years; range = 2.4–21.7 years) based on serum samples collected from 26 retired fluorochemical production workers from Alabama, USA, over a five-year period (Olsen et al., 2007). In a study conducted by Li et al. (2018) using blood samples from 106 volunteers in a Swedish population exposed to high levels of PFAS in their drinking water, the mean serum elimination half-life was estimated as 3.4 years (95% CI = 3.1-3.7 years; range = 2.2 – 6.2 years). Xu et al. (2020) analysed serum levels of four PFOS isomers in a study of 26 airport workers in Sweden exposed to drinking-water contaminated with fire-fighting foam, and reported mean half-lives ranging from 0.73 – 1.69 years after adjusting for background PFOS exposure. Lastly, based on a review of studies in occupational cohorts and exposed communities, Lin et al. (2021) reported mean PFOS half-lives ranging from 2.91 – 4.8 years.

The contribution of urinary excretion of PFOS to half-life in humans has been questioned, as renal clearance is much lower than in animals and is impacted by both the isomeric composition of the mixture present in blood and the gender/age of the individuals. Saturable renal resorption

[5] it should be noted that for derivatives which degrade to a PFAS, the non-perfluorinated portion of the compound can undergo metabolism possibly forming active metabolites that accompany the PFOA and PFOS in serum.

of PFOS from the glomerular filtrate via transporters in the kidney tubules is believed to be a major contributor to the long half-life of this compound. No studies were identified on specific renal tubular transporters for PFOS, which have been reported for PFOA. Biliary and faecal elimination of PFOS does occur, and there is evidence that PFOS undergoes extensive enterohepatic recirculation in humans. For example, in an analysis of paired serum and bile samples of four adult male and females in Japan, the biliary excretion rate for PFOS was estimated as 2.98 ml/kg bw per day compared to 0.015 ml/kg bw per day for urinary excretion, and a biliary resorption rate of 0.97 was estimated (Harada et al., 2007). Additionally, in a case study of excretion following PFOS inhalation, treatment with the bile sequestrant cholestyramine reduced PFOS serum levels from 23 ng/g to 14.4 ng/g (Genuis et al., 2010). Finally, PFOS exposed residents in Ohio and West Virginia (USA) who were also taking the bile sequestrant cholestyramine had significantly lower serum PFOS (geometric mean: 1.26 ng/mL) compared to similarly exposed non-cholestyramine users (geometric mean: 19.12 ng/mL) (Ducatman et al., 2021). It has been suggested that the enterohepatic circulation of PFOS may also contribute to its long half-life in humans. (US EPA, 2016a). In women, blood loss during menstruation can also be a significant route of excretion, possibly accounting for 30% of the elimination half-life difference between males and females (Wong et al., 2014).

Studies in animals suggest considerable species-and gender-dependent differences in the elimination half-life of PFOS, with animals showing much shorter half-lives compared to humans. In the monkey, rat and mouse, half-lives between 110 and 132 days (monkeys), 39.8 and 66.7 days (rats) and 34.2 and 39.6 days (mice), were reported, with evidence of longer half-lives for males than females in mice and monkeys, and vice-versa in rats (Chang et al., 2012). In rats and mice, the primary routes for the elimination of PFOS are via the urine (around 18%) and faeces (around 8%). Also, according to ATSDR (2021), in animals extensive enterohepatic recirculation occurs; therefore, “biliary excretion does not represent a major elimination pathway”.

3.4.2. PFOA

Similar to PFOS, estimates of elimination half-life in humans vary widely. In an occupationally-exposed cohort from Ohio, USA, a serum elimination half-life of 3.8 years for PFOA was determined (95% CI = 3.1–4.4 years; range = 1.5–9.1 years) (Olsen et al., 2007). Other estimates for PFOA half-life in humans include 3.26 years (range = 1.03–14.67; no 95% CI reported) in a German population living near a contaminated drinking-water supply (Brede et al., 2010), and 2.3 years (95% CI = 2.1–2.4 years; no range provided) in a US population exposed via drinking-water contaminated by a nearby chemical plant (Bartell et al., 2010). Additionally, in a highly-exposed Swedish population, the mean serum elimination half-life was estimated as 2.7 years (95% CI = 2.5-2.9 years; range = 1.8 – 5.1 years) (Li et al., 2018). A lower PFOA elimination half-life of 1.48 years was reported by Xu et al. (2020) among airport workers in Sweden, after adjusting for background PFOA exposure. Lastly, based on a review of studies in occupational cohorts and exposed communities, Lin et al. (2021) reported mean PFOA half-lives ranging from 1.77 – 3.9 years. It has been proposed that some PFOA half-lives reported in the literature may be overestimates due to varying degrees of unmeasured PFOA exposures from environmental media (Dourson and Gadagbui, 2021; Campbell et al., 2022a); however, this has been a subject of further debate (Post et al., 2022; Campbell et al., 2022b). Additionally, inculcation of PFOA molecules into the plasma membranes of blood cells may also be a contributor to the longer half-life since PFOA resembles the fatty acids of such membranes such that desorption time is lengthened (Dourson and Gadagbui, 2021).

Urinary excretion is a major route of elimination of PFOA, and according to the US EPA (2016b, 2021b), saturable renal resorption of PFOA from the glomerular filtrate via transporters in the kidney tubules is a major contributor to the comparatively long elimination half-life of PFOA compounds in humans. In a study of 81 whole blood-urine paired samples from 54 general adults and 27 pregnant women in China (Zhang et al., 2015), PFOA was detected in 76% of adult urine samples, with mean urinary PFOA concentrations of 0.008 ng/mL and 0.003 ng/mL in general adult and maternal urine samples, respectively. In another analysis of paired blood and urine samples from 86 adults in China (Zhang Y. et al., 2013), renal clearance rates for young females, and both older females and males, were 0.16 and 0.19 ml/kg bw-day respectively, and it was shown that major branched isomers were more efficiently excreted than corresponding linear isomers.

In women, lactation and menstrual bleeding may also be significant routes of excretion. In a Norwegian study of breast milk samples collected from eight primiparous mothers and one mother breast-feeding her second child (Thomsen et al., 2010), PFOA breast milk concentrations decreased by 7% during the first month after birth. In an analysis of 20 menstruating women of age 20-50 years and 8 post-menopausal women of age 51 or more, mean PFOA levels were significantly lower in the former group (Harada et al., 2005); with Zhang Y. et al. (2013) estimating menstrual clearance as 0.029 mL/kg bw-day.

In the monkey, rat, and mouse respectively, half-lives of between 19.5 and 30 days (Butenhoff et al., 2004a), 0.12 and 11.5 days (unpublished study cited by the US EPA, 2016b, 2021b), and 15.6 and 21.7 days (Lou et al., 2009) were reported, suggesting considerable inter-species variation with evidence of longer retention in males.

In rats, mice and monkeys, the primary route for the elimination of PFOA is via the urine, with greater clearance rates being seen in female rats than in males (Unpublished data cited by the US EPA, 2016b). Biliary and faecal excretion also contribute to the elimination of PFOA in rats, with 7.2% and 7.7% of an administered dose of PFOA of 5 and 20 mg/kg bw (respectively) recovered in the faeces after the first dose, and rising to 25% and 40% at the low and high dose (respectively) after 28 days of exposure (Cui et al., 2010). Renal reabsorption of PFOA is also thought to occur, mediated by OAT proteins on proximal tubular cells which may contribute to sex-related differences in renal clearance (Kudo et al., 2002).

3.5. PBPK Models

Application of the default approaches for interspecies extrapolation commonly used in risk assessment (for example, dosimetric scaling using body weight to the ¾ power (US EPA, 2011)) may not be sufficiently health-protective, because they do not account for interspecies differences in clearance rates and half-lives (with animals showing much shorter half-lives compared to humans), maternal-foetal transfer, or tissue partitioning that are known to be relevant to PFAS. Alternatively, physiologically based pharmacokinetic (PBPK) models can provide an improved means of conducting cross-species dosimetry for risk assessment. There are several PBPK models for PFOS and PFOA applicable to rodents and humans that include consideration of gestation and lactation (EFSA, 2020). The design of these models and their parameters have been comprehensively reviewed elsewhere (ATSDR, 2021), although some examples are briefly described in this section.

The approach taken by the US EPA (2016a; 2016b) in deriving RfDs[6] for PFOS and PFOA involved use of a two-compartment PBPK model based on saturable renal resorption described by Wambaugh et al. (2013). The model includes a serum and a deep-tissue compartment, as well as a filtrate compartment into which PFCs are either excreted or resorbed via a saturable process with a Michaelis-Menten form. The model was parameterized with published toxicokinetic data in mice, rats, and monkeys, including parameters for volume of the central and filtrate compartments, and blood flow rate to the filtrate compartment. Average serum concentrations of PFOS or PFOA associated with the candidate NOAELs/LOAELs were derived from the area under the curve (AUC) considering the number of days of exposure. The predicted serum concentrations were then converted to an oral human equivalent dose (in mg/kg bw per day) for the chosen point of departure (POD), and appropriate uncertainty factors (UFs) applied to calculate the RfD.

EFSA (2020) determined the daily dietary intake of PFOS/PFOA associated with the benchmark dose corresponding to a 10% increase in response (BMD_{10}) for the potential critical effect using a slightly modified version of the PBPK model developed by Loccisano et al., (2011), a multi-compartment model parameterized with in vitro pharmacokinetic data in mice and rats. Loccisano et al. (2011) evaluated the results of their model predictions against experimental data in humans and monkeys and indicated good agreement between model simulations and experimental data. However, the PBPK models described by Wambaugh et al. (2013) and Loccisano et al. (2011) predate more recent work on PFOA and PFOS half-life in humans (Xu et al., 2020; Dourson and Gadagbui, 2021) and on PFOA PBPK studies in humans (Elcombe et al., 2013; Dourson et al., 2019; Goeden et al., 2019; Chou and Lin (2020, 2021); Dourson and Gadagbui, 2021).

Health Canada (2018a; 2018b) suggested that confidence in the mouse PBPK model is low due to lack of sufficient toxicokinetic data to support adequate model validation, concluding "there is insufficient confidence to use precise PBPK model results as points of departure for the risk assessments." Instead, Health Canada utilized predicted dose metrics from the PBPK modelling to derive chemical-specific adjustment factors (CSAFs) to replace default uncertainty factors. For example, the Loccisano et al. (2011) PBPK model was used to predict PFOS plasma and liver steady-state concentrations for humans, monkeys, mice and rats at different PFOS oral dose levels, and CSAFs were derived from the human:animal ratios of these predicted concentrations. Subsequently, a CSAF of 10 replaced the default four-fold toxicokinetic portion of the interspecies uncertainty factor for the purposes of deriving a TDI (HC, 2018a; 2018b), as the steady-state plasma PFOS level was predicted to be ten-fold higher in humans compared to rats at a dose of 0.1 mg/kg bw per day.

More recently, Dourson et al. (2019) proposed CSAFs for PFOA of 1.3 and 14 (for single doses and for repeated doses over durations sufficient to achieve steady-state, respectively) using ratios of human:animal C_{max} values derived from the Elcombe et al. (2013) human clinical study and from a pharmacokinetic study in mice (Lou et al., 2009). Dourson et al. (2019) chose the C_{max} instead of the AUC as the basis for the CSAF derivation since the identified critical effect was a developmental toxicity endpoint, in which a single exposure at any of several developmental stages may be sufficient to produce an adverse effect. However, it is unclear whether clearance under the high-dose PFAS exposures administered in this study would scale

[6] It is expected these RfDs would be superceded as a result of the US EPA's updated assessment (US EPA 2021a,b)

to low-dose scenarios, or whether PFAS toxicokinetics in persons with cancer are relevant to the general population.

Verner et al. (2016) developed a PBPK model consisting of two compartments (maternal and child) to simulate exposure in pregnant women and transfer to the child, and to simulate placental transfer and breastfeeding. Parameters for milk-plasma partition coefficient, cord blood-maternal serum partition coefficient, elimination half-life, volume of distribution, and body weights were obtained from the primary literature, and the results of the PBPK model were validated against experimental maternal and child PFOA and PFOS serum levels (at age 6, 19 and 36 months) in mother-child dyads from Germany and Norway. This PBPK model was utilized by the US EPA (2021a; 2021b) for human dosimetric adjustments in deriving their health advisories for PFOS and PFOA based on reduced antibody response to diphtheria and tetanus vaccines in young children (see section 4.2.4).

To better account for the significant placental and breastmilk transfer of PFOA during early life, Goeden et al. (2019) developed a single-compartment PBPK model to predict serum PFOA concentrations over a person's lifetime arising from exposure to PFOA from both drinking-water and breastmilk. Parameters of the model include water intake rate and concentration, breastmilk intake rate and concentration, breastmilk transfer rate and placental transfer rate, and an additional parameter to account for age-specific differences in extracellular water volume during early childhood. Additionally, model results were compared with empirical data from published studies for validation, indicating acceptable agreement. The study authors used the model iteratively to identify the drinking-water concentration (0.035 µg/L) that resulted in a steady-state serum concentration at or below a reference serum PFOA concentration of 0.065 mg/mL derived from a 38 mg/L LOAEL in a developmental toxicity study in mice (Lau et al., 2006; see section 5.5.2), an uncertainty factor of 300, and a relative source contribution of 50%.

Chou and Lin (2020) developed parameters for a PBPK model for PFOS serum concentrations in humans by using a hierarchical Bayesian framework to pool datasets across epidemiological, animal *in vivo*, and *in vitro* ToxCast studies. The authors then derived human equivalent doses from different points of departures identified from these studies, and the lower 95th percentile of these human equivalent doses was 21.5 ng/kg bw per day. Chou and Lin (2021) subsequently added gestation and lactation parameters to the model for rats and humans using a three-compartment foetal model, and used this refined model to calculate human equivalent doses from developmental toxicity studies in rats, obtaining 5th percentile human equivalent doses ranging from 0.08 to 0.91 µg/kg bw per day.

3.6. Human biomonitoring

Several biomonitoring studies observed elevated concentrations of PFAS in blood from general populations known to be exposed to drinking water contaminated with one or more PFAS (EFSA, 2020; ATSDR, 2021). Although many studies have focused on PFOS and PFOA, there is an increasing trend to evaluate the levels of additional PFAS such as PFHxS and PFNA (for example, Li et al., 2018, Ingelido et al, 2018, 2020). Importantly the biomonitoring studies highlight that median blood concentrations of PFOS, PFOA and other PFAS are similar across the general populations of Europe and North America (CDC, 2021a; CDC, 2021b; Pollock et al., 2021; Duffek et al., 2020). However, elevated exposures can be experienced worldwide by large populations due to localised incidents resulting in contaminated surface water and ground water (EFSA, 2020; ATSDR, 2021b). Reported incidents include release from fluoropolymer-

producing plants in the mid-Ohio Valley and other areas in the United States (Frisbee et al., 2009; Graber et al., 2019) and the Veneto Region, Italy (Ingelido et al., 2018; Pitter et al., 2020a). Additional incidents have been reported from the use of PFAS-contaminated soil conditioners in North Rhine–Westphalia, Germany (Hölzer et al., 2008) and in Alabama, United States (Worley et al., 2017), and from the use of firefighting foams in Ronneby, Sweden (Li et al., 2018; Gyllenhammar et al., 2015) and in numerous communities around the United States (ATSDR, 2021b; Daly et al., 2018; Barton et al., 2020; McDonough et al., 2021). General population biomonitoring data from surveys such as the US National Health and Nutrition Examination Survey (NHANES), the German Environmental Specimen Bank, and the Canadian Health Measures Survey have shown decreasing blood levels in adults for select PFAS, including both PFOS and PFOA, following the phase-out of their use (Göckener et al., 2020; CDC, 2021a; CDC, 2021b; Pollock et al., 2021). Finally, data from the CDC (2021a) show a trend of significant reductions of blood concentrations in the US population between 1999-2000 and 2017-2018. PFOA reductions were greater than 70%, and PFOS reductions were nearly 90%.

4. EFFECTS ON HUMANS

Studies related to the human health effects of PFOS and PFOA have been comprehensively reviewed and summarized elsewhere (ATSDR, 2021; EFSA, 2020; FSANZ, 2018; HC, 2018a; HC, 2018b; Steenland et al., 2020; US EPA, 2016a; US EPA 2016b; US EPA 2021a and US EPA 2021b). Although this section highlights several studies related to the toxicological effects of PFOA and PFOS exposure in humans as illustrative examples; it is not intended to be a comprehensive summary of all the data available.

4.1. Short-term exposure

No studies or case reports were identified that fully describe or quantify the effects of acute (single-dose) exposure to PFOS or PFOA in humans. However, limited information is available on short-term exposure from a dose-escalation clinical trial (Elcombe et al., 2013; as cited by Dourson et al., 2019), in which 43 patients (24 males and 19 females) with advanced solid tumors were given weekly doses of PFOA (in 50 mg tablets; approximately 2.3 mg/kg bw per day) for six weeks as a chemotherapy drug. The test article was described as non-toxic at all dose levels tested. Some nausea and vomiting of short duration was observed, as well as relatively mild lethargy, gastrointestinal disturbance and diarrhea, although the frequency and dose group(s) in which these observations occurred were not stated.

4.2. Long-term exposure

Human studies of longer duration exposures to PFOS and PFOA have evaluated a range of health effects, including birth outcomes, effects to the immune system, endocrine effects, and cancer outcomes. Studies of birth outcomes include several studies using subjects from national birth registries. For other health effects, the dataset on long-term human exposure consists of epidemiological studies in the general population, in occupational settings such as chemical plants (particularly for studies of thyroid hormone levels), and in communities presumed to have a high level of environmental exposure including through drinking-water due to PFOS and PFOA contamination of the environment (for example: residents of the Ohio River Valley (USA) or residents of the Veneto region of Northern Italy). The analysis in these studies is typically based on correlating blood levels of PFOS and PFOA to the outcome of interest, and

identifying odds ratios based on tertile or quartile of blood PFAS concentration. The sections below highlight examples of these studies for the most commonly studied health effects, but do not necessarily reflect comprehensive summaries of the literature.

4.2.1. Fertility and pregnancy outcomes

Numerous studies have been published on the relationship between PFOS and PFOA exposure and male and female reproductive and pregnancy outcomes in humans. The overall conclusions from these reviews as well as results from a few robust studies are briefly discussed in this section.

4.2.1.1. Fecundity

Lum et al. (2017) evaluated 501 couples from Michigan and Texas (USA) upon discontinuing contraception and followed them until pregnancy or 12 months of trying, and the association between PFOS and PFOA serum concentrations and menstrual cycle length and fecundity was evaluated. The authors applied a Bayesian model and adjusted for menstrual cycle length and found no statistically significant correlation between tertile of PFOS and PFOA exposure and day-specific probability of pregnancy. Similarly, Whitworth et al. (2012) conducted a case-control study in which 416 women from a Norwegian birth registry with a time-to-pregnancy of greater than 12 months were compared to 494 controls, with the analysis adjusted for maternal age, body mass index, and maternal alcohol consumption (PFOA only). Among all subjects, there was a statistically significant trend for increasing odds ratio of time-to-pregnancy greater than 12 months. When stratified by parity, the odds ratio for time-to-pregnancy greater than 12 months was statistically significant for parous women but was not statistically significant for nulliparous women.

Crawford et al. (2017) evaluated fecundability in a cohort of 99 women (aged 30 – 44 years) in North Carolina, USA. The study participants had no history of infertility and were trying to conceive for 3 months or less, and were grouped into quartiles according to level of exposure. Mean PFOS and PFOA serum concentrations were 9.29 ng/mL and 2.79 ng/mL, respectively, and due to the relatively small sample size, the analysis was only adjusted for age and mean oestrous cycle length. The fecundability ratios comparing the highest quartile of exposure to the reference group were not statistically significantly different from the null (1.0) for PFOS or PFOA.

A Canadian study of over 1,700 women demonstrated that increasing concentrations of PFAS in serum were associated with reduced fecundity, as measured by increased time to pregnancy, and reduced fertility (Vélez et al., 2015). Median serum PFOA and PFOS levels in the study population were 4.7 and 1.7 ng/mL for PFOA and PFOS, respectively. Specifically, an increase by one standard deviation in the serum-PFOA concentration was associated with a 31% increase in the odds of infertility and an 11% reduction in fecundability; however, no significant associations between exposure and time to pregnancy and fertility outcomes were observed for PFOS.

Time-to-pregnancy (TTP) was assessed in a Danish study of 1,240 women who had achieved pregnancy (Fei et al., 2009). The women were separated into quartiles of exposure based on PFOS and PFOA plasma levels. For PFOS, the exposure quartiles were 6.4-26 ng/mL, 26.1 – 33.3 ng/mL, 33.4 – 43.2 ng/mL and ≥ 43.3 ng/mL; whereas for PFOA, the exposure quartiles

were < 3.91 ng/mL, 3.91 – 5.2 ng/mL, 5.21 – 6.96 ng/mL and ≥ 6.97 ng/mL. Pregnant women in the higher three quartiles of PFOS and PFOA exposure (based on plasma levels) showed odds ratios for infertility (defined as having a time to pregnancy of greater than 12 months or having received infertility treatment) of 1.7 (95% CI: 1.01, 2.86), 2.34 (95% CI: 1.40, 3.89) and 1.77 (95% CI: 1.06, 2.95) for the respective quartiles compared to the lowest quartile of exposure for PFOS, and 2.06 (95% CI: 1.22, 3.51), 1.6 (95% CI: 0.93, 2.78) and 2.54 (95% CI: 1.47, 4.39) for PFOA. However, this study only evaluated pregnancies that led to the birth of a child; therefore, the odds ratios stated above are not reflective of women unable to get pregnant.

4.2.1.2. Maternal Hypertension and Preeclampsia

The C8 Science Panel (2012) noted that studies evaluating the association between PFOA exposure and hypertension and preeclampsia generally showed positive associations, but a clear dose response relationship was often lacking. Similarly, the Panel concluded that there is no probable link between PFOA exposure and miscarriage, preterm birth or stillbirth; however, a more methodologically robust study with greater refinement and less misclassification of exposure and outcome did report adjusted odds ratios of 1.27 (95% CI: 1.05, 1.55) and 1.47 (95% CI: 1.06, 2.04) for pregnancy-induced hypertension for every log increase in maternal serum PFOA and PFOS, respectively (Darrow et al., 2013). Six studies reporting results on pregnancy hypertension (including the Darrow et al. (2013) study) were reviewed by EFSA (2018), from which it was concluded there is "insufficient evidence to suggest that PFOS or PFOA are associated with pregnancy induced hypertension or preeclampsia."

4.2.1.3. Preterm Births and Pregnancy Loss

According to EFSA (2018), no consistent associations were observed between PFOS and PFOA exposure and preterm delivery, and there was insufficient evidence to determine if PFOA and PFOS exposure affects time to pregnancy or risk of pregnancy loss (miscarriage). Since those opinions were issued, the Meng et al. (2018) study reported slightly elevated odds ratios of 1.5 (95% confidence interval 1.1 – 2.2) and 1.1 (95% confidence interval 0.8 – 1.5) for preterm birth (length of gestation less than 35 weeks) associated with a doubling of PFOS and PFOA exposure, respectively, with an odds ratio of 1.9 for the highest quartile of exposure. Despite this more recent report, the EFSA (2020) review upheld its previous 2018 opinion; however, more recent publications have reported significant associations for PFOA but not for PFOS, as described below.

In a case-control study nested within the Danish National Birth Cohort, Liew et al. (2020) compared 220 pregnancies ending in miscarriage during weeks 12-22 of gestation with 218 pregnancies resulting in live births, with respect to maternal PFOS and PFOA plasma concentrations. Maternal plasma concentrations in the cohort ranged from 3.03 – 59.3 ng/mL (median of approximately 24 ng/mL) for PFOS, and 0.31 – 10.8 ng/mL (median of approximately 3.8 ng/mL) for PFOA. The study authors reported odds ratios of 1.2 and 1.3 for miscarriage per doubling of PFOS and PFOA plasma concentrations (respectively) in early pregnancy, after adjustment for maternal age, socio-occupational status, maternal smoking and alcohol intake, week of blood sampling, outcome of last pregnancy and time gap since last pregnancy. However, there was no adjustment for co-exposure to other contaminants. The authors also reported a significant plasma concentration-related trend of increased odds of

miscarriage and quartile of PFOA exposure, but no such significant association was determined for quartile of PFOS exposure. The authors concluded that higher maternal levels of PFOA were associated with an increased risk for miscarriage during weeks 12-22 of gestation among parous women, although the need for larger studies to confirm this association was acknowledged.

Chu et al. (2020) evaluated 372 mother-child pairs from a Chinese birth cohort study to evaluate the association between maternal PFOS and PFOA exposure and preterm birth (< 37 weeks gestation). The analysis was adjusted for infant sex, maternal age, maternal occupation, maternal education, family income and parity. Pairs were also divided into quartiles based on ranges of maternal serum levels. The odds ratios associated with preterm birth for the highest PFOS exposure quartile (greater than 11.93 ng/mL) and second highest PFOS quartile (7.15 – 11.93 ng/mL) were 4.99 (95% CI 1.21, 16.88) and 4.52 (95% CI 1.34, 18.56) respectively, with a statistically significant trend test. Corresponding odds ratios for PFOA were slightly elevated but the associated confidence intervals included 1.00.

Sagiv et al. (2018) measured plasma concentrations of PFOS and PFOA in 1645 women in early-stage pregnancy (median gestational age of 9 weeks) from a birth cohort in Massachusetts, USA, and evaluated the association between preterm birth (less than 37 weeks gestation) and PFOS and PFOA serum concentrations. The analysis was adjusted for socioeconomic factors (maternal age at enrolment, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, pre-pregnancy body mass index, paternal education, household income, child's sex, and gestational age at blood draw), and two hemodynamic markers: plasma albumin concentration and plasma creatinine concentration, for estimating plasma volume expansion and glomerular filtration rate, respectively. The cohort was also divided into quartiles of exposure, with the highest quartile associated with serum concentrations of 34.9 – 185.0 ng/mL for PFOS and 8.0 – 49.3 ng/mL for PFOA. The odds ratios for preterm birth associated with each increase in a quartile of exposure were 1.1 and 1.0, for PFOS and PFOA respectively; however, the odds ratios associated with quartiles 2 – 4 of PFOS serum concentrations were elevated (ranging from 2.0 – 2.4) with the lower 95% confidence intervals slightly higher than 1.0. Adjustment for the two hemodynamic factors did not significantly affect the results.

Wikström et al. (2021) evaluated the association between PFOS and PFOA maternal serum levels and first trimester miscarriage in a Swedish pregnancy cohort. Median PFOS and PFOA maternal plasma concentrations were 6.09 ng/mL and 2.00 ng/mL respectively in pregnancies resulting in miscarriages, and 5.45 and 1.64 ng/mL respectively in pregnancies resulting in live births. Odds ratios for miscarriage associated with a one-unit increase in log base-2 serum levels (adjusted for parity, age and tobacco exposure) were 1.13 (95% CI: 0.82, 1.52) ($p > 0.05$) and 1.48 (95% CI: 1.09, 2.01) ($p < 0.05$) for PFOS and PFOA respectively. The study authors concluded that there is an association between PFOA exposure and miscarriage in the study cohort, although the number of miscarriages included in the cohort (n = 78) was relatively small compared to the number of live births (n = 1449).

4.2.1.4. Effects to Male Reproduction

Tarapore and Ouyang (2021) reviewed 15 studies evaluating the association between PFAS exposure and male reproductive endpoints, and correlations between increased exposure to PFOA and PFOS and changes in circulating hormones (testosterone, estradiol, LH, FSH, etc.)

have been reported in adult males (Cui et al., 2020; Di Nisio et al., 2019; Petersen et al., 2018; Vested et al., 2013; Joensen et al., 2013), in children (Lopez-Espinoza et al., 2016), and adolescents (Tsai et al., 2015). Additionally, correlations of varying strength between increased exposure to PFOA and PFOS and reduced semen quality in adult males, including lower sperm count and reduced percentage of morphologically normal sperm, were reported in several studies (Cui et al., 2020; Di Nisio et al., 2019; Pan et al., 2019; Louis et al., 2015; Vested et al., 2013; Joensen et al., 2009). Other studies found no associations or only weak associations between PFOS and PFOA exposure and changes in circulating sex hormones (Lewis et al., 2015; Raymer et al., 2012; Specht et al., 2012; Joensen et al., 2009; Olsen et al., 1998), or sperm parameters (Petersen et al., 2018; Raymer et al., 2012; Toft et al., 2012). According to Tarapore and Ouyang (2021), the reasons for these inconsistent findings may include differences in exposure and characteristics between the study cohorts, including: the composition and concentration levels of various PFAS within the exposure mixtures, the ages of the participants, racial composition of the participants, the participants' dietary intakes, and/or susceptibility windows of exposure. These studies collectively suggest an association between PFOS and PFOA exposure and changes in reproductive hormones.

Bach et al. (2016) reviewed nine studies that explored the association between PFAS exposure in men and semen parameters. For semen volume, total sperm count and sperm concentration, none of the studies found consistent associations with exposure to PFOS or PFOA; however, two of the studies (Joensen et al., 2009; Toft et al., 2012) found serum levels of PFOS and PFOA to be associated with decrements to sperm morphology only when PFOS and PFOA exposure were combined, with the association attenuated when PFOS and PFOA exposures were evaluated separately. Furthermore, subsequent studies described by Bach et al. (2016) found no evidence of such an association (e.g. Raymer et al., 2012). Bach et al. (2016) also noted that many of the studies on PFOS and PFOA exposure and male reproduction were cross-sectional, and given the relatively long half-lives of PFOS and PFOA, it is uncertain whether the outcomes evaluated in cross-sectional studies and the measured blood plasma levels are causally related.

According to EFSA (2020), based on its review of several cross-sectional studies on semen quality and sex hormones in men exposed to PFOS and PFOA, there is insufficient evidence to demonstrate that prenatal or postnatal exposures to these compounds are associated with effects on pubertal development or male fertility. Similarly, Steenland et al. (2020) noted that despite reports of decreased sperm count and quality with higher PFOA exposure, subsequent studies did not support an adverse effect on fecundability. In summary, based on the conclusions of several published literature reviews and authoritative assessments, although some studies have found a correlation between increased PFOS and PFOA exposure and changes in male reproductive hormones and sperm quality, the strength of the correlations have been inconsistent, there is insufficient data to identify a clear dose-response relationship, and evidence of causality has not been clearly demonstrated.

4.2.2. Developmental outcomes

4.2.2.1. Reduced Birthweight

Wikström et al. (2020) conducted an analysis of maternal serum PFOS and PFOA and birth outcomes in 1533 infants identified from a Swedish birth registry. The cohort was divided into

quartiles of exposure based on maternal serum concentrations, and odds ratios were calculated for small for gestational age (SGA), defined in this study as infant weight being below the 10th percentile for gestational age and sex. The analysis was stratified by infant gender, and adjusted for maternal weight, parity, cotinine levels and gestational age. For PFOS, odds ratios for SGA associated with the upper quartile of exposure (compared to the reference group) were 1.56 (95% CI: 1.09, 2.22), 2.05 (95% CI: 1.00, 4.21), and 1.30 (95% CI: 0.70, 2.40) for all infants, girls, and boys, respectively. For PFOA, the corresponding odds ratios for low birth weight were 1.44 (95% CI: 0.86, 2.40), 2.33 (95% CI: 1.00, 5.43), and 1.04 (95% CI: 0.54, 2.01) for all infants, girls, and boys, respectively. Thus, for both PFOS and PFOA, the association between maternal PFOA and PFOS exposure and low birth weight was more pronounced for girls than boys. The mean and interquartile range of maternal serum concentrations were 5.38 (3.97 – 7.60) ng/mL and 1.61 (1.11 – 2.60) ng/mL for PFOS and PFOA respectively; however, the range of maternal PFOS and PFOA levels in the upper quartile of exposure was not stated.

Chu et al. (2020) evaluated 372 mother-child pairs from a Chinese birth cohort study to evaluate the association between maternal PFOS and PFOA exposure, and low birthweight (< 2500 g). The analysis was adjusted for gestational age, infant sex, maternal age, maternal occupation, maternal education, family income, and parity. Pairs were also divided into quartiles based on ranges of maternal serum levels, with the highest quartiles having maternal PFOS and PFOA serum concentrations of greater than 11.93 ng/mL and greater than 2.63 ng/mL, respectively. The odds ratios associated with low birthweight for the highest exposure quartiles were 3.70 for PFOS and 1.00 for PFOA, although the 95% confidence interval for PFOS included 1.00. For PFOS, a significant trend of increasing odds ratios with increasing quartiles of exposure was also reported.

Sagiv et al. (2018) measured plasma concentrations of PFOS and PFOA in 1645 women in early-stage pregnancy (median gestational age of 9 weeks) from a birth cohort in Massachusetts, USA, and evaluated the association between birth-weight-for-gestational-age and PFOS and PFOA serum concentrations. The analysis was adjusted for socioeconomic factors (maternal age at enrollment, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, prepregnancy body mass index, paternal education, household income, child's sex, and gestational age at blood draw), and two hemodynamic markers: plasma albumin concentrate and plasma creatinine concentration, for estimating plasma volume expansion and glomerular filtration rate, respectively. The cohort was also divided into quartiles of exposure, with the highest quartile associated with serum concentrations of 34.9 – 185.0 ng/mL for PFOS and 8.0 – 49.3 ng/mL for PFOA. Each increase in the quartile of exposure for PFOS and PFOA was associated with a mean reduction in the birth-weight-for-gestational-age z-score of 0.04 (95% CI -0.08, 0.01) and 0.02 (95% CI -0.08, 0.03), respectively, and the highest quartiles of exposure for PFOS and PFOA were associated with mean z-score reductions of 0.13 and 0.07 respectively, indicative of a weak association. The adjustment for the two hemodynamic factors did not significantly affect the results, suggesting that they do not confound the association between early-pregnancy PFOS and PFOA exposure and birthweight outcomes.

In a study by Darrow et al. (2013), the association between maternal exposures to PFOS and PFOA and low birthweight was evaluated among approximately 1,630 births in 1,330 women living near the DuPont Washington plant in the USA. The odds ratios (adjusted for maternal age, educational level, smoking status, parity, and BMI) associated with a reduction in birthweight of greater than 2500 g was 0.94 (95% confidence interval 0.75 – 1.17) and 1.12

(95% confidence interval 0.75 – 1.67) per unit increase in maternal serum PFOA and PFOS, respectively.

With respect to maternal PFOA exposure and low birth weight, EFSA (2018) concluded that "there may well be a causal association" based on its review of 13 prospective studies and four cross-sectional studies that included more than 100 participants. An updated review (EFSA, 2020) described eight additional studies published after the 2018 opinion evaluating PFOA exposure and low birthweight. This included a study of 3535 mother-infant pairs from the Danish National Birth Cohort in which a doubling of PFOS and PFOA exposure (respectively) resulted in birthweight reductions of approximately 45 g (95% confidence interval 14 – 77 g) and 36 g (95% confidence interval 5 – 66 g) (Meng et al., 2018). EFSA (2020) noted that while the other reviewed studies did not find a significant association, the Meng et al. (2018) study included women with comparatively higher PFOA exposures. Thus, according to the updated EFSA (2020) opinion, these additional studies did not contradict their 2018 conclusion.

According to a similar literature review by Steenland et al. (2020), these studies collectively suggest that an increase of 1 ng PFOA per mL maternal serum is associated with a reduced birthweight of approximately 10 g; however, reverse causality related to the magnitude of plasma volume expansion and glomerular filtration rate may contribute to this apparent association. The ATSDR (2021) concluded that "no studies found increases in the risk of low-birth-weight infants" associated with maternal PFOS serum levels.

4.2.2.2. Other developmental outcomes

Post-natal development was assessed in the offspring of mothers with measured levels of PFOS/PFOA in serum during pregnancy, in cord blood or in breast milk, and in longitudinal studies for periods between 6 months and 22 years. A large majority of these studies assessed impacts on neurobehavioral development, including risk of ADHD/hyperactivity; however, no clear quantitative associations between the serum levels and the neurodevelopmental effects were supported (EFSA, 2020).

Other potential adverse effects from exposure during development include: increased frequency of overweight condition in early childhood and adolescence, changes in female puberty onset, and reduced semen quality in males. EFSA (2020) concluded that "support for associations between prenatal exposure to PFOS or PFOA and (being overweight during) early life was considered insufficient." Similarly, EFSA (2020) concluded that based on a review of "a number of cross-sectional studies", there was insufficient evidence that exposure to PFOA or PFOS is related to changes in puberty onset or semen quality.

Ou et al. (2021) conducted a nested case-control study in a cohort of approximately 11,500 newborns in China to evaluate the association between maternal plasma PFOS and PFOA concentrations (blood collected before delivery) and incidence of congenital heart defects (timing of evaluation not specified). Median maternal blood plasma concentrations for total PFOS and PFOA were 5.75 and 1.52 ng/mL respectively in mothers of newborns with a congenital heart defect, and were 5.74 and 1.49 respectively in controls. A statistically significant odds ratio of 1.81 (95% CI: 1.06, 3.08) for total congenital heart defects compared to controls associated with 75th percentile exposure to PFOS isomers with a linear perfluorocarbon chain was calculated. However, similar odds ratios calculated for total PFOS (i.e. combined linear and branched PFOS) and for PFOA were not statistically significantly

different from 1.0. The authors concluded that exposure to linear PFOS may be associated with septal and conotruncal defects.

4.2.3. Neurotoxicity (non-developmental)

A limited number of cross-sectional epidemiology studies investigated the association between early-life exposure to PFOS and/or PFOA and adverse neurobehavioral, neuropsychiatric and cognitive outcomes in adults and children. However, based on a comprehensive review of these studies, EFSA (2020) concluded that no consistent adverse associations with serum PFAS levels were found, with some showing inverse ('protective direction') associations.

4.2.4. Immune outcomes

Observational human studies reported associations between exposure to PFOS/PFOA and adverse immune responses including, asthma, allergies, serum antibody response to vaccination and propensity for infections. The NTP (2016) concluded that PFOA and PFOS are presumed to be human immunotoxicants, and EFSA (2020) selected immunotoxicity as the critical effect in their derivation of a tolerable daily intake for the major PFAS, further concluding that tolerable exposure limits should be based on preventing deficits of the humoral immune system in humans. The quality of the studies varies, with the more robust designs related to the end point of reduced antibody response to vaccination (ATSDR, 2021). These provide stronger evidence of a causal association between serum PFOS and PFOA concentrations and adverse effects on antibody response following vaccination, compared to other immunological outcomes (EFSA, 2020). Examples of recent studies that evaluated this association are described below.

In a cross-sectional study involving healthy 1-year-old children in Germany (Abraham et al., 2020), including 80 breast-fed and 21 formula-fed children, plasma PFOA levels were significantly inversely correlated with antibody responses to vaccines for *Haemophilus influenza* type b (HIB) ($r = -0.32$), tetanus ($r = -0.25$) and diphtheria ($r = -0.23$); however, these correlations were not significant for plasma PFOS levels. The study cohort was also evaluated for exposure to persistent organic compounds, mercury, cadmium and lead. The study authors reported significant associations between HIB antibody levels and exposures to polychlorinated biphenyls (PCBs) and dioxins, but not for heavy metals; this was attributed to the high correlation between these contaminant concentrations with PFOA. Furthermore, in a separate multivariate analysis with inclusion of dioxins and PCBs in addition to PFOA and PFOS, only PFOA had a significant effect on HIB antibody response; no significant associations were observed between other contaminants and other antibody levels. Using the data from this study, ESFA (2020) proposed a $BMDL_{10}$ of 17.5 ng/ml serum for the sum of PFOS, PFOA, PFNA and PFHxS, which was converted to a value of 0.63 ng per kg body weight per day using the Loccisano et al. (2011) PBPK model (see section 4.5).

A birth cohort study reported significant adverse impacts of PFAS exposure on indicators of vaccination efficacy in children (Grandjean et al., 2012). The study was based on 656 births in the Faroe Islands and followed 587 of the children to age 7 years. Median PFOS and PFOA maternal plasma concentrations were 27.3 and 3.2 ng/mL respectively, while median plasma concentrations in children aged 5 years were 16.7 and 4.1 ng/mL respectively. At age 7, a doubling in exposure to PFOS and PFOA based on plasma concentrations was associated with a decrease in diphtheria and tetanus antibody concentrations of 28% (95% CI: 3 – 46 %) and

24% (95% CI: 4 – 44%). Additionally, a two-fold increase in child plasma PFOS concentrations at age 5 years resulted in odds ratios of 2.38 (95% CI: 0.89 – 6.35) and 2.61 (95% CI: 0.77 – 8.92) associated with diphtheria and tetanus antibody concentrations (respectively) falling below a clinically protective level of 0.1 IU/mL. The corresponding odds ratios for PFOA were 3.27 (95% CI: 1.43 – 7.51) and 4.20 (95% CI: 1.54 – 11.44) for diphtheria and tetanus antibody concentrations, respectively. The analysis was adjusted for age, sex, PCB exposure, time since vaccination and booster type.

In 516 subjects from a Faroese birth cohort, serum-PFOA and PFOS concentrations were assessed at the ages of 7 and 13 years, and were evaluated against concentrations of antibodies against diphtheria and tetanus (Grandjean et al., 2017). Median PFOS serum concentrations were 15.3 and 6.7 ng/mL in participants aged 7 and 13 years respectively, whereas the corresponding median PFOA concentrations were 4.4 and 2.0 ng/mL respectively. After adjusting for sex, age, and PCB exposure as covariates, the authors reported that diphtheria antibody concentrations significantly decreased by about 25% for every doubling of PFOA (in 13-year old subjects) and PFOS (in 7-year-old subjects). However, the respective 95% confidence intervals of 3.0 – 42.5% reductions and -1.4 – 45.4% reductions suggest high intra-individual variability. Similar associations between elevated PFOA and PFOS plasma concentrations and reduced tetanus antibody concentrations were not as strong as for diphtheria antibody concentrations.

Dalsager et al. (2021) conducted an analysis of approximately 1500 mother-child pairs contained within the Odense (Denmark) Child Cohort to investigate the association between maternal serum concentrations of PFAS during pregnancy and incidence of childhood infections between birth and age 4. Median and 95th percentile maternal PFOS plasma concentrations were 7.52 ng/mL and 15.1 ng/mL respectively, and median and 95th percentile maternal PFOA plasma concentrations were 1.68 and 4.01 ng/mL respectively. A doubling of maternal PFOS concentrations was associated with a 23% increase in the risk of hospitalization for any infection (adjusted hazard ratio of 1.23, 95% CI: 1.05, 1.44), with the relationship being strongest for lower respiratory tract infections (adjusted hazard ratio of 1.54, 95% CI: 1.11, 2.15), but weaker for urinary tract infections, GI tract infections and other infections. Similar hazard ratios for PFOA were slightly elevated but were generally lower than corresponding ratios for PFOS. The hazard ratios were adjusted for maternal age, parity, maternal educational level, child sex and child age, but not for socioeconomic status or other environmental contaminants.

In summary, it is suggested that decreased antibody response to vaccination may lead to reduced immune system functionality. However, studies report inconsistencies in the relationship between PFAS exposure and infection propensity in early life (Antoniou et al., 2022; ATSDR, 2021; EFSA, 2020; Steenland et al., 2020; US EPA, 2021a; 2021b) and therefore, the clinical relevance of these findings is unclear. More studies, particularly with more objective measures of infections, are needed (EFSA, 2020).

4.2.5. Endocrine outcomes

According to the results of studies reviewed by the US EPA (2016a; 2016b), evidence of altered circulating thyroid hormones in studies of occupational cohorts and in the general population studies was mixed. An analysis of an occupational cohort (approximately 200 male workers) in Minnesota (Olsen et al., 1998) showed significantly elevated TSH levels in a group having

PFOA serum concentrations ranging from 10 – 30 μg/mL; however, this increase was not observed for those with greater than 30 μg/mL serum PFOA, and there was no correlation with testosterone or estradiol levels. In a pooled evaluation of 940 chemical plant employees from Minnesota (USA), Alabama (USA), and Belgium (Olsen and Zobel, 2007) adjusted for age, BMI, and alcohol consumption, there was a statistically significant negative association between serum PFOA and free T4, and a statistically significant positive association between serum PFOA and T3. In an analysis of 1181 subjects from the U.S. NHANES database, the association between serum PFOS and PFOA levels, and levels of circulating thyroid hormones (total and free T4 and T3, TSH, and thyroglobulin) was evaluated, adjusting for age, race, drinking, smoking status, and urinary iodine (Wen et al., 2013). In women, a log unit increase in serum PFOS was associated with an increased serum total T3 concentration of 6.628 ng/dL (95% CI 0.545–12.712, P = 0.035). No other significant correlations between serum PFOS and PFOA and thyroid hormones were identified in that study. Additionally, an analysis of a cohort of 551 adolescents and young adults (aged 12 to 30 years) in Taiwan that was divided into quartiles based on PFOS and PFOA exposure identified no significant associations increasing quartile of serum PFOS and PFOA and circulating free T4 and TSH (Lin et al., 2013). In that study, the highest exposure quartile had serum concentrations of greater than 13.14 ng/mL PFOS or greater than 9.71 ng/mL PFOA.

In more recent studies, Crawford et al. (2017) evaluated the association between PFOS and PFOA exposure and levels of circulating TSH, T3, T4, and antimullerian hormone (AMH), a measure of ovarian reserve, in a cohort of 99 women (aged 30 – 44 years) in North Carolina, USA. The study participants had no history of infertility and were trying to conceive for 3 months or less. The analysis was only adjusted for age and mean oestrous cycle length, and mean PFOS and PFOA serum concentrations were 9.29 ng/mL and 2.79 ng/mL, respectively. The only statistically significant association reported was a positive correlation between PFOA serum concentration and T3 levels, and there were no statistically significant associations between PFOS and PFOA levels and levels of circulating TSH, AMH, free T4 or bound T4. In a cross-sectional study of 1366 maternal blood samples collected between gestational weeks 5 and 19 from mother-child pairs in the Danish National Birth Cohort, although there were associations between increased PFOS and PFOA serum levels and increased TSH during week-specific samples taken from gestations weeks 5-10, this effect become null after week 10, and there were no apparent associations between maternal PFOS and PFOA, and TSH and free T4 in total samples (Inoue et al., 2019). Another cross-sectional study conducted on over 21,000 individuals aged 14-39 living in a PFAS-contaminated area (Veneto Region, Italy) did not detect a significant association between serum TSH and serum PFOA or PFOS among adolescents or women, whereas low levels of PFOA and PFOS in adult males was association with only a mild decrease in TSH (Gallo et al., 2022).

A limited number of human studies suggested the potential for PFOS/PFOA exposure to be linked to changes in sex hormones in men, early onset of puberty and menopause, increased incidence of endometriosis, and changes to menstrual cycle length. However, these studies provided insufficient evidence of consistent associations between PFOS/PFOA exposure and these outcomes (EFSA, 2020). Additionally, other studies have evaluated the potential for effects of PFOS/PFOA exposure on thyroid function, including in cohorts from the C8 Science Panel (2012) and from NHANES. Many studies assessed thyroid hormone levels (TSH, free T4 and free T3) in adults, with some providing combined analyses for pregnant women and their newborns. However, collectively these studies do not provide sufficient support for an association between PFOS/PFOA exposure and thyroid disease or changes in thyroid hormones

(EFSA, 2020). Additionally, according to a literature review by Steenland et al. (2020), "the evidence of an association of PFOA with thyroid disease has gotten weaker" and "evidence for a causal impact (of PFOA exposure) on thyroid hormones remains weak." In draft assessments by the US EPA (2021a; 2021b), it was noted that evidence from human epidemiological studies was inconsistent regarding associations between PFOS and PFOA exposure and endocrine outcomes, but the results are suggestive of positive associations for PFOA and TSH, especially in adults, and for PFOA and T4, especially in children.

4.2.6. Metabolic outcomes

Studies on the effects of PFOS and PFOA exposure on metabolic outcomes have largely focused on the relationship between exposure and increased serum cholesterol, and several cross-sectional studies have been conducted in a population living in a highly contaminated region (Veneto) of Northern Italy, which have found correlations between increased exposure to PFOS or PFOA and slightly elevated cholesterol and blood pressure. For example, Canova et al. (2020) reported mean total cholesterol increases of 4.99 mg/dL and 1.94 mg/dL associated with each log increase in serum PFOS and PFOA, respectively, based on a study of 16,224 adults aged 20-39 years. In a follow-up study comprising 6,669 adolescents and 2.693 children, Canova et al. (2021) found significant associations between serum PFOS and PFOA and total cholesterol, LDL cholesterol, and HDL cholesterol in adolescents, whereas in children, these associations were found for PFOS only. Although Jeddi et al. (2021) did not find positive associations between serum PFOA or PFOS and prevalence of metabolic syndrome in a study of nearly 16,000 young adults, positive associations were found for individual components of metabolic syndrome such as elevated triglycerides and blood pressure. Additionally, in a high-exposed cohort of 232 male ex-employees who had worked in a PFAS-producing factory (PFOA and PFOS median plasma concentrations of 80.8 ng/L and 8.55 ng/mL respectively), there were statistically significant associations between total PFAS serum levels and total cholesterol, LDL cholesterol and systolic blood pressure, but not for HDL cholesterol or diastolic blood pressure (Batzella et al., 2022). Lastly, Pitter et al. (2020b) evaluated blood pressure measurements in 16,224 individuals aged 20-39 years and reported that each log-unit increase in PFOS or PFOA serum concentrations was associated with increased odds of hypertension in men, with odds ratios of 1.13 and 1.06 respectively for PFOA and PFOS.

In summary, cross-sectional epidemiology studies evaluated serum lipid status in association with serum PFOS and PFOA concentrations in workers and the general population. Statistically significant positive associations between exposure to PFOS and/or PFOA and total serum cholesterol are reported; similar findings were reported for LDL cholesterol but not for HDL cholesterol. It is proposed that this finding may have clinical significance as an increase in LDL cholesterol is associated with an increase in cardiovascular risk (EFSA, 2020). However, it is unclear whether the effect of exposure on serum cholesterol levels results in an increased risk of cardiovascular disease. Further studies on the effect of PFOS and PFOA exposure on the prevalence of obesity and diabetes have largely been unable to detect a significant association.

For example, a longitudinal study of cardiovascular disease in a PFAS-exposed population was conducted by Winquist and Steenland (2014), in a cohort of over 30,000 community-exposed and over 750 occupationally exposed individuals in the U.S with a median follow-up duration of 32.6 years. Regardless of gender, age group or quintile of exposure, there was no significant correlation between PFOA exposure and onset of hypertension or cardiovascular heart disease, despite some evidence of a significant association between PFOA exposure and

hypercholesterolemia (particularly in men aged 40 – 59). Based on the results of this study, Steenland et al. (2020), suggested that increased serum cholesterol resulting from increased exposure to PFOA may have little impact on the risk of cardiovascular disease.

Caution in the interpretation of the causal relationship between increased PFOS and PFOA exposure and increased cholesterol is discussed by the authors of the individual studies and by EFSA (2020), particularly as the findings in humans are contrary to those from animal studies where there is a PPARα-mediated decrease in serum lipids at high doses of PFOS and PFOA, implying that the mode of action in humans may be unrelated to peroxisomes. In humans, increases in total serum cholesterol are possibly related to impaired lipoprotein transport rather than lipid metabolism as indicated by the variable responses in humans for LDLs, IDLs and HDLs. In their most recent evaluation, EFSA (2020) acknowledged that the uncertainty regarding causality for this endpoint may be larger than reported in the previous evaluation "due to a postulated biological process around the enterohepatic cycling of both PFAS and bile acids, the latter affecting serum cholesterol levels" (EFSA, 2020). Steenland et al. (2020) also suggested that the dose-response relationship between PFOS and PFOA exposure and elevated cholesterol may be non-linear, as the results from several cross-sectional studies indicate that the lower the range of PFOA exposure studied, the stronger the effect per unit exposure. Similarly, EFSA (2020) also noted that a maximum association with total cholesterol occurs at PFOA serum levels of 25 ng/mL and does not continue to increase as the serum level increases. Furthermore, according to the conclusion of an expert workshop, the biological mechanism underlying the association between PFAS exposure and elevated cholesterol in humans remains unclear (Andersen et al., 2021).

Studies reviewed by existing authoritative assessments have not found consistent evidence of an association between exposure to PFOS and PFOA and prevalence of diabetes or obesity. EFSA (2018) reviewed 15 studies on the associations between exposure to PFOS and PFOA and increased risk of diabetes and adiposity, and concluded there is "no evidence that PFOS or PFOA increases the risk of metabolic syndrome." Health Canada (2018b) similarly concluded that "data available from cross-sectional environmental studies conducted within the C8 Health Project suggest that there is no link between PFOA and Type II diabetes." Additionally, ATSDR (2021) cited three studies that evaluated the association between community PFOA/PFOS exposure and obesity (Barry et al., 2014; Braun et al., 2016a and 2016b), and the corresponding odds ratios and risk ratios comparing exposed and unexposed populations were not statistically significant from 1.0.

4.2.7. Other effects

4.2.7.1. Liver

EFSA (2018) reviewed several studies on associations between PFOS and PFOA and various endpoints related to liver toxicity and concluded there is likely a causal relationship between serum PFOA and serum ALT levels; however, it was also noted that elevated ALT levels rarely exceeded the normal range. Furthermore, there was no consistent association between PFOS exposure and serum ALT. Based on this review, EFSA (2018) concluded that although there is evidence for a causal relationship between PFOA exposure and elevated liver ALT activity, the adversity of this effect is unclear due to the low magnitude of the increases and lack of

associated liver disease. EFSA (2020) reviewed four additional studies evaluating PFOS and PFOA exposure and liver endpoints and determined that the results of these newer studies agree with the earlier EFSA (2018) conclusion. According to draft assessments by the US EPA (2021a; 2021b), the human epidemiological data provide consistent evidence of a positive association between both PFOS and PFOA exposure and increased ALT activity in adults; however, since the r values were not large in magnitude, it is unclear whether the observed changes are clinically adverse.

4.2.7.2. Kidney

Several cross-sectional studies indicate a strong association between serum PFOS/PFOA and a decrease (5 – 10%) in estimated glomerular filtration rate (eGFR), which may be linked to chronic kidney disease. However, according to ATSDR (2021), there is "suggestive evidence" that this association may be due to reverse causality, whereby increased PFOA/PFOS levels result from reduced eGFR (due to the presence of shared renal transporters for perfluroalkyls and uric acid), rather than vice versa. EFSA (2020) also noted that confounding by variables present in the GFR equations, such as age, sex, height and weight, may impact the ability to determine causation.

4.2.7.3 Uric acid

Several studies indicate an association between serum PFOS/PFOA and increased serum uric acid levels, which is clinically considered a potential risk factor for hypertension and an independent risk factor for stroke. Reduced GFR could confound these results by leading to increased serum uric acid (EFSA, 2020).

4.2.8. Carcinogenicity

Results from some of the most robust relevant studies, as well as conclusions from existing authoritative assessments, are discussed below.

4.2.8.1. PFOS

In general, epidemiological studies in occupationally exposed cohorts as well as case-control studies reviewed by ATSDR (2021) and U.S. EPA (2021b) found mixed associations between PFOS exposure and cancers of the breast, bladder, kidney, colon, liver, pancreas or prostate. Several occupational studies in workers at a 3M plant in Alabama, USA (Alexander et al., 2003; Alexander and Olsen, 2007) were reviewed by the US EPA (2016a) that suggested elevated standardized mortality ratios and incidence ratios, but these studies were deemed inconclusive due to the very low number of cases identified (ranging from 3-11 cases). Other population-based cohort studies reviewed in these authoritative assessments (Alexander and Olsen, 2007; Eriksen et al., 2009) reported elevated odds ratios for prostate cancer; however, the confidence intervals included the null, and the finding was not repeated in another case-control study in a Danish population (Hardell et al., 2014), or in a study that examined associations between PFOS and prostate-specific antigen, a biomarker for prostate cancer in adult males (Ducatman et al., 2015).

In three case-control studies of breast cancer risk in PFOS-exposed populations in Denmark reviewed by ATSDR (2021), a "small-scale" study of 31 cases found a slight increase in cancer

risk (Bonefeld-Jorgensen et al., 2011), but this finding was not replicated in a similar study conducted by the same authors in a larger, different population (Bonefeld-Jorgensen et al., 2014); a third case-control study (Wielsøe et al. 2017) found significantly increased odds ratios of approximately 3 and 5.5 in the second and third tertiles of exposure, respectively. In a case-control study by Mancini et al. (2020), blood samples from 194 breast cancer cases and 194 controls were analysed for PFAS, and cases were stratified by tumour hormone receptor status. Quartiles of PFOS plasma concentrations ranged from 5.8 – 13.6 ng/mL, 13.6 – 17.3 ng/mL, 17.3 – 22.5 ng/mL and 22.5 – 85.3 ng/mL, respectively. Although increasing quartile of PFOS plasma concentrations was associated with increasing odds ratios for both estrogen and progesterone receptor-positive tumours, there was no significant relationship between increasing quartile of exposure and increased risk of hormone receptor-negative tumours. The authors noted the limited power of the study when stratifying cases based on tumour hormone receptor status (n = 2 – 43 cases per exposure quartile, per group). Similarly, a Taiwanese study of 120 cases and 119 controls derived an odds ratio of 3.25 (95% CI: 1.29, 8.23) for the association between a natural log increase in PFOS plasma concentrations and incidence of estrogen receptor-positive tumours in subjects below 50 years of age (Tsai et al., 2020). Similar odds ratios calculated in this study for estrogen receptor-negative tumour cases, as well as cases in subjects greater than 50 years of age, were not statistically significantly different from the null.

Shearer et al. (2021) published the results of a case-control study in which 324 cases of renal cell carcinoma were individually matched (based on age, race, ethnicity, study center and year of blood draw) to 324 controls within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. The analysis was adjusted for smoking status, history of hypertension, and prior freeze-thaw cycles of blood samples. The odds ratio for renal cell carcinoma associated with a doubling of PFOS serum concentration was elevated at 1.39 (95% confidence interval of 1.04-1.86).

The US EPA (2016a) review noted that "limitations in design and analysis" of epidemiological studies due to the small number of cases and lack of adjustment for other perfluorinated chemicals in serum of existing studies preclude the ability to make a definitive conclusion regarding PFOS exposure and cancer risk, a conclusion that was affirmed in the US EPA (2021a) draft assessment. EFSA (2020) concluded there is insufficient support for carcinogenicity of PFOS in humans, noting that temporal changes in cancer incidence rates, risk factors, survivability, and diagnostic criteria may result in biased non-comparable outcomes when evaluating studies of PFOA and PFOS and cancer incidence reported between the 1950s and 2000 (EFSA, 2018).

4.2.8.2. PFOA

Evidence of carcinogenic effects of PFOA in epidemiology studies is derived primarily from studies that focused on a population who worked at a DuPont plant in West Virginia where PFOA was used from 1952 in the production of fluoropolymers. Emissions from the plant contaminated the drinking water in several water districts in Ohio and West Virginia, and these studies included some who had worked at the plant. The cohort consists of around 69,000 individuals (adults and children) who had consumed drinking-water contaminated by PFOA from the plant. The US EPA (2016b) noted that two of these studies showed a positive association between plasma PFOA levels and self-reported cases (Barry et al., 2013) and incident cases between 1996 and 2005 (Vieira et al., 2013) of kidney and testicular cancers,

with the strength of the association slightly stronger for testicular cancer compared to kidney cancer in both studies. In an updated review by Steenland et al. (2020), it was noted that the C8 Science Panel concluded in 2012 that "there was a probable link between PFOA and both testicular and kidney cancers" with stronger evidence for testicular cancer and "somewhat" stronger evidence for kidney cancer having become available since that time. Since the publication of the Steenland et al. (2020) review, the Shearer et al. (2021) case-control study reported that the odds ratio for renal cell carcinoma associated with a doubling of PFOA serum concentration was 1.71 (95% confidence interval of 1.23-2.37) and statistically significant, with a greater than twofold increased risk among those in the highest quartile of PFOA exposure compared to the lowest. The study authors concluded that these findings "add substantially to the weight of evidence that PFOA is a renal carcinogen."

With respect to other cancer types, Steenland et al. (2020) concluded there is "some suggestive evidence" for an association between PFOA exposure and prostate cancer, whereas for liver and pancreatic cancer, Steenland et al. (2020) concluded there is "little evidence" of an association.

Recognizing that temporal changes in PFOA production rate, industrial hygiene practices, incidence rates, diagnoses, changes in other risk factors and survival changes over time that may result in biased non-comparable outcomes, EFSA (2018) suggested that studies among background and occupationally exposed individuals provided limited evidence to suggest that exposure to PFOA (and PFOS) are associated with increased cancer risk, and noted similar temporal limitations in evaluating PFOA human exposure studies as previously described for PFOS. Thus, the relevance of these findings to interpreting the risk of cancer in the general population following exposure to these chemicals remains unclear. IARC (2016) concluded "there is limited evidence in humans for the carcinogenicity of perfluorooctanoic acid (PFOA)", noting positive associations for cancers of the testis and kidney, and classified PFOA as "possibly carcinogenic to humans (Group 2B)". The US EPA (2016b) concluded there is "suggestive evidence of carcinogenic potential" for PFOA, based on the availability of epidemiological studies that demonstrate an association between PFOA exposure and kidney and testicular tumours among highly exposed individuals. The US EPA (2021b) draft assessment affirmed this conclusion for kidney cancer, noting that the Shearer et al. (2021) study adds support for an association, whereas the US EPA (2021b) draft assessment identified no new data for testicular cancer more recent than the previous US EPA (2016b) assessment, and further reported that the association between PFOA exposure and breast cancer is unclear. More recently, based on both epidemiological and animal toxicological studies, the US EPA proposed changing the cancer designation of PFOA to a "likely carcinogen" (US EPA, 2021) which was supported by their Science Advisory Board (SAB, 2022).

5. EFFECTS ON ANIMALS AND IN VITRO TEST SYSTEMS

Studies related to toxicological effects of PFOS and PFOA in animals have been comprehensively reviewed and summarized elsewhere (ATSDR, 2021; EFSA, 2020; FSANZ, 2018; HC, 2018a; HC, 2018b; US EPA, 2016a; US EPA 2016b; US EPA, 2021a; US EPA 2021b). As such, discussion in the current background document is limited to brief descriptions of a few robust studies. Additionally, as the oral route of exposure is relevant to this background document, the discussion below focuses on oral studies.

5.1. Acute toxicity

Oral LD_{50} values suggestive of moderate acute toxicity in rats have been reported for both PFOS and PFOA in authoritative reviews by ATSDR (2021), Health Canada (2018a, 2018b), and US EPA (2016a, 2016b). For PFOS, the oral LD_{50} was 251 mg/kg bw for male and female rats combined. Noteworthy signs of toxicity included neurotoxic effects (decreased limb tone, ataxia, hypoactivity and urinary incontinence), stomach distension, lung congestion and irritation of the glandular mucosa. For PFOA, rat oral LD_{50} values ranged from 430 to 680 mg/kg bw. Non-lethal effects reported in dosed animals included ptosis, piloerection, hypoactivity, decreased limb tone, ataxia and corneal opacity. In mice, the oral LD_{50} for PFOS was 579 mg/kg bw with similar toxicological effects as those observed in the acute rat studies. No oral LD_{50} study in mice was located for PFOA.

5.2. Short-term exposure (≤ 90 days)

5.2.1. PFOS

Oral studies of short and intermediate duration (90 days or less) for PFOS were conducted in rats and mice. These studies consistently reported liver effects (including increased relative liver weight and liver transaminase activities) and reduced serum T4 levels at doses as low as 0.27 mg/kg bw per day in rats exposed for 90 days. Application of the criteria developed by Hall et al. (2012) to evaluate the human relevance of the liver changes suggested that several hepatic findings (including increases in liver weight, hepatocellular hypertrophy and alterations in serum lipid levels in the absence of other degenerative lesions) were not relevant for human risk (ATSDR, 2021).

PFOS studies in rats

In Sprague Dawley rats, PFOS was administered in the diet at doses of 0, 0.05, 0.2, 0.4 and 1.5 mg/kg bw per day for 14 weeks (Seacat et al., 2003). Males in the highest dose group showed increases in absolute and relative liver weight, increased numbers of segmented neutrophils in peripheral blood, decreased blood cholesterol, and increased serum alanine aminotransferase and urea nitrogen. In females in the highest dose group, increases in relative liver weight and blood urea nitrogen were apparent. Histological evaluation showed hepatic hypertrophy and cytoplasmic vacuolisation in males at the two highest doses and in females at the highest dose only and a NOAEL of 0.4 mg/kg bw per day was derived.

Groups of 8-10 male Sprague Dawley rats were administered PFOS in drinking water at concentrations of 0, 1.7, 5.0 or 15.0 mg/L (equivalent to doses of 0, 0.27, 0.79 or 23.7 mg/kg bw per day, according to ATSDR, 2021 for 91 days (Yu et al., 2009). At 0.27 mg/kg bw per day, there was a 42% decrease in total T4 levels, with further dose-dependent decreases in T4 at higher dose levels. No dose-dependent changes were reported in total T3, free T4, or TSH levels.

Additionally, in a study designed to evaluate neurotoxic effects, Wistar rats administered PFOS in the diet for 13 weeks showed significantly increased relative liver and brain weights at doses equivalent to approximately 1.5 – 3.1 mg/kg bw per day or greater; however, no neurotoxic symptoms were reported (Kawamoto et al., 2011).

Similar effects were seen in several repeated dose studies of 28 days in duration or less. For example, in a 28-day gavage study carried out by NTP (2019a) in male and female Sprague-Dawley rats, a dose of 0.312 mg/kg bw per day PFOS was associated with significantly increased liver weight and reduced serum T4 levels, with hematological effects and liver hepatocellular hypertrophy occurring at higher doses. In other repeated dose studies of similar duration, dietary exposure to 1.5 mg/kg bw per day PFOS was associated with increases in relative liver weight and blood glucose levels in male Sprague-Dawley rats (Seacat et al., 2003). Additionally, the US EPA (2016a) identified a LOAEL of 1.33/1.43 mg/kg bw per day from a 28-day feeding study in SD rats (Curran et al., 2008) based on a statistically significant increase in absolute (females) and relative (males and females) liver weight, and a decrease in serum T4 in both sexes. Elcombe et al. (2012) reported a 28-day study in male Sprague Dawley rats in which increased relative liver weights and serum cholesterol were at a PFOS dose of 1.7 mg/kg bw per day.

In a repeated dose study of Sprague Dawley rats administered doses of PFOS of 0, 1.25, 5, 10 mg/kg bw per day by oral gavage for 28 days (Kim et al., 2011), increased relative liver weight in males and females was apparent at doses of 5 mg/kg bw per day and above while in males there was an increase in serum aspartate aminotransferase (AST) at the same dose. The most sensitive endpoint was an increase in liver transaminase in males in the lowest dose group (1.25 mg/kg bw per day).

In a 28 day study carried out by NTP, male and female Sprague-Dawley (Hsd:Sprague Dawley SD) rats (n = 10/dose) were administered PFOS by gavage at doses of 0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg bw per day (NTP, 2019a). Serum levels of PFOS were similar in males and females. There were no statistically significant treatment-related clinical observations in male or female rats. However, dose-related and significant increases in absolute and relative liver weights were reported in males and females at all doses, when compared with controls. In males, cholesterol levels were significantly reduced at all doses and in females at the highest dose only. These correlated with histopathologic changes in both sexes with decreased extramedullary haematopoiesis and hypocellularity reported at doses ≥ 1.25 mg/kg bw per day and hepatocellular alteration (hypertrophy and/or cytoplasmic alterations) at doses ≥ 2.5 mg/kg bw per day. Serum levels of free and bound T4 were reduced in males and females at PFOS doses ≥ 0.312 mg/kg bw per day and T3 levels increased at doses ≥ 0.625 mg/kg bw per day. The lowest dose of 0.312 mg/kg bw per day was associated with increased liver weight and co-occurring histopathological changes in liver histopathology.

PFOS studies in mice

No subchronic repeated dose studies in mice with PFOS exposure durations of 90 or more days were located. In several shorter-duration studies (10 – 30 days) in mice, similar hepatic effects were observed but generally at higher PFOS doses compared to rats (approximately 2 – 5 mg/kg bw per day in mice). For example, following a 30-day administration of PFOS by oral gavage at doses of 0, 2.5, 5, 10 mg/kg bw per day to C57Bl/6 mice, statistically significant increases in relative liver weight compared to control (accompanied by increases in serum ALP, AST, ALT and GGT) were reported in all three treatment groups, with increases of 55%, 91%, and 155% above the control mean in the three respective doses (Xing et al., 2016).

In a 21-day study, Wan et al. (2012) administered PFOS to male CD-1 mice by oral gavage, and significantly increased liver weights, significantly increased liver triglycerides, and a

"yellowish coloration" of the liver were reported in groups exposed to 5 or 10 mg/kg bw per day, along with histopathological evidence of macrovesicular steatosis.

In a 14-day study in male Balb/c mice administered PFOS by oral gavage at doses of 0, 5 or 20 mg/kg bw per day, Wang et al. (2014) reported statistically significant increases in absolute and relative liver weights and hepatic lipid concentrations at doses of 5 and 20 mg/kg bw per day compared to controls.

Qazi et al. (2009) reported effects following 10 days exposure of male C57Bl/6 (H-2) mice to PFOS in the diet at doses equivalent to 2, 10, 40 mg/kg bw per day, with significantly increased relative liver weight occurring at the lowest dose tested (2 mg/kg bw per day), and decreased spleen, thymus, body and body fat weights occurring at higher doses.

In summary, these studies indicate that the primary effects of PFOS are on the liver and biochemical parameters associated with lipid metabolism. Other effects including body weight changes, endocrine, neurological and immunological effects were also noted, and these are further discussed in the following sections.

5.2.2. PFOA

Oral studies of various durations (ranging from 14 days to 90 days) have been conducted in PFOA-exposed monkeys, rats, and mice. Studies documenting the toxicity of PFOA after short-term oral exposure confirmed that the liver is the main target organ for PFOA induced toxicity, potentially occurring through PPARα-mediated peroxisome proliferation, enhanced lipid peroxidation, or other mechanisms. Some alterations in the kidney and serum thyroid hormone levels were observed at higher PFOA doses. Changes in serum levels of markers of liver damage occur mainly in mice and include increased ALT, AST, GGT and ALP. In rats, increased ALP was also been reported (HC, 2018b). Several studies in rats and mice found increased kidney weight at exposure levels of PFOA ≥ 1 mg/kg bw per day (Goldenthal et al., 1978; Butenhoff et al., 2004b; Cui et al., 2009; Yahia et al., 2010), with most showing relative, but not absolute changes. Some of the weight changes were associated with histological effects (Butenhoff et al., 2004b; Cui et al., 2009; Yahia et al., 2010).

PFOA studies in primates

The lethality of PFOA was seen in a 90-day oral toxicity study in rhesus monkeys, with all animals exposed to doses of 100 mg/kg bw per day (highest dose) being dead within 5 weeks (Goldenthal, 1978). According to ATSDR (2021) the LOAEL for this study was 3 mg/kg bw per day (lowest dose tested) based on increased absolute liver weight reported in all three dose groups.

PFOA studies in rats

A 13-week repeated dose study was conducted in groups of 15 male Crl:CD BR rats exposed to PFOA in the diet equivalent to doses of 0, 0.06, 0.64, 1.94, and 6.5 mg/kg bw per day (Perkins et al., 2004). Statistically significant increases in mean relative liver weight were reported at 1.94 and 6.5 mg/kg bw per day, as well as increased incidence of mild hepatocellular hypertrophy at doses of 0.64 mg/kg bw per day and above. At doses of 1.94 and 6.5 mg/kg bw per day, slightly increased incidences of coagulative necrosis in the liver compared to controls

were also reported. These changes were accompanied by statistically significant increases in palmitoyl-CoA oxidase activity (a biomarker of peroxisome proliferation) at doses of 0.64 mg/kg bw per day (at week 4 only) and 1.94 and 6.5 mg/kg bw per day (at weeks 4, 7 and 13). A NOAEL of 0.64 mg/kg bw per day was identified for this study by the authors of the US EPA (2016b) review based on a conclusion the liver weight and palmitoyl-CoA responses were associated with the activation of PPARα and were not accompanied by significant dose-related changes suggestive of an adverse response.

In a 28-day study carried out by NTP (2019b), male and female Sprague-Dawley (Hsd:Sprague Dawley SD) rats (n = 10/dose) were administered PFOA by gavage at doses of 0, 0.625, 1.25, 2.5, 5, or 10 mg/kg bw per day (males) or 0, 6.25, 12.5, 25, 50, or 100 mg/kg bw per day (females) (NTP, 2019b). In males, statistically significant reductions in serum cholesterol and T3 were reported at all dose levels except for the highest dose (10 mg/kg bw per day), whereas statistically significant reductions in triglycerides and free and total T4, as well as statistically significant increases in albumin/globulin ratios, were reported at all dose levels tested. Histopathological changes at all doses in males were described as cytoplasmic changes of hepatocytes plus degeneration and inflammation of the olfactory epithelium. Similar effects were seen in females, but generally at higher dose levels (≥ 25 mg/kg bw per day) than in males.

Cui et al. (2009) also described increased relative liver weights in Sprague Dawley rats following 28 days exposure by oral gavage to PFOA at doses of 5 or 20 mg/kg bw per day. The increased liver weight was accompanied by increased incidence of hepatocellular hypertrophy, fatty degeneration, acidophilic lesions, gross dilation and congestion in the hepatic sinusoid or central vein.

Loveless et al. (2008) administered doses of 0, 0.3, 1, 10 and 30 mg/kg bw per day PFOA by gavage to groups of 10 male CD rats for a 29-day period. Total cholesterol, non-HDL cholesterol, HDL cholesterol and triglycerides were all significantly reduced at doses of 0.3 mg/kg bw per day or greater, although these effects were not clearly dose-dependent. Significant increases in relative liver weight, in the incidence of hepatocellular hypertrophy of moderate severity, and in the incidence of focal necrosis were reported at doses of 10 mg/kg bw per day or greater. The authors of the US EPA (2016b) assessment proposed a LOAEL of 10 mg/kg bw per day for this study.

PFOA studies in mice

In two 14-day studies, male Balb/c and male Kunming mice exposed to PFOA for 14 days by oral gavage showed increased relative liver weights at doses of 5 and 2.5 mg/kg bw per day respectively. Serum ALT levels were also increased in Kunming mice at the lowest dose of 2.5 mg/kg bw per day (Wang et al., 2013; Yang et al., 2014). Yang et al. (2014) also showed increased hepatic malondialdehyde levels in mice exposed at 2.5 mg/kg bw per day, which the authors attributed to enhanced lipid peroxidation.

Loveless et al. (2008) administered doses of 0, 0.3, 1, 10 and 30 mg/kg bw per day PFOA by gavage to groups of 20 male CD-1 mice for a 29-day period. The most sensitive effects included increased absolute and relative liver weight and moderate-to-severe hypertrophy and cell necrosis, occurring at doses of 1 mg/kg bw per day and higher. The authors of the US EPA (2016b) assessment proposed a LOAEL of 1 mg/kg bw per day for this study.

Four-week-old male ICR mice were exposed to doses of 0, 0.49, 2.64, 17.63 and 47.21 mg/kg bw per day via their drinking-water for 21 days (Son et al., 2008). In all dose groups, relative liver weight was significantly increased in a dose-dependent manner, and plasma ALT activity was significantly increased at doses of 2.64 mg/kg bw per day or greater. At higher doses (17.63 mg/kg bw per day or greater), reduced tumour necrosis factor-alpha expression, elevated transforming growth factor-beta expression, increased eosinophil infiltration and enlarged hepatocytes with acidophilic cytoplasm were reported in the liver histopathology. The US EPA (2016b) proposed a LOAEL of 0.49 mg/kg bw per day, the lowest dose tested, based on the changes in liver weights.

Following four weeks exposure to PFOA by oral gavage at doses of approximately 0, 5.4, 10.8 or 21.6 mg/kg bw per day, male 29S4/SvlmJ mice showed increased relative liver weight at all doses, including the lowest dose of 5.4 mg/kg bw per day (Minata et al., 2010). Additionally, in the low-dose group, total bilirubin was significantly reduced, total triglycerides were significantly increased, and there was a significant increase in the incidence of hepatocellular hypertrophy compared to controls. At higher dose levels, increased plasma AST, reduced total cholesterol, increased bile duct epithelial thickness, and increased apoptosis in hepatic cells and in the bile duct epithelium were reported. The US EPA (2016b) proposed a LOAEL of 5.4 mg/kg bw per day (lowest dose tested) based on the liver weight changes reported in low-dose animals.

The changes seen in liver weights following short-term exposure to PFOA are supported by histological changes in the liver in both rats and mice and include: cytoplasmic enlargement of hepatocytes and cytoplasmic vacuolation in rats; as well as single cell and focal necrosis, increased mitosis and mild calcification in mice (HC, 2018b; ATSDR, 2021). Application of the criteria developed by Hall et al. (2012) to evaluate the human relevance of the liver changes suggested that doses associated with liver hepatocyte hypertrophy and cytoplasmic vacuolation in the absence of any necrotic changes were not relevant for human risk (ATSDR, 2021).

5.3. Chronic exposure

5.3.1. PFOS

In male and female Cynomolgus monkeys administered PFOS at doses of 0, 0.03, 0.15, or 0.75 mg/kg bw per day by oral intubation for 183 days, compound-related mortality was reported in 2 of 6 male monkeys at the highest dose. In the remaining animals, adverse effects were apparent including: decreased body weights, increased liver weights, lowered serum total cholesterol and high-density lipoproteins (HDL), increased TSH levels, lowered triiodothyronine (T3) concentrations, and lowered oestradiol levels (male animals). Due to varying levels of significance for these effects at different time points in the study, the authors proposed a NOAEL of 0.15 mg/kg bw per day based on the changes in thyroid hormones and in HDL levels. However, following re-analyses of the data by authoritative bodies, a NOAEL of 0.03 mg/kg bw per day has been proposed for the decreases in cholesterol (ATSDR, 2021).

A chronic toxicity (and carcinogenicity) 2-year feeding study of PFOS was carried out in male and female Crl:CD(SD)IGS BR rats (Thomford, 2002; Butenhoff et al., 2012a). At dietary concentrations equivalent to approximately 0.2 – 0.3 mg/kg bw per day (mid dose group), increased incidence of several histopathological effects in the liver were reported, including:

hepatocellular centrolobular hypertrophy (males and females), centrilobular eosinophilic granular cytoplasm (females only), hepatocellular midzonal/centrilobular vacuolation (males only), and cystic degeneration (males only). The only noteworthy clinical chemistry finding was increased serum urea nitrogen in males and females at week 53; no other significant clinical chemistry changes were reported at week 53 or study termination at this dose. At dietary concentrations equivalent to approximately 1.0 - 1.3 mg/kg bw per day (high dose group), PFOS was associated with similar liver histopathological effects as those seen at the mid dose, in addition to increased incidence of hepatocyte necrosis and reductions in body weight. Additionally, statistically significant increases in hepatocellular adenoma were reported in high-dose males and females, as well as a single incidence of hepatocellular carcinoma in a high-dose female. No PFOS-related toxicity to thyroid, kidney or bladder was evident. Based on the findings of hepatotoxicity, a NOAEL for PFOS of approximately 0.1 mg/kg bw per day in male and female rats may be identified.

5.3.2. PFOA

The NTP (2020) conducted chronic PFOA exposure studies in Sprague-Dawley rats which were designed to assess the contribution of combined gestational and lactational exposure (i.e. perinatal exposure) to systemic toxicity and carcinogenicity. Groups of 12-week-old female rats were exposed to dietary concentrations of 0, 150, or 300 ppm PFOA from GD 6 through PND 21. Litters were standardized to eight pups per litter (four males and four females where possible). After weaning, groups of 60 male and female F1 rats were exposed to dietary concentrations of 0, 150 or 300 ppm PFOA (males) or 0, 300 or 1000 ppm PFOA (females). Additional groups of 50 male and 50 female F1 rats that were not exposed perinatally were exposed to similar dietary concentrations of PFOA in their diet for two years. At week 16 of dosing, interim observations and analyses were conducted in groups of ten F1 perinatally-exposed rats per sex per dose group, and the remaining groups of 50 animals continued PFOA exposure for two years. Due to excess toxicity in the male F1 rats, a second iteration of the study was conducted. The methodology of the second iteration of the study was similar to the first, except that in the second study there were only two exposure groups in the F0 females (0 or 300 ppm), and F1 males were exposed to 0, 20, 40 or 80 ppm post-weaning instead of 0, 150 or 300 ppm. According to the authors of the NTP (2020) report, after weaning, average PFOA consumption was 1.1, 2.2 and 4.6 mg/kg bw per day for F1 male rats and 29.6 and 98.6-104.4 mg/kg bw per day for F1 female rats. Observations during and after the two-year exposure included monitoring for clinical signs of toxicity, body weights, feed consumption and histopathology (including neoplastic and non-neoplastic lesions). Observations at the 16-week interim evaluation observations included organ weights, clinical chemistry, histopathology, (non-neoplastic lesions), internal plasma and liver PFOA levels, liver acyl-CoA oxidase (biomarker of PPARα induction) activity, and liver aromatase activity.

Survival rates were unaffected in all groups of exposed rats compared to controls after two years of exposure. Among male and female F1 rats there were statistically significant and dose-related reductions in body weight, as well as dose-related and statistically significant increases in the incidence rates of several non-neoplastic lesions in the liver (including hepatocyte cytoplasmic alteration, liver cell hypertrophy, pigmentation and necrosis), and in the pancreas (acinar cell hyperplasia). The incidence rates for all of these lesions were significantly increased at 1.1 mg/kg bw per day in males. Similar lesions were seen in exposed F1 females, as well as in the kidney and forestomach; however, they received comparatively higher PFOA dose levels than males.

In the 16-week interim evaluation of perinatally-exposed F1 animals, toxicity was observed in the liver, glandular stomach, kidney, and thyroid gland in males and in the liver, kidney, and thyroid gland in females. Plasma concentrations of PFOA were consistently higher in males compared to females and were not significantly affected by perinatal exposure. Acyl-CoA oxidase activity in the liver was consistently elevated in males and females, with higher activity in males. Also, female rats generally had less severe toxicological outcomes at comparable dose levels, which reflects the lower internal plasma concentrations of PFOA in female rats relative to male rats. In general, very few significant differences were observed between the responses of groups of animals exposed to PFOA only after weaning, compared to groups with both perinatal and postweaning exposures, and most of these differences were considered sporadic.

Two other chronic toxicity studies were conducted in animals that evaluated the toxicity of PFOA via the oral route and are described below:

Sprague Dawley rats were administered PFOA in the diet for 2 years at levels equivalent to 0, 1.3 or 14.2 mg/kg bw per day for males and 0, 1.6 or 16.1 mg/kg bw per day for females (Butenhoff et al., 2012b). The following statistically significant non-neoplastic effects were reported in animals exposed to 14.2 – 16.1? mg/kg bw per day: increased hepatocellular hypertrophy portal mononuclear cell infiltration, cystoid degeneration and hepatocellular vacuolation (without hepatocellular necrosis), increases in serum ALT, AST, and ALP that persisted to the end of the exposure period (males only), presence of alveolar macrophages and haemorrhage in the lungs (males only), ovarian tubular hyperplasia, sialadentitis (males only), and vascular mineralization in the testis and epididymis. At the mid dose of 1.3 – 1.6 mg/kg bw per day, increased incidence of ovarian tubular hyperplasia and sialadentitis (males only) were the only non-neoplastic histopathological effects reported. Conversely there were no treatment-related increases in the incidence of non-neoplastic lesions in the thyroid, pituitary, adrenal gland, kidney, and uterus. The only potential treatment-related neoplastic lesion reported was increased incidence of Leydig cell adenomas in high-dose males.

In another chronic study, male Sprague Dawley rats received PFOA in the diet for 2 years, corresponding to an overall mean daily intake of 13.6 mg/kg bw per day (Biegel et al., 2001). A statistically significant increase in relative liver weight was reported in the exposed group compared to the pair-fed control group but not to the *ad libidum* control group. This was also associated with significantly increased hepatic β-oxidation activity (a biomarker of peroxisome proliferation) in exposed animals compared to *ad libidum* and pair-fed controls. In addition, statistically significant increases in the incidence of liver carcinomas, Leydig cell hyperplasia, Leydig cell adenoma, acinar cell hyperplasia and acinar cell adenoma were observed in the exposed animals compared to either the *ad libidum* control or pair-fed control groups. However, there was no exposure-related increase in liver cell proliferation as measured by 5-bromo-2-deoxyuridine (BrdU) labelling.

5.4. Neurotoxicity (non-developmental)

The data pertaining to neurotoxicity of PFOS and PFOA are limited and preclude a rigorous evaluation of dose-response and mode of action. However, existing studies have shown that PFOS appears to have neurotoxic effects in rats at doses as low as 5 mg/kg bw per day for at least two weeks, based on clinical evidence including cachexia, lethargy and tonic convulsions;

and in mice at doses as low as 2.15 mg/kg bw per day for three months, based on reduced performance in a water maze test and other histological evidence.

5.4.1. PFOS

Evidence of neurotoxicity was reported in rats following 28 days of repeated oral exposure to PFOS (via intragastric intubation), presenting as cachexia, lethargy, and reduced activity at doses of ≥ 5 mg/kg bw per day, and histological effects in the brain (including focal hyperplasia of cerebral gliocytes and focal demyelination of nerve fibres) at 20 mg/kg bw per day (Cui et al., 2009).

Male Sprague Dawley rats exposed to PFOS at levels of 0, 1.7, 5.0 and 15 mg/L in drinking water for 91 days had corresponding levels in the brain cortex of 0.56, 3.25, and 17.21 μg/g, respectively (Liu et al., 2010). Dose-related increases in calcium-signalling molecules (CaMKIIα, cAMP-response element binding protein, c-fos, and c-jun) were reported, and the authors suggested that PFOS-induced neurotoxicity may be mediated via Ca^{2+} modulation.

In Sprague Dawley rats administered PFOS in the diet at levels equivalent to 0.1-0.2, 0.4-0.8, 1.5-3.5 and 5.6-13.9 mg/kg bw per day for 13 weeks (Kawamoto et al., 2011), tonic convulsions were reported in five of six high-dose rats during week five of the study when a brief ultrasonic stimulus was applied to the animals biweekly; ultrasonic stimulations were discontinued thereafter. No tonic convulsions occurred in any of the other dose groups, and no tonic convulsions were induced by PFOS exposure without ultrasonic stimulation. The study authors also examined samples of brain tissue that were stained to detect any damage to neuronal or glial cells, and no changes to these cells were detected. Similarly, in PFOS-treated rats there were no ultrastructural abnormalities of the neurons in the cortex and hippocampus, or abnormalities in the neurons and granular cells of the cerebellum.

C57/BL/6 mice were given oral doses of 0, 0.43, 2.15, or 10.75 mg/kg bw per day PFOS for a duration of three months and neurological function was evaluated by measuring escape latency and time spent in the target quadrant in a Morris water maze test (Long et al., 2013). Group mean escape latencies increased in a dose-dependent manner and were significantly increased in the mid- and high-dose groups. Additionally, the time spent in the target quadrant of the maze was significantly reduced in all exposure groups., Exposure to PFOS was also associated with increased incidence of apoptosis in the hippocampal neural cells (mid- and high-dose groups) as well as significantly increased glutamaterelease in the hippocampus in high-dose animals. Increased expression of CaM-KIIα, pCREB, c-fos and c-jun was also observed in rat cortex and hippocampus at a dose of 0.238 mg/kg bw per day (Liu et al., 2010a).

5.4.2. PFOA

Goldenthal et al. (1978; as cited by US EPA, 2016b) administered gavage doses of 0, 3, 10, 30, or 100 mg/kg bw per day PFOA to groups of two male and female Rhesus monkeys for 90 days. Effects related to the nervous system include a statistically significant increase in relative pituitary gland weight in males exposed to 3 mg/kg bw per day as well as a significant decrease in absolute brain weight in females exposed to 10 mg/kg bw per day. Based on the information available in the secondary summary, it is unclear whether these effects were dose-related.

In rhesus monkeys exposed to lethal levels of PFOA (≥30 mg/kg/day by gavage for 90 days) no treatment-related changes were seen in the brain (Butenhoff et al. 2002).

In rats, repeated doses of PFOA of up to 110 mg/kg bw per day via the diet for 90 days was not associated with gross or microscopic alterations in the brain, spinal cord, or peripheral nerves (Griffith and Long 1980). However, reduced activity, cachexia and increased lethargy were reported in rats following oral exposure to PFOA at levels of 5 or 20 mg/kg bw per day (via intragastric intubation) for two weeks, as part of a 28-day repeated dose study (Cui et al 2009).

In a two-year study in which rats were exposed to approximately 15 mg/kg/day PFOA in the diet, there was a small but statistically significant increase in relative brain weight compared to controls in the male low dose group, but not the high dose group. Relative brain weights were unaffected by exposure in females, and there were no gross or microscopic alterations in the brain, spinal cord, or peripheral nerves reported (Butenhoff et al., 2012).

In summary, the data pertaining to neurotoxicity of PFOS and PFOA are limited and preclude a rigorous evaluation of dose-response and mode of action. However, existing studies have shown that PFOS appears to have neurotoxic effects in rats at doses as low as 5 mg/kg bw per day for at least two weeks, based on clinical evidence including cachexia, lethargy and tonic convulsions; and in mice at doses as low as 2.15 mg/kg bw per day for three months, based on reduced performance in a water maze test and other histological evidence.

5.5. Reproductive and developmental effects

5.5.1. PFOS

Reproductive effects

The reproductive and developmental toxicity database for PFOS is extensive, with studies described in monkeys, rats, mice and rabbits.

Reproductive toxicity was evaluated in Cynomolgus monkeys administered up to 0.75 mg/kg/day PFOS by the oral route for 183 days. No significant morphological alterations in the sex organs were reported, however there were significant decreases in levels of serum oestradiol in males on days 62, 91, and 182 (Seacat et al. 2002).

A two-generation study has been described in rats administered PFOS by oral gavage at doses of 0, 0.1, 0.4, 1.6 and 3.2 mg/kg bw per day for 42 days prior to mating in males and females, and during pregnancy and lactation in females. At the highest dose, gestation length and the number of implantation sites were significantly reduced in F0 dams, followed by a reduction in litter size that was not statistically significant. In the F1 offspring, pup body weight and survival were also reduced in the two highest dose groups, with 26% mortality within 4 days at a dose of 1.6 mg/kg bw per day and 45% mortality within one day at 3.2 mg/kg bw per day. Surviving pups in the F1 generation showed transient delays in reflex and physical development indicative of neurotoxicity. An overall NOAEL of 0.1 mg/kg bw per day was proposed by the authors of the study based on systemic effects (reduced food consumption and body weight gain) in the F0 dams occurring at doses of 0.4 mg/kg bw per day or greater, whereas a NOAEL for reproductive and developmental effects of 0.4 mg/kg bw per day was

proposed by the authors of the study based on reduced survival and body weight gain, as well as delayed eye opening, air righting, surface righting, and pinna unfolding in F1 pups at 16 mg/kg bw per day (Luebker et al., 2005a).

Zhao et al. (2014) investigated the effects of pre-natal exposure to PFOS on male reproductive parameters. Pregnant Sprague Dawley rats were exposed to PFOS at doses of 5 and 20 mg/kg bw per day from GD 1 – 19. Reduced maternal body weight was reported at the highest dose, which was also seen in male offspring at both doses. Testosterone levels in male pups were reduced at the highest dose and progesterone levels were reduced at both doses. Decreased testosterone levels were associated with a decrease in the number of Leydig cells and an increased apoptosis rate. In addition, the testis weight (total) and anogenital distance in male offspring were reduced in the high-dose group.

Dietary exposure of rats to PFOS at levels between 1.3 and 1.8 mg/kg/day for 4 or 14 weeks was not associated with any gross or microscopic alterations in the sex organs of males or females (Seacat et al. 2003). Similarly, in a 2-year dietary study in rats, administration of up to 1.04 mg/kg/day PFOS did not induce gross or microscopic alterations in the reproductive organs (Butenhoff et al. 2012b; Thomford 2002b).

Reproductive performance (assessed as number of litters, gestation length, number of implantation sites, or potential resorptions) was not affected in rats administered 1 mg/kg/day PFOS throughout gestation and lactation (GD0 to GD20) (Butenoff et al. 2009).

A significant decrease in serum testosterone levels and epididymal sperm count was observed in mice administered 10 mg/kg/day PFOS for 21 days, with no decreases seen at the lower dose of 5 mg/kg bw per day or at the higher dose for the shorter period of exposure of 14 days (Wan et al. 2011).

Developmental effects

The effects of PFOS gavage exposure on maternal toxicity and birth outcomes in rats and mice were evaluated by Thibodeaux et al. (2003), with a follow-up assessment of developmental outcomes in the offspring conducted by Lau et al. (2003). Maternal rats were given 1, 2, 3, 5 or 10 mg/kg bw per day from GD2 – GD21, whereas maternal mice were given 1, 5, 10, 15 and 20 mg/kg bw per day from GD 1-18. Pup survival rate was significantly reduced in rats exposed to ≥ 2 mg/kg bw per day, and in mice exposed to ≥ 10 mg/kg bw per day. A $BMDL_5$ of 0.58 mg/kg bw per day was calculated for survival of rat pups at PND 8 by Lau et al. (2003). In rats, maternal toxicity was reported at PFOS doses ≥ 2 mg/kg bw per day, as well as marked reduction in maternal serum T4 and T3 at doses of ≥ 1 mg/kg bw per day, whereas at a dose of 10 mg/kg bw per day, reduced foetal body weight and increased incidence of cleft palate and anasarca were reported. Also in rats, post-natal growth rate and the average age at eye opening were significantly delayed at doses of PFOS ≥ 2 mg/kg bw per day, and PFOS-exposed neonates showed reductions of T4, but not T3 or TSH, in all dose groups. In addition to the BMD_5 for pup survival rate, Thibodeaux et al. (2003) also derived $BMDL_5$ values for reduced maternal body weight at term (0.15 mg/kg bw per day for rats; 3.14 mg/kg bw per day for mice), reduced maternal serum T4 (0.046 mg/kg bw per day for rats; 0.352 mg/kg bw per day for mice), and increased frequency of foetal sternal defects (0.122 mg/kg bw per day for rats; 0.016 mg/kg bw per day for mice). Increased incidence of foetal cleft palate was also modelled in rats and mice but the $BMDL_5$ values indicate that it is a less sensitive endpoint than the other

developmental effects modelled. Therefore, for most developmental endpoints evaluated, the rat was more sensitive than the mouse, except for the increased incidence of foetal sternal defects, which corresponded to the most sensitive reported $BMDL_5$ value when evaluated in mice.

The critical prenatal exposure window for PFOS in rats was evaluated by Grasty et al. (2003). Groups of timed-pregnant Sprague-Dawley rats were given gavage doses of 25 mg/kg bw PFOS (as a potassium salt) for four consecutive days, during various stages of gestation. Based on pup mortality rates, the most sensitive exposure window was reported to be GD 17-20, and appeared to coincide with maturation of the lung.

Prenatal developmental toxicity of PFOS was assessed in pregnant rats administered PFOS by oral gavage at doses of 0, 1, 5 and 10 mg/kg bw per day on GD 6-15. Developmental toxicity was evident in offspring as an increase in abnormalities of the lens of the eye at doses of 1 mg/kg bw per day or greater, although the increase in the incidence of this effect was statistically significant only at the 10 mg/kg bw per day dose. Thus, a LOAEL of 10 mg/kg bw per day was proposed by the authors for developmental toxicity. Maternal toxicity was also evident as reduced body weight gain, with a NOAEL of 5 mg/kg bw per day also proposed for this endpoint (HC, 2018a).

Several developmental toxicity endpoints were observed in the offspring of pregnant rats administered PFOS by oral gavage between GD 6 and 15. For example, a LOAEL for developmental effects of 5 mg/kg bw per day was proposed for rats based on reduced birth weight as well as increased incidence of visceral anomalies, delayed ossification and skeletal variations. In the same study, maternal toxicity was evident as reduced body weight gain with a NOAEL of 1 mg/kg bw per day; both PODs were proposed by the study authors (Wetzel, 1983).

Luebker et al. (2005b) reported the effects of exposure to PFOS in rats by oral gavage at doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6 and 2.0 mg/kg bw per day from 6 weeks prior to mating until day 4 of lactation. Gestation length and pup viability were significantly reduced at doses of $\geq$ 0.8 mg/kg bw per day. Based on these effects, $BMDL_5$ values ranging from 0.27 to 0.89 mg/kg bw per day were calculated by the authors of the study.

A statistically significant increase in mortality (22%) was also described at PND 3 in the offspring of Sprague Dawley rats exposed during pregnancy to PFOS at a level of 2 mg/kg bw per day. This was associated with a statistically significant decrease in body weight at birth and was also observed in surviving pups at PND 7 – 21. Using transmission electron microscopy, morphological changes of the mitochondria in the heart of pups were reported (Xia et al., 2011).

Yahia et al. (2008) reported developmental defects in ICR mice exposed to PFOS at doses of 0, 1, 10 and 20 mg/kg bw per day by oral gavage during GD 0 – 18. Mean litter size was unaffected, but pup survival was reduced to 55% of the rate in the control group at 10 mg/kg bw per day, and all pups in the high-dose group died within hours after birth, predominately from lung atelectasis and severe dilatation of intracranial blood vessels. At all doses, a statistically significant increase in the incidence of sternal defects (not further described) was apparent ($p<0.01$ for all doses). Additionally, statistically significant increases in the frequency of several developmental effects were reported in the 10 and 20 mg/kg bw per day dose groups, including: reduced body weight, cleft palate, delayed eruption of incisors, wavy ribs, curved

fetus, spina bifida occulta, and delayed ossification (the latter effect was reported in the 20 mg/kg bw per day dose group only). Maternal toxicity was also evident, based on reduced body weight gain from PND 11 in high-dose dams (which was concurrent with reduced food consumption and increased water intake), as well as statistically significant increases in liver weight in dams exposed to 10 or 20 mg/kg bw per day.

The potential effect of PFOS on placental hormone (PRL family) production was investigated as a mechanism for developmental growth retardation in CD-1 mice. There was a correlation between decreased placental levels of prolactin family members and fetal weight (Lee et al., 2015).

In a developmental toxicity study, New Zealand white rabbits were exposed to PFOS by oral gavage at doses of 0, 0.1, 1.0, 2.5 and 3.75 mg/kg bw per day from GD 6-20 (Case et al., 2001). Abortions were reported (between GD 22 and 28) in around 50% (10 of 22) of pregnant rabbits receiving PFOS at a dose of 3.75 mg/kg/day by gavage on GDs 6–20. In maternal animals, there were statistically significant decreases in body weight gain compared to controls in the 1 mg/kg bw per day and 2.5 mg/kg bw per day dose groups during GD 7-21; however, this effect did not persist beyond the dosing period. Mean foetal weights on a per-litter basis were also significantly reduced in the 2.5 and 3.75 mg/kg bw per day dose groups, with reductions of approximately 10% and 24% compared to controls, respectively. There were no treatment-related effects in the incidence of external, soft tissue, or skeletal abnormalities. Delayed ossification in "certain bones" and a "slight" increase in the incidence of cleft palate were also reported in the offspring of high-dose dams. A NOAEL of 1 mg/kg bw per day was proposed by ATSDR (2021) for this study, based on decreased foetal body weight, and a NOAEL of 0.1 mg/kg bw per day was identified for maternal toxicity based on decreased weight gain.

In summary, PFOS affects developmental processes with impacts that include (from most to least sensitive): increased incidence of foetal sternal defects ($BMDL_5$ of 0.016 mg/kg bw per day in mice), reduced maternal serum T4 ($BMDL_5$ of 0.046 mg/kg bw per day in rats), reduced maternal body weight ($BMDL_5$ of 0.15 mg/kg bw in rats), increased maternal liver weight (0.3 mg/kg bw per day), changes in glucose homeostasis (0.3 mg/kg bw per day), and altered placental physiology (0.5 mg/kg bw per day).

Neurodevelopmental effects

Neurochemical and neurobehavioral markers were evaluated in the offspring of rats following prenatal PFOS exposure (Lau et al., 2003). No effect on learning and memory behaviours were identified; however, the authors reported statistically significant deficits in the developmental patterns for choline acetyltransferase activity, with a LOAEL of 1 mg/kg bw per day.

Butenhoff et al. (2009) reported a significantly increased motor activity and decreased habituation in male rat offspring at PND17 following maternal gestational and lactational exposure to PFOS at 1.0 mg/kg bw per day, with no impact on learning and memory.

In Crl:CD (SD)IGS BR VAF rats, no effects on learning and memory or passive avoidance behaviour in F1 pups was reported following exposure to PFOS at a level of 0.4 mg/kg bw per day (Luebker et al., 2005b).

Acute oral exposure of 10-day old mice to PFOS at doses of 0.75 or 11.3 mg/kg bw was associated with impaired performance in behavioural tests at 2 and 4 months, with no indication

of clinical toxicity. The authors considered these effects to be mediated via the cholinergic system (Johansson *et al*., 2008).

A significant increase in escape latency was apparent in the offspring of pregnant rats exposed to PFOS via drinking-water at 15 mg/L during gestation and lactation, who were cross-fostered with either control or treated dams, and continued exposure at the level of their lactational dam (Wang et al., 2015). Dose levels normalized by body weight were not reported, and body weights and drinking water consumption levels were not evaluated.

PFOS induced apoptosis in cerebellar granule cells derived from 7-day old Sprague Dawley rats, acting via a protein kinase and extracellular signal-regulated kinase (ERK) pathways (Lee et al., 2013). Wang et al. (2010) found that pre-natal exposure to 3.2 mg/kg/day of PFOS in the feed had some effect on gene expression associated with neuroactive ligand-receptor interaction, calcium signalling pathways and PPAR signalling. Cultured hippocampal neurite growth and branching were suppressed by exposure to 50 µmol PFOS. The authors hypothesised that this was a consequence of PFOS incorporation into the neuronal lipid bilayer membrane. PFOS was the only member of the sulfonate family to exhibit this effect. (Liao et al. 2009).

5.5.2. PFOA

Reproductive effects

An intermediate-duration study in which Cynomolgus monkeys were administered PFOA at doses of 0, 3,10 or 20 (originally 30) mg/kg bw per day for 4 or 26 weeks, did not report any gross or histologic alterations in the sex organs at termination (Thomford 2001; Butenhoff et al. 2002). At 4 weeks, serum levels of oestradiol and oestriol were not significantly altered; however, buto-estrone was reduced in both dose groups. No exposure-related changes were reported in serum oestrone, oestriol, oestradiol, or testosterone at 26 weeks, indicating that the reduced serum oestrone levels in the 4-week study was transitory. Similar absence of gross and histologic findings was reported in Rhesus monkeys administered PFOA at up to 100 mg/kg bw per day for 13 weeks (Griffith and Long 1980).

In a two-generation study, Sprague-Dawley rats were administered PFOA at doses of 0, 1, 3, 10 and 30 mg/kg bw per day by oral gavage from the age of 6 weeks for at least 70 days before mating and up to weaning. At 3 weeks following weaning, F1 pups received the same dose as their parents. Developmental/reproductive effects in the F1 generation were evident at the highest dose (30 mg/kg bw per day), including reduced birth weights and increased pup mortality during PND 2-8 in males and PND 2-4 in females in animals, and an increased time to sexual maturity (i.e. delay in preputial separation in males or vaginal patency in females). Both F0 and F1 males showed exposure-related toxicity at all doses, including significantly increased relative liver and kidney weights, and significantly reduced terminal body weight and body weight gain in F1 males. The authors proposed a NOAEL of 30 mg/kg body weight per day by the authors for reproductive function and of 10 mg/kg body weight for developmental toxicity (Butenhoff et al. 2004). The US EPA (2016b) proposed a LOAEL of 1 mg/kg bw per day for systemic effects in F0 and F1 males based on the body weight and organ weight effects.

In the NTP (2020) chronic study with combined gestational and lactational exposure, mean body weights during lactation were significantly reduced in F1 males and female pups born to F0 females exposed to 300 ppm PFOA in the diet (approximately 21.7 mg/kg bw per day), although the magnitude changes were less than 10% compared to the control means. The mean litter size and survival ratio of F1 rats during lactation were not significantly impacted by exposure of F0 females to 0, 150 or 300 ppm of PFOA in the diet (equivalent to approximately 10.9 and 21.7 mg/kg bw per day) from GD 6 to PND 21 (NTP, 2020); however, no other reproductive or developmental toxicity endpoints were evaluated in this study.

The effect of PFOA exposure on male reproductive parameters was assessed in 8-week-old Balb/c mice administered PFOA at levels of 0.31, 1.25, 5 and 20 mg/kg bw per day for 28 days. A dose-related decrease in absolute testis weight was reported, which reached statistical significance compared to controls ($p < 0.01$) at the highest dose tested; however, relative testis weight was unchanged. Sperm count and the percentage of teratosperm were statistically significantly decreased ($p<0.05$ and $p<0.01$ respectively) in the 5 mg/kg bw per day group. A statistically significant increase was seen in sperm motility and sperm progression at the same dose ($p<0.01$ for both parameters) (Zhang et al., 2014b).

Li et al. (2011) reported sperm abnormalities, but not reduced sperm count, in both wild-type 129/Sv mice (mPPARα) and 129/Sv mice expressing humanized PPARα (hPPARα) exposed to PFOA at 1 mg/kg bw per day for 42 days, from 8 weeks of age. These findings were not apparent in PPARα-null 129/Sv mice, and the authors commented that PFOA might disrupt testosterone biosynthesis by lowering the delivery of cholesterol into mitochondria and decreasing the conversion of cholesterol to pregnenolone and androstandione in the testis of mPPARα and hPPARα mice and that this may, in part, be related to NH_4^+PFOA-induced mitochondrial damage (HC, 2018b).

In a two-year study in rats, PFOA administered at 15 mg/kg bw per day in the diet was associated with an increased incidence of vascular mineralisation in the testes (3M, 1983). Similarly, in rats exposed to PFOA for two years at a dose of 13.6 mg/kg bw per day, a significant increase in the incidence of Leydig cell hyperplasia was reported (Biegel et al., 2011).

Chronic exposure (two years) to PFOA at a level of 15 mg/kg bw per day was associated with increased incidence of gonadal stromal hyperplasia of the ovaries (Grade 3 and above) in female rats (Mann and Frame, 2004).

Developmental effects (morphological alterations)

Developmental toxicity following exposure to PFOA was reported in CD-1 mice exposed to doses of 1, 3, 5, 10, 20, or 40 mg/kg bw per day PFOA by oral gavage daily from GD 1 to 17 (Lau et al., 2006). Maternal toxicity, presenting as a dose-related decrease in body weight gain, was reported, which reached statistical significance at the highest dose (p value not stated by the authors). In addition, a number of foetal skeletal developmental effects were apparent, including: reduced ossification of forelimb proximal phalanges ($p<0.05$) at all doses except the 5 mg/kg bw per day group), reduced ossification of hindlimb proximal phalanges ($p<0.05$) at all doses except 3 and 5 mg/kg bw per day, reduced ossification of the calvaria and enlarged fontanel ($p \leq 0.05$) in dose groups 1, 3, and 20 mg/kg bw per day, and reduced ossification in the supraoccipital bone ($p \leq 0.05$) at doses $\geq$ 10 mg/kg bw per day. Statistically significant

increases ($p \leq 0.05$) in minor limb and tail defects were also reported in the fetuses at doses ≥ 5 mg/kg bw per day. A prenatal developmental LOAEL of 1 mg/kg bw per day was proposed by the study authors based on increased skeletal defects, and a NOAEL was not established.

In utero exposure to 0.3 mg/kg bw per day PFOA resulted in morphometrical alterations in the femur (increases in the periosteal area) and decreases in bone mineral density in the tibia of 13- or 17-month-old mice exposed to 0.3 mg/kg/day in the diet on GDs 1–21 (Koskela et al., 2016). The study authors also described a companion *in vitro* study of osteoclasts and osteoblasts, which provided mechanistic support for the *in vivo* findings.

Developmental effects (mammary gland development)

The effect of PFOA on mammary gland development was compared in Balb/c and C57BL/6 mice. Doses of 0, 1, 5, 10 mg/kg bw per day were administered by oral gavage from PND 21 for 4 weeks. Three parameters were defined to assess mammary gland development: ductal length, number of terminal endbuds and number of terminal ducts. A significant reduction of all three parameters was observed in Balb/c mice for the two highest dose groups (5 and 10 mg/kg bw per day). In C57BL/6 mice a decrease in all three parameters was observed at the highest dose only (10 mg/kg bw per day). At 5 mg/kg bw per day, PFOA exposure was associated with a significant increase in number of terminal end buds and stimulated terminal ducts. Additionally, PFOA exposure was associated with delayed vaginal opening at 1 mg/kg bw per day in the Balb/c strain and at 5 mg/kg bw per day in the C57BL/6 strain, with no vaginal opening occurring in higher dose groups (Yang et al., 2009).

A study in pregnant CD-1 mice dosed with 5 mg/kg bw per day PFOA (the only dose level tested) reported that the mammary gland showed changes suggesting substantial delay (possibly up to 10 days) in gland differentiation on PND 20 and alterations in milk protein gene expression on PND 20 (White et al. 2007). Subsequent studies by this group support the finding of delayed mammary gland differentiation. For example, mammary gland development in CD-1 mice was also inhibited following in utero and postnatal PFOA exposure at levels of 5 mg/kg bw per day (White et al., 2009).

The same research group carried out a three-generation reproduction study reporting compromised weaning-induced mammary gland involution on PND 22 in groups of CD-1 mice exposed to either 1 mg/kg bw per day from GD 1 to GD 17 or 5 ppb PFOA in drinking water (equivalent to approximately 0.00045 mg/kg bw per day) from GD 7 until the end of the study (about 12 weeks), as did subsequent F1 and F2 offspring (White et al. 2011). In the group receiving 5 ppb PFOA in their drinking water, decreased weaning-induced mammary involution was reported. Furthermore, mammary gland development scores were significantly decreased in all treated F1 pup groups on PND 22, 42 and 63, indicating lack of full development of the mammary glands; although PFOA-induced effects on mammary gland development were less evident in F2 pups. Similar findings were reported in the groups in which P0 dams received 1 or 5 mg/kg bw per day PFOA from GD 1 to GD 17.
Macon et al. (2011) administered PFOA by oral gavage to pregnant CD-1 mice during full- and late-gestation stages, with doses of 0, 0.3, 1.0, 3.0 mg/kg bw per day on GD 1-17, or 0, 0.01, 0.1, 1.0 mg/kg bw per day on GD10-17). Pups were evaluated up to 12 weeks postnatally, with female pups showing stunted mammary gland development at all doses, in both the full- and late-gestation arms of the study.

PFOA is an agonist of PPARα and PFOA has been shown to have varying effects on mammary development in mice, depending on the strain. For example, Zhao et al. (2010) reported that in C57Bl/6 mice, peripubertal treatment with 5 mg/kg bw PFOA had a stimulatory effect on mammary gland development in both wild-type and PPARα knockout strains, thus showing that this effect is independent of PPARα expression in this strain. To further determine the effect of PPARα expression on the relationship between PFOA exposure and mammary gland development in different strains of peripubertal mice, Zhao et al. (2012) administered doses of 0 or 2.5 mg/kg bw per day PFOA to groups of five wild-type and PPARα-knockout Balb/c mice, and doses of 0 or 7.5 mg/kg bw per day PFOA to groups of five wild-type and PPARα-knockout C57Bl/6 mice, five days per week for four weeks, beginning at peripubertal age. Exposure to PFOA at these dose levels was associated with inhibition of mammary gland development, including significant reductions in mean ductal length, number of terminal end buds and number of stimulated terminal ducts in wild-type mice from both strains, with Blab/c mice being more sensitive. However, there were no significant reductions in these endpoints in the PPARα knockout mice.

Thus, whereas PFOA-induced mammary gland stimulation is independent of PPARα expression, PPARα expression does appear to contribute to PFOA-induced mammary gland inhibition in these strains. However, the reasons for the inconsistent effects of PFOA on mammary gland development in different strains and dose levels, as well as their relevance to humans, are unknown

Developmental effects (placenta, uterus, liver)

Necrosis was observed in the placenta of mice administered via gavage 10 or 25 mg/kg bw per day PFOA on GDs 11–16 (Suh et al. 2011); no alterations were observed at 2 mg/kg bw per day.

In a developmental study conducted by Yang et al. (2009), groups of five 21-day-old Balb/c mice and additional groups of five 21-day-old C57BL/6 mice were administered gavage doses of 0, 1, 5 and 10 mg/kg bw per day PFOA for 4 weeks. Significant delays in vaginal opening were observed at the lowest dose in Balb/c mice (with no opening at the higher doses) and at 5 mg/kg bw per day in C57BL/6 mice (with no opening at 10 mg/kg bw per day). Uterine development was also affected, with a dose-dependent decrease in absolute and relative uterine weight in Balb/c mice. In C57BL/6 mice, decreased uterine weight was apparent at the highest dose of 10 mg/kg bw per day.

Low-dose effects of PFOA on uterine weight were reported in CD-1 mice, using an immature uterotrophic assay. A statistically significant increase (1.48-fold) in total and relative (1.46-fold) uterine weight was detected at a PFOA dose of 0.01 mg/kg bw per day (Dixon et al., 2012). EFSA (2020) noted that when compared with E2-treated mice (500 µg/kg bw/day), the estrogenic effects (primarily in the uterus) were only minimally, if at all supported by an extended histological analysis of uterine, cervical and vaginal tissue.

Developmental hepatotoxicity was compared in CD-1, 129/Sv wildtype and PPARα knockout mice exposed from GD 1-17 to PFOA at levels of 0, 0.01, 0.1, 0.3, 1 or 5 mg/kg bw per day. The authors reported dose-dependent increases in Ito cell and centrilobular hepatocyte hypertrophy at the highest dose in CD-1 mice. Bile duct hyperplasia and bile duct hyaline droplet accumulation were seen in 129/Sv wildtype and PPARα knockout mice. Due to the

occurrence of non-neoplastic liver lesions in all three strains, it was concluded that these occur via a PPARα independent mechanism (Filgo et al., 2015).

Developmental effects (neonatal survival)

The effects of PFOA exposure on neonatal survival were addressed by several studies in mice. For example, exposure of CD-1 mice from GD-1 to birth to PFOA at doses of 1, 3, 5, 10, 20, 40 mg/kg bw per day by oral gavage was associated with increased resorption of litters at the highest dose compared to controls. In addition, there was a reduction in the number of live pups and body weights at the two highest doses. Post-natal survival was significantly reduced at doses ≥ 5 mg/kg bw per day group, and dose dependent growth deficits seen at doses ≥ 3 mg/kg bw per day. Significant delays in eye opening and accelerated sexual maturation in male offspring were noted at doses ≥ 5 mg/kg bw per day (Lau et al., 2006). Yahia et al. (2010) exposed pregnant ICR mice on GD 0 -17 to PFOA by oral gavage at doses of 0, 1, 5 and 10 mg/kg bw per day. Maternal toxicity was evident as a dose-dependent increase in liver weight reaching significance at the two highest doses; changes in some serum biochemical parameters were seen at all doses. Fetotoxicity was evident as reduced survival of offspring, with 100% mortality within 6 hours of birth at the highest dose. Furthermore, Abbott et al. (2012) reported increased mortality in the offspring of CD-1 mice exposed via gavage from GD 1-17 to PFOA at a dose of 5 mg/kg bw per day, with 49% of all pups born alive surviving to PND7.

Several studies provided evidence that pup survival rate is dependent on a PPARα-dependent mechanism (Abbott et al., 2007; Albrecht et al., 2013; Nakamura et al., 2009). Abbott et al. (2007) exposed both wild-type and PPARα-null mice from GD 1-17 to ≥ 5 mg/kg bw per day PFOA and found that full litter resorptions occurred in both groups; however, postnatal survival was significantly decreased, and eye-opening was significantly delayed, in PFOA-exposed wild-type offspring compared to controls, but not in the corresponding PPARα-null offspring. In a similar experiment using wild-type, PPARα-null mice and PPARα-humanized mice exposed to a single dose of 3mg/kg PFOA, Albrecht et al. (2013) also found that the frequency of litter resorptions was independent of PPARα status, whereas PPARα status had no effect on the timing of eye-opening in neonates. Albrecht et al. (2013) further reported that pup survival was decreased only in PFOA-exposed wild-type mice, but not in PFOA-exposed PPARα-null or PPARα humanized mice. Furthermore, Nakamura et al. (2009) showed that increased expression of PPARα-related genes was observed in wild-type mice, but not in humanized PPARα or PPARα-null mice, exposed for 2 weeks to ≥ 0.1 mg/kg bw per day.

In conclusion, impacts to mammary gland development is considered the most sensitive developmental outcome (EFSA, 2020), which was reported in the offspring of mice administered doses as low as 0.01 mg/kg bw per day PFOA administered during late gestation (GD 10 to GD 17), and at doses as low as 0.00045 mg/kg bw per day in mice when administered through multiple generations (P0 dams from GD 7 – PND 22; P1 and P2 offspring from PND1 – PND 63). PFOA exposure in various strains of mice has also been linked to changes in uterine weight in dams, delayed vaginal opening in the offspring, and decreased neonatal survival. Both PFOA-induced mammary gland inhibition and reduced neonatal survival in mice appears to be at least partially dependent on PPARα expression and phenotype, with PPARα knockout mice being less susceptible to mammary gland inhibition, and humanized phenotypes being less susceptible to neonatal mortality. The relevance of these effects in the tested strains of mice to humans is therefore uncertain and likely requires further study.

Neurodevelopmental toxicity

Male neonatal NMRI mice exposed to single gavage doses of PFOA at levels of 0.58 or 8.7 mg/kg bw on PND 10 (noted as the approximate peak time of rapid brain growth in mice) showed reduced locomotion and rearing within a minute period at the highest dose. At two months, mice in both dose groups showed reduced total activity, which was more pronounced at four months (Johansson et al., 2008, 2009).

5.6. Immunological effects

5.6.1. PFOS

Studies designed to identify the effects of PFOS on the immune system were primarily conducted in mice. The studies evaluated several related endpoints including: mortality from infection, changes in levels of immunoglobulins and cytokines, activity levels of immune cells, and lymphocyte phenotype and proliferation. Evidence of general immune system toxicity has also been reported, including decreases in white blood cell counts, body weight and histological changes in the spleen and thymus. Immune system effects observed at the lowest levels indicate that immunosuppression is likely the most sensitive human-relevant effect reported in animal studies (EFSA, 2020; Pachkowski et al., 2019).

In B6C3F1 mice, following a 28-day exposure by oral gavage to PFOS, a dose-dependent decrease in immune function, evaluated as the suppression of T-dependent antigen response (TDAR) for IgM using sheep red blood cell (SRBC) as an antigen, was reported. NOELs of 0.00017 mg/kg bw per day in males and 0.003 mg/kg bw per day in females (corresponding to serum PFOS concentrations of 17.8 and 123 ng/g respectively) were identified by the authors (Peden-Adams et al., 2008). Based on findings of similar studies reported by Dong et al. (2009, 2011, 2012a), EFSA reported a LOAEL for an impaired response to sheep red blood cells of 0.083 mg/kg bw per day in mice exposed to PFOS for 60 days, with the highest NOAEL being 0.0167 mg/kg bw per day.

Host resistance to Influenza A virus was reduced in female B6C3F1 mice following oral exposure to PFOS at doses of 0, 0.005 or 0.025 mg/kg bw per day for 21 days, resulting in an increased mortality from infection at the highest dose (Guruge et al., 2009).

PFOS-induced increases in serum levels of IgG and IgE have been reported. In male rats administered PFOS at doses between 0.14 and 0.634 mg/kg bw per day, a significant trend for increased total serum IgG2a, IgG2c and a secondary T-dependent IgG response was apparent (Lefebvre et al., 2008). In male C57BL/6 mice, increased SRBC-specific IgE and IgG were observed at PFOS exposure levels of 0.833 mg/kg bw per day (Dong et al., 2011).

Increased splenic natural killer (NK) cell activity was observed in male B6C3F1 mice exposed to PFOS at levels between 0.017 and 0.166 mg/kg bw per day for 28 days. In female mice, no changes in activity were observed at either dose (Peden-Adams et al., 2008). Dong et al. (2011) reported non-monotonic changes in NK activity in male C57BL/6 mice exposed to PFOS, with increases at 0.083 mg/kg bw per day, no effects at 0.417 mg/kg bw per day and decreases at 0.833 and 2.083 mg/kg bw/ day. Splenic NK cell activity was decreased in male and female mice born to maternal animals exposed to PFOS from GD 1-17 at doses of ≥ 1 mg/kg bw per

day (male pups) and 5 mg/kg bw per day (female pups) (Keil et al., 2008), and in adult male mice exposed to ≥ 20 mg/kg bw per day for 7 days (Zheng et al., 2009).

Evidence of increased apoptosis in the spleen and thymus was also been reported in rats following exposure to PFOS at levels ≥ 3.21 mg/kg bw per day (Lefebvre et al., 2008) and in mice following exposure to levels ≥ 0.0833 mg/kg bw per day (Wang et al., 2011b; Dong et al., 2012a; Zhang et al., 2013). Histological effects in thymus and spleen were seen in rats and mice at PFOS levels of ≥ 18 and ≥ 5 mg/kg bw per day respectively (Goldenthal et al., 1978a; Cui et al., 2009). Decreased absolute and/or relative weight of thymus and spleen were reported at doses of 0.984 mg/kg bw per day in male rats (Butenhoff et al., 2012b) and at ≥ 0.417 mg/kg bw per day in male mice (Dong et al., 2009, 2012a; Qazi et al., 2009b; Zheng et al., 2009, 2011).

Rats administered PFOS at levels of 1.56 mg/kg bw per day for 14 weeks, or 1.04 mg/kg bw per day for two years did not show significant morphological alterations in the spleen, thymus, and mesenteric lymph nodes (Butenhoff et al. 2012b; Lefebvre et al. 2008; Seacat et al. 2003; Thomford 2002b).

5.6.2. PFOA

Short-term studies to assess immunotoxic potential of PFOA were carried out in mice. Vetvicka and Vetvickova (2013) showed significant inhibition of phagocytosis and natural killer (NK) cell activity, and decreased antibody responses compared to controls in BALB/c mice exposed to PFOA for 7 days at a dose of 20 mg/kg bw per day. Cellularity was significantly decreased in the thymus, but not in the spleen, however a significant suppression of T-lymphocyte proliferation, inhibited B-lymphocyte proliferation, inhibited phagocytosis and reduced NK were noted in the spleen. As simultaneous hepatotoxicity was also apparent, the effects have the potential to be indirect rather than direct effects.

DeWitt et al. (2008) also showed a significant decrease in IgM synthesis following a challenge with sheep red blood cells (SRBC) in female C57BL/J mice exposed to PFOA at a level of 30 mg/kg bw per day for 15 days. The effects were also apparent in the recovery group that had not been exposed beyond 10 days. Significant increases in relative liver weights were evident in both the treated and the recovery group, with decreased body weights in the treated group only. In a follow-up study, the authors reported that the SRBC-specific IgM synthesis was suppressed at PFOA levels ≥ 3.75 mg/kg bw per day in a dose-dependent manner in female C57BL/6N mice. No effects on delayed hypersensitivity were seen, indicating that the humoral arm of the immune system is affected (DeWitt et al., 2009a).

In a series of studies, Qazi et al. (2009a, 2009b, 2012) reported on the effects of oral exposure to PFOA on circulating neutrophils and the inflammatory response of macrophages following lipopolysaccharide (LPS) stimulation in male (C57BL/6 (H-2b)) mice. In their evaluation of the studies, EFSA concluded that effects on cellular composition of the thymus, spleen, and bone marrow were seen, corresponding to an immunosuppressive and inflammatory response to PFOA exposure. It was noted that some of these effects are similar to those seen following food restriction and thus may be related to inhibition of food consumption. In addition, it was noted that food consumption and body weight after terminating exposure were similar to controls, while effects on immune parameters, once established, were still evident (EFSA, 2020).

Vetvicka and Vetvickova (2013) also reported effects in female Balb/c mice exposed to PFOA for 21 days which showed significantly reduced NK splenic activity, total antibody production in response to ovalbumin and the formation of IgM directed against trinitrophenol (TNP) following exposure to PFOA at a level of 20 mg/kg bw per day.

Immunotoxic changes were reported in male ICR mice exposed via drinking water to PFOA at doses of 0, 0.49 ± 0.04, 2.64 ± 0.15, 17.63 ± 1.15, 47.21 ± 3.57 mg/kg bw per day for 21 days. In the spleen, CD8+ lymphocyte populations were decreased by approximately 50% compared with controls at all dose levels and CD4+ lymphocyte populations were increased at the highest two doses (43% and 106%, respectively). At the highest dose, CD8+ lymphocytes were increased (110%) in the thymus, whereas CD4+ levels were unchanged. Atrophy was apparent with decreased thickness of the cortex and medulla, and more densely arranged lymphoid cells in the cortex. Exposure to PFOA at the highest dose was also associated with increased expression of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 in the spleen but not in the thymus, and C-myc expression was increased in both the spleen and the thymus (Son et al., 2009).

The immunotoxic effects of PFOA exposure by oral gavage at doses of, 0, 0.3, 1, 10, 30 mg/kg bw per day for 29 days was compared between male CD-1 mice and male CD rats. Immune system-related effects were apparent in mice at the two highest doses, as reduced IgM titres to SRBCs, decreased spleen and thymus weights and numbers of thymocytes and spleen cells. An increase in blood neutrophils and monocytes was also noted in the two highest dose groups, and a decrease in peripheral blood lymphocytes at the highest dose. In rats, no effects on SRBC-IgM titres were reported, although increased haematopoiesis in the spleen was reported in the high-dose group. No changes in total spleen or thymocyte cell number was evident. Effects on the spleen in rats included a significant reduction in haemoglobin and haematocrit at the two highest doses. An increase in reticulocytes at the highest dose was associated with extra-medullary haematopoiesis (Loveless et al., 2008). EFSA (2020) proposed a NOAEL of 1 mg/kg bw per day based on suppression of anti-SRBC IgM titre in this study.

5.7. Genotoxicity

Available studies provide inconclusive data as to the genotoxicity of PFOS and PFOA. In 2008, based on a comprehensive review of a large dataset of in vitro and in vivo genotoxicity assays, EFSA concluded that the observed carcinogenicity of PFOS in rodents is unlikely to be related to a direct genotoxic mode of action, but instead may be attributable to a non-genotoxic mode of action that may involve oxidative stress. This conclusion was upheld in a more recent EFSA review (EFSA, 2020).

5.7.1. PFOS

PFOS is negative in mutagenicity studies with five strains of *Salmonella* (TA100, TA1535, TA1537, TA1538 and TA09 strains; *Escherichia coli* (WP2uvrA,) with and without metabolic activation (S9) and in the mitotic recombination test in *Saccharomyces cerevisiae* (D4 strain,) (EFSA, 2018?; NTP, 2019a; EFSA, 2020). PFOS did not show mutagenic activity in the *umu* test when tested up to 1,000 μM (Oda et al., 2007).

Assays for the induction of ROS by PFOS in human hepatoma HepG2 cells have shown conflicting results, with two studies reporting no generation of DNA single strand breaks or

micronuclei formation (Florentin et al., 2011; Eriksen et al., 2010). However, in a further study in HepG2 cells, a non-dose-dependent increase in DNA strand breaks was associated with a concomitant increase in ROS production (Wielsoe et al., 2015). PFOS also induced micronuclei and DNA strand breaks in rat bone marrow and peripheral blood (Celik et al., 2013; Ele and Celik, 2016), and increased mutation frequencies at redBA/gam gene loci in gpt delta transgenic mouse embryonic fibroblasts (Wang et al., 2015). DNA damage following exposure to PFOS has also been reported in *Caenorhabditis elegans*, green mussels, earthworms and zebrafish (EFSA, 2018).

In the 28-day NTP study, female rats at the highest dose (5 mg/kg bw per day) showed a significantly increased frequency of micronucleated polychromatic erythrocytes. However, as this was within the historical control range, the finding was considered by the authors to be equivocal. No increase was seen in males. A significant dose-dependent decrease in the percentage of polychromatic erythrocytes in the peripheral blood of both sexes was observed. The authors suggested it was indicative of bone marrow as a target for PFOS-induced cytotoxicity (NTP, 2019a).

PFOS was negative in the *in vivo* bone marrow mouse micronucleus assay at single oral doses of 237.5, 450 and 950 mg/kg bw (with sampling at 24, 48 and 72 hours), and several PFOS precursors were found negative in different *in vivo* tests (ATSDR, 2021).

5.7.2. PFOA

PFOA did not show mutagenic effects in the Ames test, using four strains of *Salmonella typhimurium* in the presence or absence of rat S9 metabolic activation system (Fernandez Freire et al., 2008; Buhrke et al 2013). NTP reported equivocal findings in strain TA98 without S9 and negative findings with S9. Negative findings were also reported in TA100 and E.coli with and without S9. In the absence of S9 assays utilised PFOA at concentrations up to 1000 µg/plate. In the presence of S9, PFOA concentrations up to 5000 µg/plate were used and for the *E. coli* assay, a dose up to 10,000 µg/plate was tested. PFOA has shown no mutagenic activity in the *umu* test at levels up to 1000 µM (Oda et al., 2007). However, PFOA increased mutation frequency at CD95 loci in human hamster hybrid cells at the highest (cytotoxic) concentration (200 µM) after long-term (16 days) incubation, with mitochondria-dependent ROS being shown to play an important role (Zhao et al., 2010; Zhao et al., 2011b).

Conflicting results were reported regarding DNA damage. PFOA did not increase oxidative DNA damage (as measured by a comet assay) in rat testicular cells exposed to 100 and 300 µM for 24 hours (Lindeman et al., 2012). Four studies reported no genotoxic effects (DNA strand breaks, micronuclei) after PFOA exposure (up to 400 µM for 24 h) in HepG2 (Eriksen et al., 2010; Florentin et al., 2001), V79 (Buhrke et al., 2013) and SHE cells (Jacquet et al., 2012). Conversely, increased frequency of micronuclei, strand breaks and 8OHdG (8-hydroxy-2'deoxyguanosine, a biomarker of oxidative stress and carcinogenesis) were reported in HepG2 cells (Yao and Zhong, 2005; Wielsoe et al., 2015) and TK6 cells (Yahia et al., 2014). PFOA induced ROS has also been reported in the absence of detectable DNA damage in HepG2 cells, at concentrations between 0.4 and 2000 µM (Eriksen et al., 2010). Assays to assess clastogenic effects of PFOA were negative in three cell lines (two hamster and one human) (ATSDR, 2021).

Three *in vivo* bone marrow micronucleus tests in mice (doses of 237.5. 450 and 950 mg/kg bw

per day) with sampling at 8, 24 and 72 hrs were negative (Environment Canada and Health Canada, 2012). NTP also reported no increases in micronucleated reticulocytes in peripheral blood of female rats (Sprague-Dawley) administered PFOA (6.25 to 100 mg/kg bw per day) by gavage once daily for 28 days. Although a significant increase was noted in male rats at all doses (6.25 to 10 mg/kg bw per day), this was within the laboratory's historical control range (95% confidence limits). The percentage of immature erythrocytes in peripheral blood of male or female rats was also unchanged, suggesting that PFOA did not induce bone marrow toxicity.

5.8. Carcinogenicity

5.8.1. PFOS

Neoplastic effects were evaluated as part of the chronic toxicity study in rats reported by Thomford et al. (2002) and re-evaluated by Butenhoff et al. (2012b) (see Section 6.3.1). Statistically significant increases in hepatocellular adenoma were observed in males and females at the highest dose (1.42 – 1.49 mg/kg bw per day respectively). In this group a single hepatocellular carcinoma was also evident in one female. Findings related to increased incidence of thyroid follicular cell adenomas and carcinomas in both male and female rats did not reach significance or show dose-response. Similarly, the apparent increase in combined thyroid follicular cell adenoma and carcinoma and mammary gland tumours (primarily combined fibroma adenoma and adenoma) in the female rats lacked dose-response for all tumour classifications (evaluated using logistic regression tumour progression analysis). In summary, findings from the long-term carcinogenicity study confirm that the liver is a potential target organ for chronic toxicity and carcinogenicity, which may be attributable to a mode of action involving activation of PPARα and other PPARα-independent modes of action (see Section 7).

5.8.2. PFOA

Carcinogenicity studies for PFOA indicate that exposure can lead to liver adenomas (Biegel et al. 2001), Leydig cell adenomas (Biegel et al. 2001; Butenhoff et al. 2012), and pancreatic acinar cell tumours (PACT) (Biegel et al. 2001) in rats (HC, 2018b).

Liver tumours

Biegel et al. (2001) reported an increased incidence of liver adenomas in male CD rats compared to controls, at a PFOA oral exposure level of 20 mg/kg bw per day for 2 years. Butenhoff et al. (2012) reported an increase in liver carcinomas in male and female Crl:CD(SD)IGS BR rats following dietary exposure to PFOA at doses of 14.2 mg/kg bw per day (males) or 16.1 mg/kg bw per day (females) for 2 years; however, incidences were not statistically significant from controls and no dose-response relationship was observed.

More recently, in the NTP (2020) chronic study in rats, the incidence of liver adenomas in male rats was significantly increased after exposure to dietary concentrations of 40 and 80 ppm PFOA (~2.2 and 4.6 mg/kg body weight per day) for 2 years. In females, there were slight increases in the incidences of hepatocellular carcinoma in the high-dose groups (1,000 ppm); however, the increases were not significantly different from controls. The incidence of these neoplasms was not influenced by the presence or absence of perinatal exposure during gestation and lactation.

Leydig cell tumours

An increased incidence of testicular Leydig cell tumours (LCTs) was also reported in the studies carried out by Biegel et al. (2001) and Butenhoff et al. (2012a). In the latter study, statistical significance was reached at doses of 14.2 and 13.6 mg/kg bw per day respectively when compared to controls. However, the relevance of this tumour to humans is unclear (ATSDR, 2021) and its incidence in PFOA-exposed male rats may be facilitated by a non-genotoxic mode of action (Butenhoff et al., 2012a).

Pancreatic acinar cell tumours

Pancreatic acinar cell tumours were observed in the Biegel et al. (2001) study, with an incidence of 11% at 20 mg/kg bw per day compared to controls (incidence of 0%). Although these tumours were not reported in the Butenhoff et al. (2012) study, a slight increase in pancreatic acinar hyperplasia was reported at the higher dose of 14.2 mg/kg bw per day. Pancreatic acinar cell tumours were also significantly increased in male rats in the NTP (2020) study, at dietary concentrations as low as 20 ppm PFOA (~1.1 mg/kg body weight per day). The incidence of pancreatic neoplasms was elevated at the highest dietary concentration tested in females (1000 ppm; approximately 100 mg/kg bw per day). These effects were also not influenced by the presence or absence of perinatal exposure during gestation and lactation.

The authors of the NTP (2020) report concluded PFOA exposure is associated with clear evidence of carcinogenic activity in male rats based on the increased incidence of liver adenomas and pancreatic acinar cell tumours. It is therefore not possible using current evidence to exclude PFOA as a human carcinogen.

6. MODE OF ACTION

The primary effects observed following exposure to PFOS and/or PFOA in animal studies are liver toxicity, developmental toxicity, and immune toxicity (ATSDR, 2021). The potential modes of action for each of these endpoints are discussed below, however as some endpoints have been extensively studied and reported elsewhere, a summary of evidence only is provided here.

6.1. Hepatotoxicity

Several adverse effects on the liver have been reported in animal studies which are considered to result from a combination of PPARα-dependent and independent changes (ATSDR, 2021); however, findings in PPARα-null mice indicate that these effects are not fully dependent on PPARα activation. Other suggested PPARα-independent mechanisms of PFAS liver toxicity include changes in the expression of proinflammatory cytokines, including increases in IL-6, IL-1β, tumor necrosis factor-α (TNFα), C-reactive protein, and COX-2 at higher perfluoroalkyl doses (Fang et al. 2012b, 2012c; Yang et al. 2014 as cited in ATSDR, 2021), and decreases in TNFα, interferon-γ (IFN-γ), IL-4, and IL-6 levels at lower doses (Fang et al. 2012b; Qazi et al. 2013 as cited in ATSDR, 2021). It has been suggested that the release of these cytokines activate the NFκB p65 pathway, causing suppression of PPARα promoter activity and resulting in increased liver triglyceride levels and steatosis (Fang et al., 2012c; as cited in ATSDR, 2021).

In rodents, the effects reported on lipid parameters are suggested to occur due to the ability of PFOS and PFOA to impair the release of cholesterol and/or triglycerides from the liver. Lipid homeostasis altered through PPARα activation, results in an upregulation of genes related to fatty acid oxidation, resulting in a reduction in lipid levels, hypertrophy, lowered serum cholesterol and/or triglyceride concentrations. This mode of action is considered to occur through PPARα activation which can happen in both rodents and humans; however, the downstream responses (i.e. increases in hepatocyte proliferation and liver weight, and under chronic exposure conditions, liver tumour formation) of this activation are attenuated and sometimes not observed in humans (Felter et al., 2018). Further, it is feasible that the higher levels of peroxisomal ß-oxidation eventually cause cell death and enhanced release of liver transaminases. It is not clear whether this mode of action is relevant to increased serum ALT in humans (ATSDR, 2021).

6.2. Developmental Toxicity

Several developmental effects have been observed in rodents exposed to PFAS, generally in the absence of overt maternal toxicity. PPARs are expressed in the embryos of both rodents and humans, and based on inconsistent findings in studies comparing developmental effects in wild type and PPARα-null mice, some developmental effects of PFAS exposure observed in experimental species, including PFOA-induced inhibition of mammary gland development and increased neonatal mortality in mice, may be mediated by PPARα activation, with the degree of the effects differing between PFAS (ATSDR, 2021). However, the effect of PFOA on mammary gland development has not been studied in other animals or in humans, and the mode of action is unknown.

The ability of PFOA to disrupt metabolism by altering the expression of genes involved in homeostatic control of lipids and glucose has been postulated to explain the decreased neonatal survival and body weights associated with PFOA exposure (Abbott et al., 2012). Neonatal mortality may also occur from PFOS-induced alterations in the structure of lung surfactants when exposure occurs during the gestational period of lung maturation (Chen et al. 2012; Grasty et al. 2003, 2005), possibly leading to death because of poor oxygen uptake. Lastly, the potential for lowered birth weights associated with PFOA exposure in humans has been suggested to be linked to IGF-1 in cases where an inverse relationship with growth occurred. In rodents, PFOS and PFOA-related reduced body weight has been linked to several parameters, including the loss of white adipose tissue and the up-regulation of uncoupling protein-1 (UCP-1) with subsequent effects on energy expenditure and regulation of food consumption.

6.3. Immunotoxicity

Following a systematic review of evidence, NTP (2016) concluded that PFOS and PFOA are "presumed to be immune hazards to humans", with both being strongly associated with a suppression of antibody response. Other reported immunotoxic effects, including PFOA-induced impairment of infectious disease resistance, increased hypersensitivity-related outcomes, and increased autoimmune disease incidence, and PFOS-induced suppression of natural killer cell activity, were considered to have a weaker evidence base. A mode of action of immunotoxicity by PFOS and PFOA has not been established. Although some mechanisms of immunotoxicity may be shared, given that different lymphoid cell cytokine profiles are

reported for PFOS and PFOA, other mechanisms may be present that differ. It is suggested that the shared PFOA/PFOS mechanisms include gene modulation via PPARs, NF-κB transcription and regulation of apoptosis (Liang et al., 2022). A T-cell dependent antibody response (TDAR) assay in female wild-type and PPARα knock-out mice showed that PFOA suppressed TDAR in both genotypes, indicating that the mechanism for antibody response suppression is independent of PPARα activation (DeWitt et al. 2016). Modulation of cell-signalling responses critical to antibody production (for example, c-Jun, NF-κB, and IL-6) has been proposed as a potential mode of action for PFOA (DeWitt et al., 2012; Corsini et al., 2014).

6.4. Endocrine Disruption

PFAS exposure in rats was associated with induction of changes in thyroid hormone levels and in human studies, associations between levels of serum PFAS and thyroid hormone levels have also been reported. Mechanisms remain undefined although changes to thyroid function may be mediated by binding to the thyroid hormone receptor, and/or by altering expression of genes involved in thyroid function or thyroid hormone regulation. In vitro data suggests that PFOS and PFOA may be androgen receptors and ERα antagonists, however in vivo data is either unclear or not available to support these findings (ATSDR, 2021).

6.5. Carcinogenicity

There is a strong evidence base to indicate that PFOS and PFOA are not DNA reactive compounds. Butenhoff et al. (2012a; 2012b) noted that the ability of PFOS and PFOA to activate the xenosensor nuclear receptors PPARα, constitutive androstane receptor (CAR), and pregnane X receptor (PXR) in producing liver tumours in rodents is well established. However, since evidence from dose response studies indicates that hepatocellular adenoma and liver proliferation precede PPARα activation in rodents, liver tumours may not be driven by a peroxisome proliferation mode of action, and thus their potential human relevance cannot be dismissed (HC, 2018a; 2018b). Leydig cell adenomas induced by PFOA in rat testis are proposed to be caused by reduced serum testosterone levels which is compensated for by the release of luteotrophic hormone, leading to the growth stimulation of Leydig cells and tumour formation. Although the tumour type is common in aging rats, it is rare in humans; therefore, these findings are not considered relevant for human risk assessment. Pancreatic hyperplasia following exposure to PFOA is considered a precursor tumour event, also considered to be mediated through PPARα. Altered composition and output of bile acids is considered to lead to enhanced secretion of cholecystokinin, which, following binding to acinar CKK1 receptor, stimulates growth of this cell type. It is not considered a relevant mode of action for humans (HC, 2018b).

7. SUMMARY OF HEALTH EFFECTS

A large proportion of the available toxicity data for PFOS and PFOA (and some other perfluoroalkyls) is from epidemiology studies in humans, with oral exposure being the assumed route of exposure. For PFOS and PFOA specifically, this database comprises evaluations of health outcomes in subjects exposed in occupational settings, and residents living near a PFOA plant who had relatively high exposure via drinking-water and other environmental sources.

There are differences in the toxicokinetics of perfluoroalkyls between humans and experimental animals. Correlating serum measurements in both humans and animals with dose

requires the application of toxicokinetic models when deriving estimates for the human equivalent doses from the animal studies and when converting human serum measurements to human external doses. Toxicokinetic considerations are summarized in section 7.1, and adverse effects for animal and human toxicity are summarized in section 7.2.

7.1. Toxicokinetics

- PFOS and PFOA are readily absorbed through the gastrointestinal tract in mammals, including humans, and distributed predominantly to the plasma and liver without metabolism via binding to proteins. No metabolism occurs and excretion is via urine and faeces.

- Due to the non-linear nature of the toxicokinetics of PFOS and PFOA and the large differences in clearance rates between humans and other species, PBPK models have been used to refine dose conversions in several recent assessments (EFSA, 2020; Health Canada, 2018a and 2018b; Goeden et al., 2019; Dourson et al., 2019; Chou and Lin, 2020; 2021).

- Large interspecies and intraspecies differences in biological half-lives of both PFOS and PFOA have been described, possibly due to differences in renal clearance. Published estimates for the elimination half-lives of PFOA and PFOS in humans range from approximately 1.8 – 3.9 years and 2.9 – 4.8 years respectively (see section 3.4), compared with days or hours in rodents.

- Saturable renal resorption from the glomerular filtrate via transporters in the kidney tubules, as well as enterohepatic and gastrointestinal recirculation, may contribute to the relatively long half-lives of PFOS and PFOA in humans.

- Placental and lactational transfer occurs for both PFOS and PFOA.

7.2. Toxicity in animal studies

Toxicity studies for PFOS and PFOA were carried out in multiple species, including monkeys, rats, and mice. Adverse effects reported for PFOS included developmental toxicity (increased incidence of foetal sternal defects, reduced maternal serum T4, reduced maternal body weight, increased maternal liver weight, changes in glucose homeostasis, and altered placental physiology), liver toxicity (increased liver weight, decreased cholesterol and hepatic steatosis), immune effects, and increased incidence of hepatocellular adenoma. For PFOA, adverse effects included developmental toxicity (including delayed mammary gland development in mice), liver toxicity (hypertrophy, necrosis and effects on the metabolism and deposition of dietary lipids), kidney weight changes, immune effects, and increased incidence of neoplastic lesions (including liver adenomas, Leydig cell adenomas, and pancreatic acinar cell tumours).

The applicability of the adverse health effects reported in animals to human health is uncertain, recognizing species and sex-related differences in the toxicokinetics of PFAS. In addition, the mode(s) of action for PFOS and PFOA-induced toxicities are not fully elucidated, although both PPARα- dependent and -independent pathways have been proposed. As the limitations in the animal data were considered differently among existing authoritative assessments, a range of endpoints have been used to develop national or regional health-based drinking-water values (see section 10.1 and A.1 for more information).

7.2.1. Repeated dose toxicity

- In rodents, the liver is the major systemic target organ for PFOS and PFOA, with a dose-dependent increase in the relative liver weight starting at 0.49 mg/kg bw per day for PFOS and increased absolute and relative liver weight and hepatic peroxisomal ß-oxidation at 0.64 mg/kg bw per day for PFOA. However, increased liver weight is not by itself an adverse effect unless accompanied by necrosis, fibrosis, steatosis or other clinically relevant signs of liver damage (Hall et al., 2012). Non-neoplastic lesions in the liver, including hepatocyte cytoplasmic alteration, hepatocyte hypertrophy, pigmentation and necrosis, were reported in rats exposed to dietary concentrations as low as 20 ppm for two years (~1 mg/kg bw per day).

- The effects of PFOS in rodents, i.e. increased organ weight, hypertrophy of hepatocytes and focal necrosis with induction of peroxisomal ß-oxidation are mediated through both PPARα-dependent and independent modes of action.

- PFOA-induced transactivation of PPARα is also apparent as related to the hepatic effects, with enhanced liver peroxidation and elevated liver enzymes in serum seen at 2.5 mg/kg bw per day in mice. PPARα-independent modes of action also occur.

7.2.2. Reproductive and developmental toxicity

- In rodents, the most sensitive developmental effect following PFOS exposure was increased incidence of foetal sternal defects, which was associated with a $BMDL_5$ of 0.122 mg/kg bw per day in rats and 0.016 mg/kg bw per day in mice. Additionally, evidence of maternal toxicity endpoints includes reduced maternal body weight at term ($BMDL_5$ values of 0.15 mg/kg bw per day for rats; 3.14 mg/kg bw per day for mice) and reduced maternal serum T4 (0.046 mg/kg bw per day for rats; 0.352 mg/kg bw per day for mice).

- In rodents, the most sensitive developmental effect following PFOA exposure was impaired development of the mammary gland in one specific mouse strain (CD-1), after chronic exposure of the dams to 5 ppb PFOA in drinking water (equivalent to approximately 0.00045 mg/kg bw per day) beginning at GD7 in P0 dams and continuing through the F1 and F2 generations. In treated P0 dams, normal weaning-induced mammary involution was delayed, whereas in the F1 offspring, delayed mammary gland development was reported in the PFOA-exposed group. Similar effects were seen in mice exposed to doses as low as 0.01 mg/kg bw per day during late-stage gestation (GD 10 – GD 17). Other strains (i.e. C57Bl or Balb/3) experienced similar effects but at much higher PFOA doses (1 or 5 mg/kg); the reason for this difference is not known.

- A prenatal developmental LOAEL of 1 mg/kg bw per day was proposed for PFOA based on accelerated onset of puberty in males, as well as increases in the incidence of several skeletal defects, including: reduced ossification of forelimb proximal phalanges, reduced ossification of hindlimb proximal phalanges, reduced ossification of the calvaria and enlarged fontanel, and reduced ossification in the supraoccipital bone.

- In adult male mice exposed to PFOA for 28 days, damage to the seminiferous tubules and decreased testosterone and progesterone levels were reported at 1.25 mg/kg bw per day.

7.2.3. Neurotoxicity

- PFOS was shown to have neurotoxic effects in rats at doses as low as 5 mg/kg bw per day for at 28 days, based on clinical evidence including cachexia, lethargy and tonic convulsions. Similar effects were reported in rats exposed to PFOA at doses of 5 mg/kg bw per day for two weeks. In mice, doses as low as 2.15 mg/kg bw per day for three months was associated with reduced performance in a water maze test and other histological evidence (including increased incidence of apoptosis in the hippocampal neural cells). PFOA exposure was also associated with developmental neurotoxicity in male neonatal mice exposed to single gavage doses as low as 0.58 mg/kg bw on PND 10, which resulted in reduced total activity after two months.

7.2.4. Immunotoxicity

- Structural and functional immune parameters are affected by PFOS in mice. The most sensitive effects include suppression of the T-cell dependent antibody response to immunisation, with a LOAEL for an impaired response to sheep blood cells of 0.083 mg/kg bw per day in mice exposed to PFOS for 60 days. Additionally, host resistance to Influenza A virus was reduced in female PFOS-exposed mice, resulting in an increased mortality from infection at 0.025 mg/kg bw per day.

- Male mice exposed to doses of PFOA as low as 0.49 mg/kg bw per day for 21 days had an approximate 50% reduction in CD8+ lymphocyte populations in the spleen compared to controls. Otherwise, PFOA-induced immunotoxicity in mice and rats was generally reported at higher doses than doses associated with PFOS-induced immunotoxicity.

- In summary, there is evidence to support the conclusion that PFOA is immunotoxic in rodents with an association between PFOA exposure and dysregulation of the immune system, and with different influences on innate versus acquired immunity. As effects were usually seen at doses that also induced general toxic effects including those related to food intake and body weights, indirect effects of PFOA on the immune system cannot be ruled out.

7.2.5. Genotoxicity

- No evidence to support a direct genotoxic mode of action for both PFOS and PFOA was identified. However, there is some evidence for a role of oxidative stress induced by both PFOA and PFOS.

7.2.6. Carcinogenicity

- For PFOS, doses as low as approximately 1.5 mg/kg bw per day (via dietary exposure) were associated with significantly increased hepatocellular adenoma in both male and female rats exposed for two years. For PFOA, the most sensitive tumour effects included increased incidence of liver adenomas in male rats exposed to doses as low as 2.2 mg/kg bw per day (via the diet) for two years, and increased incidence of pancreatic acinar cell tumours in male rats exposed to approximately 1.1 mg/kg bw per day (via the diet) for two years. However, statistically significant increases in the incidence rates of these tumours were not reported in female rats exposed similarly to PFOA.

7.3. Human toxicity endpoints

Some of the most common adverse effects reported in humans following exposure to PFOS/PFOA are listed below:

- Epidemiological studies suggest that exposure to PFOS and PFOA adversely affects antibody response to vaccination against diphtheria and tetanus in children, with evidence of PFOA having a stronger association compared to PFOS. However, there is limited evidence of an association between PFOS and PFOA serum levels and increased incidence of illness in children; for example, according to CDC (2019) data the number of new cases of diphtheria in the United States over a 40-year period was less than one per year on average. Additionally, a mode of action has not been established for immunotoxicity, and this endpoint is associated with high intraindividual variability. Thus, further studies are needed to determine whether this association leads to increased infection rates. The immune system effects were proposed by EFSA (2020) to be the most robust among those reported for the risk assessment of PFOS and PFOA; thus, EFSA (2020) referred to this endpoint in humans to derive a tolerable weekly intake (TWI) for a group of four PFAS (PFOA, PFNA, PFHxS and PFOS). Similarly, the US EPA (2021a; 2021b) derived updated draft health advisories for PFOA and PFOS based on immune system effects, an approach that was supported by their Science Advisory Board (SAB, 2022).

- There is support for an association between exposure to PFOS and PFOA and increased serum levels of total and LDL cholesterol from epidemiology studies. This association may be mediated by inter-individual variability in the degree of intestinal reabsorption and enterohepatic circulation of PFOS/PFOA. It was suggested by EFSA (2020) that the maximum association with total cholesterol occurs at PFOA serum levels of 25 ng/mL and does not continue to increase as the serum level increases.

- Although epidemiological studies provide evidence for an association between exposure to PFOS and PFOA and increased serum ALT, the magnitude of the associations is small, with ALT levels rarely being outside the reference range and no evidence of liver disease.

- Several reproductive outcomes in PFOS and PFOA-exposed human cohorts, including fecundity, maternal hypertension and preeclampsia, preterm births and pregnancy loss, and effects to male sperm parameters, have been studied. Small but statistically significantly elevated odds ratios for associations between elevated serum levels of PFOA and reduced fecundability, increased risk of miscarriage, and altered sperm morphology have been reported. These associations were generally weaker for PFOS. However, due to the cross-sectional design of some of these studies, there is potential for confounding and reverse causality that requires the results to be interpreted with caution.

- Data seem to support an association between PFOS and PFOA and decreased birth weight which may increase risk for future disease. An increase of 1 ng/mL maternal plasma PFOA is associated with a reduced birthweight of approximately 10 g; however, the overall association may be confounded by the magnitude of plasma blood volume expansion and glomerular filtration rate, with the strength of the association being overestimated in studies where glomerular filtration rate is not accounted for (Steenland et al., 2020).

- Studies evaluating the association between PFOS exposure and cancers of the breast and prostate do not consistently support a causal relationship. However, there is stronger evidence of an association between PFOA exposure and kidney and testicular cancer incidence in community and occupationally exposed populations, with a recent case-control study (Shearer et al., 2021) finding a significantly elevated odds ratio of 1.71 associated with renal cell carcinoma incidence resulting from a doubling of PFOA serum concentration.

- Many studies assessed thyroid hormone levels (TSH, free T4 and free T3) in adults, with some providing a combined analysis for pregnant women and their newborn infants. Evidence of the association between PFOS/PFOA exposure and thyroid disease or changes in thyroid hormones such as TSH, T4 and T3 is inconsistent. The US EPA (2021a; 2021b) reports evidence of positive associations for PFOA and TSH in adults and PFOA and T4 in children, whereas Steenland et al. (2020) stated that the evidence for a causal impact (of PFOA exposure) on thyroid hormones "remains weak."

8. PRACTICAL CONSIDERATIONS

8.1. Monitoring

PFOS and PFOA impact both ground and surface waters, including those used as sources of drinking-water, as described in section 2.5. In general, routine monitoring of drinking-water is not recommended but where contamination is suspected based on system hazard analysis, investigative monitoring should be carried out by water providers to assess PFOS and PFOA concentrations in source waters. Where these chemicals are present at levels exceeding the country/regional guideline value, quarterly surface water and semi-annual groundwater monitoring should be conducted when resources allow. Monitoring in finished drinking-water should also be conducted if treatment is put in place for removal. Conversely, if PFOS and PFOA are not detected or are found at concentrations close to or below levels of concern in source water, monitoring frequencies can usually be reduced. In areas where no inputs can be identified upon investigation, monitoring would also usually not be necessary. The exception is where fluorinated precursors (e.g. polyfluoroalkyl amide (FA) and sulfonamide) are found in source water, and chlorine, ozone or advanced oxidation processes are applied as part of water treatment. In such situations, PFOS and PFOA generation have been demonstrated (Xiao et al., 2018) and appropriate monitoring may therefore need to be conducted.

Where contamination with PFAS is suspected, such as near manufacturing sites or where there is contamination with AFFF[7], monitoring for other PFAS should also be considered. A range of PFAS have been identified that can contribute to the total amount of PFAS present, for example, the Drinking Water Directive (EC, 2020) recommends a group of 20 PFAS for monitoring if the risk assessment shows that PFAS are likely to be present in a drinking-water source. However, since monitoring for PFAS substances is difficult (see section 9.2), investigative efforts in resource limited areas may be difficult or not possible. In such cases, consideration should be given to possible sources using water safety plan hazard identification processes and, where appropriate, continuing inputs should be stopped or better handled by

[7] AFFF is a mixture of many PFAS compounds that are likely to co-occur near impacted sites (HC, 2018a, 2018b).

industry and appropriate pollution control authorities in conjunction with stopping of non-essential material uses.

8.2. Analytical methods and achievability

Standard analytical methods are available for the determination of PFAS in water matrices by international standards organization (ISO, 2019) and by the US EPA (2019, 2020). These methods are based on tandem mass spectrometry coupled with liquid chromatography, usually achieving a limit of quantification of 10 ng/L. A limit of quantification as low as 1 ng/L can be achieved through sample pre-concentration (with a range of resins available that adsorb more or less polar compounds).

The US EPA Method 533 includes 25 PFAS including several precursors with the lowest concentration minimal reporting level (LCMRLs) ranging from 1.7 to 13 ng/L. The US EPA Method 537.1 includes 18 PFAS and reports a LCMRL in drinking water of 2.7 ng/L for PFOS and 0.82 ng/L for PFOA. In total, 29 different PFAS can be evaluating using the two methods, recognizing some overlap between them. The Method 533 LCMRLs for PFOA and PFOS are 3.4 ng/L and 4.4 ng/L, respectively. Method 533 also includes the analysis of multiple short-chain PFAS that cannot be measured by Method 537.1. The limit of quantification in the ISO method for most of the compounds to which the method applies is ≥ 0.2 ng/L, but actual detection levels can depend on the blank levels realized by individual laboratories. Due to the ubiquitous nature of PFAS, sample contamination through the presence of trace levels in reagents, labware, sample collection implements, and instrumentation is possible. Quality control procedures are therefore essential to ensure accurate analysis (Van Leeuwen et al. 2006; Yamashita et al. 2004; US EPA, 2020).

Methods continue to be refined and detection and quantification limits continue to improve. The typical list of substances that can be measured includes PFOA (335-67-1) Perfluoro-n-octanoic acid, PFOS (1763-23-1) Perfluoro-1-octanesulfonate, PFBA, (357-22-4) Perfluoro-n-butanoic acid, PFPA (2706-90-3) Perfluoro-n-pentanoic acid, PFHxA (307-24-4) Perfluoro-n-hexanoic acid, PFBS (375-73-5) Perfluoro-1-butanesulfonate, PFHpA (375-85-9) Perfluoro-n-heptanoic acid, 6:2PTS (27619-97-2) Perfluoro-octane sulfonate 6:2, PFHxS (355-46-4) Perfluoro-1-hexanesulfonate, PFNA (375-92-8) Perfluoro-n-nonanoic acid, PFHpS (375-92-8) Perfluoro-1-heptanesulfonate, PFDA (335-76-2) Perfluoro-n-decanoic acid, PFUnA (2058-94-8) Perfluoro-n-undecanoic acid, PFDoA (307-55-1) Perfluoro-n-dodecanoic acid, PFOSA (754-91-6) Perfluoro-octanesulfonamide, PFDS (335-73-3) Perfluoro-1-decanesulfonate, PFPeS (2706-91-4) Perfluoro-1-pentanesulfonate.

These are all specialist methods requiring advanced analytical equipment that is not likely to be available in low-income settings. Analysis of total organic fluoride is an emerging approach and may prove valid as a screening method.

8.3. Source control

It is increasingly recognized that there is a need to reduce and manage PFAS in the environment as one class. This is considered appropriate due to their similar molecular structures, environmental properties, and/or biological hazards (Kwiatkowski et al., 2020; Cousins et al., 2020a, 2020b).

Where PFAS contamination is ongoing of both ground and surface water sources as identified under hazard analysis in WSPs, it is important that such inputs are stopped or better handled by industry and appropriate pollution control authorities in conjunction with stopping non-essential uses. Where the source inputs are historical such as previous use of AFFFs resulting in heavy soil contamination, it is appropriate to identify means of either preventing contaminant spread by physical barriers or diversion away from drinking-water sources.

Blending or diluting PFAS contaminated source water with uncontaminated water may be a cost-effective and viable option for some water systems, in conjunction with stopping non-essential uses. Source substitution could also be considered. When considering the use of an alternative source or blending as control options, the water utitity should assess the water quality of new sources and the blended water to ensure that it does not interfere with the existing treatment processes, impact the distribution system, and/or cause other water quality issues.

8.4. Treatment methods and performance

8.4.1. General overview

The removal efficiency of PFAS, including PFOS and PFOA, from source waters depends on variables such as influent concentrations, background contaminants in the water matrix, available treatments and the range and characteristics of the PFAS species present (Table 9.1). Many water treatment systems are not typically optimised for removal of PFAS, so removal rates can be very variable. PFAS substances are highly stable molecules that are resistant to chemical and biological oxidation. As a result, commonly used drinking water treatment processes such as coagulation-clarification-filtration, ozonation and disinfection are ineffective at removing PFAS to any significant degree. Removal is reported to be in the range 0-5% for these processes (Crone et al., 2019). Enhanced coagulation using optimised dose and pH conditions may be able to increase removal of PFOA and PFOS to levels around 30%, but this will be highly dependent on the water matrix (Xiao et al., 2013).

Advanced oxidation processes (AOP; for example, UV/H_2O_2) are reported to provide some limited PFAS degradation of up to 15% using application conditions that are considered feasible for drinking water treatment systems. Water companies should also be aware that oxidative processes (for example ozone, chlorination or AOPs) may oxidise polyfluorinated precursor chemicals present in the raw water, which could result in an increased concentration of PFAS in the finished water (Xiao et al., 2018, HC, 2018a, 2018b). Recently, reductive processes have emerged as promising methods for degradation of a range of PFAS (Cui et al., 2020; Chen et al., 2020). However, it should be noted that many of the PFAS removal studies have been carried out at bench-scale, showing variable results and efficacy (Horst et al, 2018). Other processes such as foam fractionation have also shown the potential for a high level of removal of a range of PFAS, but these have not been as widely studied or applied for continuous scale drinking water treatment as other technologies (Burns et al., 2021; Buckley et al., 2021). As there is less available data for these processes, they have not been considered further here, but their importance may increase as more information becomes available.

The processes that have been reported to provide enhanced removal of some PFAS (>90%) are high pressure membrane processes, adsorption and ion-exchange (IEX) (Crone et al., 2019). These processes are further described in the following sections.

8.4.2. High pressure membranes

High pressure membrane processes include nanofiltration (NF) and reverse osmosis (RO) technologies. NF membranes are characterised by pore sizes between 1-10 nm, while RO membranes typically have pore sizes of less than 1 nm (Lee et al., 2022). RO and NF operate by solution diffusion, excluding contaminants through size exclusion, electrostatic repulsion and hydrophobicity. RO can filter out smaller molecules than for NF, with respective molecular weight cut-offs of 200 and 500 Da, respectively. Both RO and NF are effective for removal of a range of PFAS, particularly charged species (including short chain compounds). Increased removal efficiency is typically seen when using RO due to its smaller molecular weight cut-off when compared with NF (Lee et al., 2022). RO and NF treatment provides greater stability and reliability for PFAS removal when compared to adsorptive processes such as activated carbon adsorption (AC) or ion exchange (IEX) (see below) as they provide an absolute barrier for removal when operated correctly (Lee et al., 2022). This enables effective treatment even with fluctuating PFAS concentration in raw water. Both membrane processes show less effective removal of neutral PFAS. The efficiency of high-pressure membrane technology to achieve the treatment goal varies depending on the membrane properties and physicochemical properties of the PFAS (including molecular weight/geometry, functional group, and hydrophobicity). This can be affected by water quality parameters such as the pH, temperature, background anions/cations, and natural organic matter (NOM) content. The literature has reported that high-pressure membranes are capable of reducing low PFOS and PFOA concentrations by >99% for PFOS and by 92 ->97% for PFOA (Lipp et al., 2010; Thompson et al., 2011, Flores et al., 2013; Applemans et al., 2014; Franke et al., 2019; Crone et al., 2019). Membrane systems must be effectively maintained to ensure that they continue to meet these high levels of removal, to reduce the impacts of fouling and loss of membrane integrity.

Operation of high-pressure membranes requires significant energy and water resources. Typically, 80-85% water recovery is observed in membrane process operation for PFAS removal (Appleman et al., 2014; Crone et al., 2019). This means that 15-20% of the feed flow ends up as a concentrated retentate stream. This high-volume waste requires further treatment and can therefore be costly and difficult to dispose of. Particularly in the case of RO, re-mineralisation of treated water is also required to reduce the corrosion potential of the water.

8.4.3. Adsorptive processes

Adsorptive processes such as AC (granular and powdered activated carbon – GAC and PAC, respectively) and IEX are processes where contaminants are removed from the aqueous phase onto solid media. The media is then either reused (after regeneration) or replaced upon exhaustion. For both AC and IEX, the highest levels of removal reported in Table 9.1 would be expected under conditions where competition with other water contaminants is minimised, through either pre-treatment, or where background water quality is good.

8.4.4. Activated carbon

AC can be used either in granular (1-2 mm in diameter) (GAC) or powdered (<0.1 mm in diameter) (PAC) form. In most applications for micropollutant removal in conventional treatment processes, PAC is dosed into the water at an early stage of water treatment and removed by coagulation, clarification and filtration processes. GAC is typically used in filtration beds downstream of clarification and depth filtration processes (Crone et al., 2019).

PAC is usually only used once prior to disposal with the water treatment sludge, while GAC media is thermally regenerated after exhaustion. Both GAC and PAC have been shown to be effective for removal of PFAS from water (Crone et al., 2019). The majority of research has considered the use of GAC, perhaps reflecting the more widescale application of GAC filtration when compared to PAC dosing. AC treatment has proven to be effective for removal of a range of PFAS compounds (Du et al., 2014). PFOS and PFOA have a good to moderate potential for adsorption onto AC. Adsorption is particularly effective for high molecular weight and hydrophobic PFAS, while lower removal for neutrally charged and hydrophilic short chain PFAS is observed. Correlations have been observed between removal efficiency by sorption and PFAS chain length, adjusted by functional group (Du et al., 2014). PFAS with sulfonate functional groups show increased removal when compared to those with carboxylate groups (McCleaf et al., 2017). Adsorption increases with increasing chain length based on the following functional group, with removal increasing in the following order: fluorotelomer sulfonic acids (FTSAs) < perfluoroalkyl carboxylates (PFCAs) < perfluoroalkane sulfonates (PFSAs) < perfluorooctanesulfonamide (FOSA) (Sörengård et al., 2020). Greater removal of linear PFAS is typically observed than when compared to branched species (McCleaf et al., 2017).

Concerns with adsorption for PFAS removal includes the increased regeneration frequency that is usually required for GAC beds when compared to their conventional operation, along with competition between PFAS, natural organic matter (NOM) and other water contaminants. There are also indications that hydrophobic PFAS and other hydrophobic compounds in the water matrix can displace adsorbed hydrophilic PFAS. Therefore, the design of a GAC system is important for achieving successful PFAS treatment. It requires consideration of the water quality to be treated, the appropriate AC media selection, adequate bed depth, moderate or low hydraulic loading rates, as well as effective operation and maintenance (including the age of the media and the frequency of replacement/regeneration of the media).

8.4.5. IEX

IEX has been shown to be effective for removal of charged PFAS species. Most PFAS species found in drinking water sources are anions (negatively charged) at pH relevant to drinking water treatment (Crone et al., 2018). As a result, most research has investigated the application of anionic exchange resins. It should be noted, however, that if cationic (positively charged) PFAS are present, then these will be poorly removed by anionic exchange resins and may require specific removal by cationic exchange resins. For the more prevalent anionic PFAS, preferential removal of high MW species is typically seen when IEX is used, although functional groups on the IEX resin can be changed to target smaller chain length PFAS. Secondary mechanisms of removal of PFAS on IEX media can also occur as a result of hydrophobic and van der Waals interactions. Anionic exchange resins typically have higher removal capacities than GAC for negatively charged PFAS. As with GAC, competition from other ions and compounds in the water matrix can negatively impact PFAS removal when using IEX. Unlike conventional IEX processes, when PFAS is being targeted, regeneration is often very poor resulting in one-off use of the resin, making the process operationally costly. Research is ongoing to establish whether more effective regeneration can be achieved using organic solvents (Du et al., 2015).

8.4.6. Examples of PFAS removal from full-scale and pilot studies

A long-term study comparing the removal of 15 PFAS by GAC across a group of full-scale drinking water treatment works (WTWs) in Sweden showed removal of between 92-100% for young GAC filter beds. The efficacy of treatment decreased to 7-100% for GAC filters that had been in service for approximately 1 year, treating 29,000 bed volumes (Belkouteb et al., 2020). In this case, average inlet concentrations of 5 PFAS compounds were <100 ng/L (PFHxA: 11.0±0.1 ng/L; PFOA: 6.6±0.1 ng/L; PFBS: 12.0±0.1 ng/L; PFHxS: 85.0±0.1 ng/L; PFOS: 11.0±0.1 ng/L). The PFAS compounds that broke through the GAC first were the shorter chained PFAS, PFHxA and PFBS, which broke through around 10,000 BV. The removal of longer chain PFAS continued longer. For example, PFOS was still 80-100% removed after 30,000 BV.

In an unpublished report from Italy, a WTWs treating groundwater contaminated with PFAS from a nearby factory was able to effectively reduce a range of 12 short- and long-chained PFAS (PFBA, PFBS, PFOA, PFOS, PFPeA, PFHxA, PFHpA, PFHxS, PFNA, PFDeA, PFUnA and PFDoA) following a range of remedial actions, including installation and improvement of GAC filtration at the WTWs (L Lucentini, Istituto Superiore di Sanità, F Russo, Veneto region, personal communication, May 2022). Over the duration of the investigation, the total PFAS in the raw water decreased from a median of 726 ng/L (max 4701 ng/L) in 2013 to 0 ng/L (max 511 ng/L) in 2021. In treated water, the total PFAS decreased from a median of 613 ng/L (max 3520 ng/L) in 2013 to 0 (max 50 ng/L) in 2021 following treatment optimisation (alongside the closure of the pollution source). Doubling the GAC filtration capacity played an important role in ensuring that many of the PFAS were reduced to levels below the limits of detection. PFBA, PFBS and PFOS were effectively removed under optimal conditions, with median residual concentrations in treated drinking water ≤LOQ (5 ng/L). For PFOA, good removal was seen, with a median residual concentration of ≤LOQ (max 10 ng/L).

A study on the occurrence and removal of 23 PFAS across 15 WTWs in the US saw good removal of long chain PFAS across GAC and IEX, while RO was effective in reducing all measured PFAS to below method reporting limits (Appleman et al., 2014). Concentrations of PFAS in the source waters were all <100 ng/L, with most in the low ng/L range. Treated water PFAS concentrations were typically below reporting limits, although notable exceptions at some sites were for PFOA (11.0-57 ng/L), PFBA (<5.0 – 27 ng/L), PFPeA (9.2 – 43 ng/L), PFHxA (7.7 – 62 ng/L) and PFHpA (4.1 – 34 ng/L). Two pilot-scale NF membranes in series reduced the total concentration of 8 PFAS from 212 ng/L (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFBS, PFHxS, and PFOS) to approximately 0.022 ng/L, an average removal of 99% (Franke et al., 2019). Others have seen >93% removal of 9 spiked PFAS substances (concentration range 330-937 ng/L) by nanofiltration (Appleman et al., 2013). Pilot-scale GAC columns achieved average reductions of 62% for 14 PFAS (PFCA, PFSAs and FOSA) spiked into a real water source in the range 0.64-1.6 µg/L (McCleaf et al., 2017).

Less effective removal of PFAS has been observed in other GAC studies at full and pilot scale. In an assessment of a Spanish WTWs, GAC filtration was ineffective for PFAS removal, although in this case, the influent concentrations were very low (maximum concentration reported was 22.3 ng/L for PFBA) and the usage state of the GAC was not reported (Borrull et al., 2021). Negative removal of short chain PFAS compounds was observed at the end of GAC column experiments, indicating displacement of these compounds from the adsorbent by more strongly adsorbing substances (Appleman et al., 2013; McCleaf et al., 2017).

8.4.7. Implications and considerations for PFAS removal processes

The processes considered effective for PFAS removal require regeneration, disposal or treatment of either a spent sorbent media or a highly concentrated reject waste stream containing PFAS and other contaminants. Care must be taken to effectively manage these waste streams during disposal to prevent contamination of the local environment. Treated water from these processes may also need further conditioning to reduce the corrosion potential of the water. It is also important to consider that the performance of the processes identified to be effective for PFAS removal will be strongly dependent on water quality and effective pre-treatment. For example, high pressure membranes typically require significant pre-treatment (for example clarification and filtration) for effective operation. Likewise, adsorption and ion-exchange require removal of bulk organic matter from the water to reduce competition effects with micropollutants. Contaminants such as iron and manganese can also disrupt processes and hence may need prior removal.

The degree of PFAS removal from drinking water can vary depending on the treatment method, class of perfluorinated chemical, and water quality. For example, regeneration frequencies of between 3-24 months were reported for GAC systems where PFAS removal was monitored (Crone et al., 2019). An understanding of the concentration range and type of PFAS species present in a water source is recommended to enable water providers to be able to estimate the operational conditions of treatment processes. Subsequently, a pilot-scale evaluation is recommended. This will provide data on the treatment performance at a specific time under specific operational environments and so should be suitably extensive in order to evaluate removal over a range of realistic water quality conditions. Systems used for the removal of PFAS should be designed, operated, and maintained specifically with consideration of the PFAS mixture and its individual influent concentrations, water pre-treatment and the overall treatment objectives for any given contaminated water source. This must also consider important operational costs associated with the treatment processes used for PFAS removal. In the case of GAC, this includes provision for media regeneration and top-up, alongside appropriate backwashing systems. For membrane filtration, this includes facilities for membrane backwashing and cleaning, as well as replacement of membrane modules. In both cases, the costs of PFAS sampling and analysis can be significant and needs to be considered.

Under optimised conditions and operation, it is reasonable to assume that RO and GAC treatment can reliably reduce PFOS and PFOA concentrations to below 0.1 µg/L (100 ng/L). (Appleman et al., 2014; Belkouteb et al., 2020). The picture for IEX is less certain, but where charged species of PFAS are predominant, IEX should be effective. The co-occurrence of PFAS compounds in water sources varies from source to source with respect to the species and concentrations present. Most studies undertaken at real drinking water systems often have low concentrations of PFAS mixtures at the inlet, often well below 0.5 µg/L (although this is dependent on the number of species measured). When high pressure membrane processes or GAC operating under optimised conditions are exposed to higher total PFAS concentrations (within the range typically observed in the environment), they would be expected to reduce total PFAS concentrations to below 0.5 µg/L. This has been established based on a limited data set from studies that have investigated water with PFAS > 0.5 µg/L, either spiked or environmental concentrations (Appleman et al., 2013; McCleaf et al., 2017; L Lucentini, ISS, personal communication, May 2021).

It is important to recognise that for resource limited water supply systems, installing, operating, and monitoring complex water treatment processes such as those listed above for PFAS removal may be very challenging. In these circumstances, prioritisation should be given to more imminent water quality risks and that expenditure for removal of contaminants such as PFAS should be justifiable and achievable.

Table 8.1: Main treatment processes for removal of PFOS and PFOA from water sources

Treatment Method	Treatment Process	Range of removal rates achievable, including under optimised conditions	Application	Advantages	Disadvantages
Activated Carbon	Granulated activated carbon (GAC) or Powdered activated carbon (PAC)	PFOS 0 to ≥ 90% PFOA 0 to ≥ 90% (Depending on age of GAC)	Surface water, Groundwater, PWSs, Households (POU/POE)	Widely used; high removal rates possible; household applications possible	Variable removal efficiency observed; competitive adsorption with e.g. natural organic matter; PAC is used only once before disposal; GAC requires thermal regeneration and media top-up; Disposal of waste carbon required; optimisation required for PFAS removal
Ion-Exchange	Ion exchange media (resins or petrochemical compounds) which can remove ions from water of opposite charge to functional groups on the resin	PFOS ≥ 90% PFOA 10-90%	Surface water, Groundwater, PWSs, Households (POU/POE)	Good removal of PFOS – sorption rates dependent on polymer matrix and porosity; some removal of PFOA possible	Single use of IEX resin after exhaustion makes process expensive; disposal of used resin required; rate of exchange influenced by many parameters, including influent PFAS concentration; competition for removal between other water contaminants; surface water may need clarification or filtration prior to use; less effective for removal of uncharged, positively charged and short-chain PFAS
Membrane Filtration	Reverse Osmosis (RO) Nanofiltration (NF)	PFOS ≥ 99% PFOA ≥ 92-99% >93% for range of species	Surface water, Groundwater, PWSs, Households (RO) (POU/POE)	High levels of removal; can be combined with GAC for higher removal rates; effective for multi-contaminant removal; household applications possible	Waste must be treated before disposal; high capital and running costs; susceptible to fouling and pre-treatment and post treatment may be needed; RO is preferable to NF due to higher removal efficiencies
Advanced Oxidation Process (AOP)	UV/H_2O_2 $UV/S_2O_8^{2-}$	PFOS 10 – 50% PFOA <10%	Surface water, Groundwater	Can oxidise numerous contaminants to degradation products using reactive hydroxyl radicals	Less effective than other methods; significant energy input needed to achieve moderate removal; may oxidise polyfluorinated precursor chemicals present in the raw water, which could result in an increased concentration of PFOS and PFOA in the finished water

PWSs – private water supplies; POU – point of use; POE – point of entry

9. CONCLUSIONS

9.1. Considerations in establishing health-based values

Due to the potential adverse health effects reported in both humans and animals following higher level exposure to PFOS and/or PFOA, a WHO GV for drinking water is warranted. However, following a review of the available data presented and discussed in previous sections, WHO considered that the uncertainties in identifying the key endpoint applicable to human health following exposure to PFOS and/or PFOA are too significant to derive a HBGV with confidence. Although the reduced antibody response following vaccination has been considered by some agencies 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 as discussed earlier, 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 HBGV is evident from the diverse range of endpoints utilised by other agencies to derive tolerable daily intakes or similar values, and the resulting range in proposed drinking-water values described in Table A.1 (see appendix). Although the values derived by several different organizations vary significantly, all have margins of safety. Data analysis also shows that science on PFAS is evolving very rapidly in various areas.

Health-based drinking water values derived by authoritative agencies range from 0.05 to 0.6 µg/L for PFOS and from 0.05 to 0.56 µg/L for PFOA.[8] By applying WHO default parameters for body weight (60 kg), daily drinking-water intake (2L) and allocation factor (20%) to the corresponding TDIs or equivalent to drinking-water, these are equivalent to health-based values ranging from 0.1 to 0.4 µg/L for PFOS and 0.1 to 1 µg/L for PFOA. In addition, general health-based values have been derived by ATSDR (2021) and EFSA (2020).

The ATSDR (2021) proposed intermediate-duration minimal risk levels of 2 ng/kg bw/day and 3 ng/kg bw/day for PFOS and PFOA, respectively, based on delayed eye opening and decreased pup body weight in a rat developmental study for PFOS, and based on increased incidence of skeletal alterations in pups in a developmental study for PFOA. By applying WHO default parameters, these values can be converted to drinking-water values of 0.012 µg/L and 0.018 µg/L for PFOS and PFOA (respectively) for an adult, and 0.003 µg/L and 0.004 µg/L for PFOS and PFOA (respectively) for a child.

Multiple U.S. states have their own health-based PFAS guideline levels, including PFOS and PFOA. According to Post (2021), 11 states have issued health-based regulations or guideline levels for PFOA and/or PFOS as of May 2020. The range is 10 to 70 ng/L for PFOA, and 8 to 70 ng/L for PFOS, compared to the US EPA's health advisory of 70 ng/L for combined

[8] Excluding draft US EPA (2021a; 2021b) interim updated health advisories for PFOS and PFOA released on June 15 2022 (see Appendix)

PFOA/PFOS (US EPA, 2016a; 2016b)[9]. In addition, guideline levels ranging from 400 – 560 ng/L (PFOS) and 130 – 1000 ng/L (PFOA) were identified in four other states; however, these states now follow the US EPA's health advisories for PFOS and PFOA (Cordner et al., 2019). According to Cordner et al. (2019), diverse risk assessment approaches were used to derive the HBGVs with key variations not only in the choice of toxicity endpoint and use of uncertainty factors (in particular AK_{UF} used to account for interspecies kinetic differences), but also in the selection of drinking water consumption levels and relative source contribution.

9.2. Derivation of the provisional guideline values

Acknowledging the significant uncertainties and absence of consensus with identifying the critical health endpoint to calculate a HBGV and the rapidly evolving science, a **pragmatic solution is therefore proposed for the derivation of provisional guideline values (pGVs).**

The pGVs are derived with the objective of reducing human exposure and therefore risk. In deriving the pGVs, global data on occurrence including co-occurrence of PFAS, available analytical methods and treatment achievability were considered.

Individual pGVs of 0.1 µg/L for PFOS and PFOA are proposed based on the following considerations:

- These values correspond to greater than 90% removal achievability with high pressure membrane filtration (NF and RO), activated carbon adsorption or ion-exchange (section 9.4), considering that upper-bound concentrations detected in drinking-water sources have mostly been in the low µg/L range (section 3.1)
- These individual pGVs for PFOS and PFOA should therefore be achievable, where these technologies are available and have been optimised for PFAS removal**.**
- Although these pGVs were not derived based on adverse health effects studies, the values fall within the range of most health-based values derived through national risk assessments (see appendix).

In addition, a combined pGV of 0.5 µg/L is proposed for total PFAS based on the following considerations:

- Approximately 30 members of the PFAS family, including PFOS and PFOA are measurable by currently available methods.
- PFOS and PFOA are likely to co-occur together with other PFAS (i.e. as a mixture) in the environment, and the PFAS studied to date demonstrate high persistence, accumulation potential and/or hazards to the environment and/or human health. Therefore managing PFAS as a class can be an effective means of reducing exposure to these substances (Kwiatkowski et al., 2020; Cousins et al., 2020a, 2020b).
- As described in section 9.4, available data, although limited, indicates that 0.5 µg/L for total PFAS should be achievable. Most studies undertaken on real drinking water systems often have low concentrations of PFAS mixtures in the inlet, often well below 0.5 µg/L (although this is dependent on the number of species measured). When high pressure membrane processes (NF and RO) or GAC operating under optimised conditions are exposed to higher total PFAS concentrations (within the range typically

[9] As noted, on June 15, 2022, US EPA released interim updated health advisories for PFOA and PFOS (see Appendix).

observed in the environment), they would be expected to reduce total PFAS concentrations to below 0.5 µg/L.

- Given the unique challenges related to PFAS and summarized in this section, water suppliers should make every effort to achieving overall levels as low as reasonably practical.

It must be noted that from a practical perspective, the recommendations are limited by available analytical methods and treatment capabilities, although methods continue to be refined and detection and quantification limits continue to fall while data are still emerging. However, monitoring and removing PFAS in drinking-water can be costly and complex as described in section 8 and may be unfeasible to implement in many low- and middle-income settings.
The pGV for each of PFOS and PFOA should not be exceeded when calculating the combined pGV.

9.3. Considerations in applying the provisional guideline values

Application of WHO GVs, including parameters selected and associated limits, should consider local circumstances, including practical achievability and affordability (WHO, 2019). The pGVs for PFOS, PFOA and total PFAS are intended to provide a marker for further investigation for a broad range of countries and water suppliers, particularly where resources are limited.

Where it is not feasible to put in place treatment technologies that can effectively remove these chemicals in low- and middle-income areas, a staged approach may be needed to achieve the pGVs, starting with higher individual PFAS limits or interim values for PFOS and PFOA (e.g. 0.4 µg/L, which is 4x the WHO pGVs and corresponds with the upper range of most health-based values derived for PFOS through national risk assessments) and progressively reducing to the WHO pGVs as available resources allow. This approach encourages incremental improvement and is consistent with WHO recommendations under the Framework for Safe Drinking-water. In such settings, switching the source or blending of source waters may be the only feasible option (see section 9.3 for considerations), with conventional treatment ineffective at removing these chemicals. However, the pGVs should not be considered as licenses to allow contamination and Member States should strive to achieve concentrations that are as low as reasonably practical, even when lower than the pGVs stated above. This includes preventing further contamination of water sources from existing sources of contamination wherever possible and preventing new sources of contamination. This should be done in conjunction with stopping non-essential uses of PFAS.

In addition, to PFOS and PFOA, other PFAS should be monitored and managed, focusing on those chemicals that are expected to be the most relevant to a country context based on an understanding of the potential sources of contamination (as part of the hazard identification phase of water safety planning) and investigative monitoring. However, the pGV for total PFAS recognises that there are reliable analytical methods to measure up to or around 30 PFAS in drinking-water, although these methods may be unavailable in resource-constrained settings (see section 9.2).

Due to the persistence of PFAS and concerns on their environmental and human health impacts, as knowledge and capacities increase, along with development of more cost-effective methods for analysing and controlling PFAS, national standards should be adjusted.

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APPENDIX

Table A.1: Health-based values (HBVs) for PFOS and PFOA in drinking water (current as of 2021)

Authoritative Assessment	PFOS HBV (µg/L)	PFOS WHO Eq GV (µg/L)[a]	Comments	PFOA HBV (µg/L)	PFOA WHO Eq GV (µg/L)[a]	Comments	Combined GV / Comment
Health Canada (2018)	0.6[b]	0.4	Key study: Butenhoff et al. (2012) Critical effect: hepatocellular hypertrophy in rats. $NOAEL_{HED}$: 0.0015 mg/kg bw per day UF: 25	0.2[b]	0.2	Key study: Perkins et al, (2004) Critical effect: hepatocellular hypertrophy in rats. $NOAEL_{HED}$: 0.0006 mg/kg bw per day UF: 25	The sum of the ratios of the detected concentrations to the corresponding maximum acceptable concentration for PFOS and PFOA should not exceed 1.
US EPA (2016a,b)	0.07[c]	0.1	Key study: Luebker et al. (2005) Critical effect: decreased pup body weight in rats. $NOAEL_{HED}$: 0.0005 mg/kg bw per day UF: 30	0.07[c]	0.1	Key study: Lau et al. (2006) Critical effect: developmental effects in rats (reduced ossification; accelerated puberty in males). $LOAEL_{HED}$: 0.005 mg/kg bw per day UF: 300	0.07 µg/L for PFOS and $PFHxS^{5}$ combined

Authoritative Assessment	**PFOS HBV (µg/L)**	**PFOS WHO Eq GV (µg/L)[a]**	**Comments**	**PFOA HBV (µg/L)**	**PFOA WHO Eq GV (µg/L)[a]**	**Comments**	**Combined GV / Comment**
US EPA (2021a,b)[j]	2.0×10^{-5}	6.0×10^{-5}	Key study: Grandjean et al., 2012; Budtz-Jorgensen et al., 2018 Critical effect: suppression of the response to the diphtheria vaccine in children. $BMDL_{5(HED)}$: 1.05×10^{-7} mg/kg bw per day UF: 10	4.0×10^{-6}	9.0×10^{-6}	Key study: Grandjean et al., 2012; Budtz-Jorgensen et al., 2018 Critical effect: suppression of the response to the tetanus vaccine in children $BMDL_{5(HED)}$: 1.49×10^{-8} mg/kg bw per day UF: 10	Not stated
FSANZ (2017)	0.07[d]	0.1	Same as US EPA (2016a)	0.7[d]	1	UF: 30; all other parameters same as US EPA (2016b)	0.07 µg/L for PFOS and PFHxS[10] combined
The German Drinking Water Commission (2016)	0.1[d]	0.2	HBV derived from a POD of 20 ng/ml (serum concentration) based on epidemiological studies with consideration of animal studies. Equivalent oral dose calculated from POD multiplied by clearance (0.00075 L/kg bw/day) and divided by half-life correction factor of 0.527.	0.1[d]	0.2	POD: 90 ng/ml (serum concentration) based on inhibition of antibody response in humans. Equivalent oral dose calculated from POD multiplied by clearance (0.0001 L/kg bw/day) and divided by half-life correction factor of 0.527.	Not stated

[10] perfluorohexane sulfonate is a member of the perfluoroalkyl family of chemicals and commonly occurs with PFOS in the environment.

Authoritative Assessment	PFOS HBV (µg/L)	PFOS WHO Eq GV (µg/L)[a]	Comments	PFOA HBV (µg/L)	PFOA WHO Eq GV (µg/L)[a]	Comments	Combined GV / Comment
UK DWI (2021)[e]	Not derived	0.1	Whilst an HBV was not derived a series of trigger or guideline values were proposed. A value of 0.1 µg/L was judged to be broadly in line with other national evaluations and if exceeded action is required to reduce concentrations.	Not derived	0.1	See comments for PFOS.	Precautionary approach recommended, but no quantitative criteria.
Danish Ministry of the Environment (2015)	0.1[f]	0.2	Key study: Thomford et al. (2002) Critical effect: Liver hypertrophy in rats. $BMDL_{10}$: 0.033 mg/kg bw per day UF: 1230	0.3[f]	0.6	Key study: Palazzolo et al. (1993) Critical effect: Increased absolute and relative liver weight in rats. $BMDL_{10(HED)}$: 0.003 mg/kg bw per day UF: 30	PFOA (conc. µg/L) / 0.3 µg/L + PFOS (conc. µg/L) / 0.1 µg/L + PFOSA (conc. µg/L) / 0.1 µg/L < 1
Swedish National Food Agency (2014)	0.09[g]	0.2[h]	Key study: Seacat et al., 2002 Critical effect: Increased TSH, reduced T_3 and reduced HDL in Cynomolgus monkeys NOAEL: 0.03 mg/kg bw per day UF: 200	-	-	-	0.09 µg/L for total PFAS (PFBS, PFHxS, PFOS, 6:2 FTS and PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA)
Ministry of Health, Labour and Welfare, Japan (2020)	0.05[i]	0.1	Same as US EPA (2016a)	0.05[i]	0.1	Same as US EPA (2016b)	0.05 µg/L (provisional) (Based on the US EPA decision to compare the summed detections of PFOA and PFOS to the health advisory values)

AF = Allocation factor; $BMDL_{10}$ = lower confidence bound on the benchmark dose with a 10% benchmark response; GV = guideline value; HED = human equivalent value; HBV = health based value; LOAEL = lowest observed adverse effect level; NOAEL = no observed adverse effect level; POD = point of departure; UF = uncertainty factor

[a]WHO equivalent values based on HBV, human body weight of 60 kg, adult drinking water intake of 2 L/day, and allocation factor of 20%, unless otherwise stated.
[b]HC (2018) HBVs calculated based on human body weight of 70 kg, adult drinking water intake of 1.5 L/day, and allocation factor of 20%.
[c]US EPA (2016a,b) HBVs calculated based on drinking water intake rate for lactating women (0.054 L/kg bw/day) and allocation factor of 20%.
[d]FSANZ (2017) and German Drinking Water Commission (2016) HBVs calculated based on human body weight of 70 kg, adult drinking water intake of 2 L/day, and allocation factor of 10%.
[e]UK value was established based on consideration of reviews from EFSA, 2008; COT, 2009; US EPA, 2016; FSANZ, 2017; EFSA 2018; HC, 2018; COT, 2020; WHO (undated).
[f]Danish Ministry of the Environment (2015) HBVs based on drinking water intake rate of 0.03 L/kg bw/day and allocation factor of 10%
[g]Swedish National Food Agency (2014) HBVs based on infant body weight of 4.2 kg, drinking water intake rate for infants (0.7 L/day), and allocation factor of 10%.
[h]Based on bottle-fed infant body weight of 5 kg, bottle-fed infant drinking water intake rate of 0.75 L/day, and allocation factor of 20%.
[i]Ministry of Health, Labour and Welfare, Japan (2020) based on body weight of 50 kg, drinking water intake rate of 2 L/day, and allocation factor of 10%.
[j]In 2022, the US EPA (2021a,b) released **updated, interim (draft)** health-based values **of 0.02 ng/L for PFOS and 0.004 ng/L for PFOA** based on suppression of the response to the diphtheria vaccine (PFOS) and to the tetanus vaccine (PFOA) in children (Grandjean et al., 2012; Budtz-Jorgensen et al., 2018). These values are based on $BMDL_{5(HED)}$ values of 1.05×10^{-7} and 1.49×10^{-8} for PFOS and PFOA (respectively), an uncertainty factor of 10, a drinking water intake level of 0.0701 L/kg-day (for children < 5 years of age), and an allocation factor of 20%.

In addition to the health-based values derived from the drinking water risk assessments listed above, WHO equivalent health-based values of **0.012 µg/L and 0.018 µg/L** (for PFOS and PFOA, respectively) for adult exposure may be derived from the ATSDR minimal risk values for oral, intermediate exposure. In addition, a WHO equivalent health-based value of **0.004 µg/L** (adult exposure) for the sum of PFOA, PFNA, PFHxS and PFOS may be derived from the EFSA (2020) tolerable weekly intake. The Netherlands National Institute for Public Health and the Environment (RIVM, 2021) also established a guideline value of 0.004 µg/L (adult exposure) for the sum of PFOA, PFNA, PFHxS and PFOS based on the EFSA (2020) health-based value. In addition, according to RIVM (2021), the PFOS, PFNA and PFHxS detections are to be multiplied by relative potency factors of 2, 10 and 0.6, respectively.