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Review

What are the effects of PFAS exposure at environmentally relevant concentrations?

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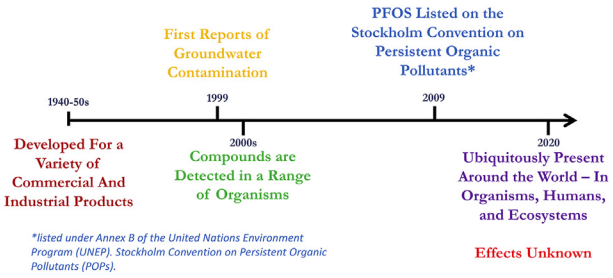
HIGHLIGHTS

- Data on the effects of PFAS at environmentally relevant concentrations is limited.
- Toxicity occurs at higher concentrations than PFAS occur in the environment.
- Hazard Quotient Analysis generally gives values of <1 for PFOA and PFOS.
- Unknown PFAS, their degradation products and precursors may be higher risk.
- Molecular markers of sublethal PFAS exposure are needed for risk assessment.

GRAPHICAL ABSTRACT



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ABSTRACT

The group of synthetic chemicals known as poly and per-fluoroalkyl substances (PFAS) are currently of high concern to environmental regulators and the public due to their widespread occurrence, resistance to degradation and reported toxicity. However, little data exists on the effects of exposure to PFAS at environmentally relevant concentrations and this hampers the effective management of these compounds. This paper reviews current research on the occurrence and ecotoxicology of PFAS at environmentally relevant doses to assess their potential biological impacts. Hazard Quotient (HQ) analysis was undertaken as part of this assessment. Most PFAS detected in the environment were found to have a HQ risk value of <1 meaning their reported concentrations are below their predicted no effect concentration. This indicates many reported toxic effects of PFAS are, theoretically, unlikely to occur outside the laboratory. However, lack of information on new PFAS as well as their precursors and degradation products, coupled with lack of knowledge of their mixture toxicity means our understanding of the risks of PFAS is incomplete, especially in regard to sub-lethal and/or chronic effects. It is proposed that the development of molecular markers for PFAS exposure are needed to aid in the development of environmental PFAS regulations that are effective in fully protecting the environment.

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1. Introduction

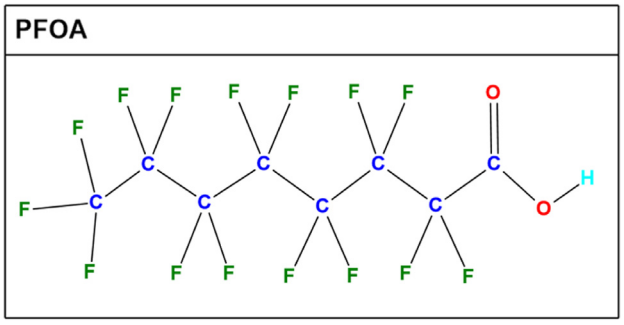
Poly and perfluoroalkyl substances (PFAS) are a group of synthetic organofluorine compounds that have been used since the 1940s for a wide variety of industrial and commercial applications (Ding and Peijnenburg 2013; Ateia et al., 2019). This includes their original use in Teflon® and Scotchgard™ as well as more modern uses such as food packaging, cosmetics, waterproof textiles and aqueous film forming foams (AFFF) used to suppress flammable liquid fires (Pelch et al., 2019).

The term PFAS is a deceptively short term for a large and diverse group of over 4700 compounds whose common feature is multiple fluorine atoms attached to alkyl chains of varying length. Shorter chain compounds tend to be more persistent but longer chain compounds are still very stable and when they do break down they often degrade into the shorter chained (and more persistent) PFAS (Coggan et al., 2019a,Coggan et al., 2019b).

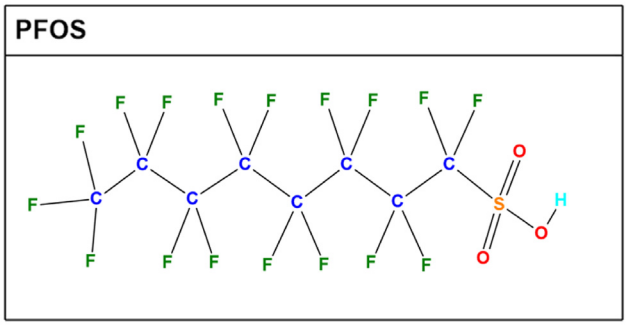
PFAS were originally thought to be inert and non-toxic and little regard was given to their environmental fate or potential human and ecological health impacts. In recent years however, PFAS have become a serious global health concern due to their ubiquitous presence in the environment, high stability and increasing reports of toxicity in both humans and animals. Of the thousands of compounds that make up PFAS, perfluorooctanoic acid (PFOA) and perfluoro-octane sulfonic acid (PFOS) (Fig. 1.a and b) are the most common and so have been the most well documented and studied worldwide. These compounds are therefore the focus of this review but related compounds are discussed where relevant.

PFOS (and its salts) and perfluorooctane sulfonyl fluoride (PFOSF) were added to Annex B of the Stockholm Convention in 2009 (HEPA, 2018) and both PFOS and PFOA are banned or are being phased out in many countries. This has led to increased use of related compounds such as the perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs), and fluorotelomer alcohols (FTOHs). These compounds are now also receiving widespread attention for their potential (eco)toxicological effects (Ruan and Jiang 2017). There is also significant concern that new PFAS are in use by industry that have yet to be detected in the environment or fully assessed for environmental effects (Pelch et al., 2019).

A significant cause of the public and regulatory apprehension over PFAS is due to the fact that they are remarkably persistent - with a reported half-life of >92 years in water (US EPA, 2014) due to the C–F bond being one of the strongest in organic chemistry. For



a.



b.

Fig. 1. a. Chemical structure of Perfluorooctanoate (PFOA; C7 fluorocarbon), and b. Perfluorooctane sulfonate (PFOS; C8 fluorocarbon).

this reason, they are often referred to as “Forever Chemicals”. PFAS are considered highly mobile and move thorugh the environment via landfill leachate, groundwater, runoff, streams and oceans, and through dust particulates. They can bioaccumulate within organisms and humans across the food web (Trojanowicz and Koc 2013) and have been found in almost every environmental compartment.

Concerns over PFAS toxicity have led to strict regulation but the lack of detailed information on their ecotoxicity have led to differing guidelines being implemented in different countries. For example the current PFAS environmental quality limits (EQLs) for water in Australia are essentially the limit of detection in the environment (in the pg/L range with a 500 mL sample). These levels are at least 1000 times lower than limits such as the United States Environmental Protection Agency (US EPA) guidelines for PFOA and PFOS (US EPA, 2016).

Industry and government alike face potentially high financial liabilities from PFAS, whether it be to dispose of PFAS contaminated waste; being fined for not following the regulatory limits; or for remediation of an environment that has been contaminated (Environmental Protection Authority Victoria 2018). In some cases the actual polluter is not clear. For example, water utilities are responsible for what their wastewater treatment plants (WWTPs) discharge to the environment but PFAS in wastewater is likely to have come from industrial use in the local catchment not the water utility which ultimately discharges it to the environment.

There has been an increase in detection of PFAS but despite the strict regulations and potentially high financial penalties for its release to the environment, there is little detailed information on the concentration of PFAS that cause harm in an ecosystem and no consistent evidence of adverse health effects in animals or humans at environmentally relevant exposure levels. There is also a lack of information on the toxicity of PFAS at low doses over time (chronic exposure) compared to high doses of a single compound (acute toxicity) which is more common in toxicity tests. There is therefore a significant need for more information on PFAS toxicity (Grandjean 2018; Pelch et al., 2019). The current review focuses on the effects of PFAS in aquatic ecosystems to help understand the mechanisms of PFAS toxicity and the doses at which this toxicity occurs. The review will also discuss the possible linked effects to human health, identify knowledge gaps of PFAS risk evaluation and highlight key future research areas.

2. Occurance of PFAS

PFAS have been in use since the 1940s but only started to draw large scale environmental attention in the early 2000s. They have since been detected in water, land, food and even human blood samples. Indeed wherever an analytical chemist cares to look one can find PFAS (CRC CARE, 2016). In part this increased interest has been due to advances in analytical instrumentation, particularly Liquid Chromatography-Mass Spectrometry (LC-MS), which has allowed the development of methods for the detection of trace amounts (nanogram per litre and lower) of perfluorinated compounds in matrices as diverse as tissue samples, drinking water, biosolids and food packaging (Lau et al., 2007). However, detecting a compound tells us nothing about its toxicity and while high levels of exposire have been implicated in a variety of health effects, including cancer, there are very limited data on what the effect of PFAS may be at environmentally relevant concentrations and exposure routes. There is also currently a poor understanding of the mechanism/s by which PFAS could have adverse health effects.

Table 1
Summary of measured PFOS and PFOA concentrations in drinking water[a] (Thompson et al., 2011a,b).

Location	PFOS (ng/L)	PFOA (ng/L)	Original Source
Australia	0–16	0–9.7	Thompson et al. (2011)
Germany	<10	<10 - 68	Wilhelm et al. (2010)
Brazil	0.58–6.7	0.81–2.8	Quinete et al. (2009)
China	<0.1–14.8	<0.1–45.9	Jin et al. (2009)
Japan	<0.1–6.9	2.3–84	Takagi et al. (2008)
India	<0.03–8.4	<0.005–2	Yim et al. (2009)
USA	<1 - 57	<5 - 30	Quiñones and Snyder (2009)

[a] A range of concentrations were measured from a number of drinking water facilities across each country.

2.1. Drinking water

Drinking waters have been routinely monitored for PFAS around the world and data on this is summarised in Table 1. These concentrations can provide an indication of the rate of exposure to PFAS across countries and have led to the development of drinking water guidelines. It can be seen that, in general, PFAS concentrations in drinking water are at the ng/L level or below. However the presence of these compounds in drinking water means that people are potentially being exposed to this substance for their entire lives; we do not know what the effects of this might be.

2.2. Groundwater

One of the main uses of PFAS is aqueous film forming foams (AFFF) used to put out fires. Firefighter-training areas and military air force bases and domestic airports routinely use AFFFs containing PFAS which can then leach into surrounding groundwater. Indeed the presence of PFAS in groundwater was first highlighted over 20 years ago in a paper by Moody and Field (1999) that in essence has led to modern interest in the area. AFFF groundwater contamination is also major source of drinking water contamination if groundwater is used for potable supplies. This has been identified as a nationally significant challenge in countries such as the United States and Sweden (Sunderland et al., 2019).

PFAS may also enter groundwater via landfill leachate. A recent Australian study showed that PFAS concentrations varied from 26 to up to 5,200 ng/L near a potential industrial point-source and that PFAS show conservative behaviour (low sorption and reactivity) during subsurface transport (Hepburn et al., 2019). Compounds present in groundwater can also leach into other waterbodies such as lakes and rivers and affect the organisms living in those areas. Unfortunately studies of PFAS in groundwater are lacking which limits our understanding of the fate, transport and physicochemical properties of these substances in this compartment (Kucharzyk et al., 2017).

2.3. Freshwater

Freshwater systems provide a range of ecosystem services as well as move water, energy, nutrients, organisms and sediment between different compartments (thereby linking groundwater, marine, and terrestrial systems). PFAS have been detected in many freshwater sites, both from a direct point source such as firefighting-training areas, or indirectly from contaminated groundwater sources. A starting point for information on PFAS in global surface waters could be the Stockholm Convention global monitoring reports conducted by parties to the Stockholm convention. This information could be used to understand where these compounds are detected but also their concentrations and longevity in surface waters and to identify trends. For example, the most often detected compounds (PFOS and PFOA) have been measured in surface water samples at concentrations of up to hundreds of nanograms per litre (Zhou et al., 2017). In contrast

concentrations in biota and sediments range from pg/g to a few ng/g (Arvaniti and Stasinakis 2015).

A further issue is that there is a clear lack of baseline environmental levels of PFAS in freshwater systems that is not associated with wastewater treatment plants, landfills or other one-off events of PFAS contamination. This is due to the fact that PFAS are persistent and can theoretically be linked back to many possible commercial and industrial practices coupled with the fact that is not well understood how they move or degrade within a system, which adds difficulty to mapping sources. It is also difficult to accurately and efficiently identify new point sources if there is little baseline measurement for comparison.

2.4. Marine water

PFAS has been detected in marine water and biota for several years. Maximum concentrations of up to 58 ng/L have been measured in onshore waters but the concentration declines to levels of 0.11 ng/L further offshore in common with most pollutants (Mhadhbi et al., 2012). PFAS has also been detected in marine mammals e.g. in the plasma of the West Indian manatee *(Trichechus manatus)* where PFOS was measured at 166 ng/L (Palmer et al., 2019) and the southernmost marine mammal, the Weddell seal *(Leptonychotes weddellii)* where level of PFOS 0.06 ng/L were found (Routti et al., 2015). Houde et al. (2006), reviewed biological monitoring programs and found (by using archived as well as recently collected samples) that concentrations of PFAS have increased in marine wildlife since the 1990s. Unfortunately, as previously mentioned, reporting a concentration does not provide information as to how toxic it is. Although there are many reports highlighting the detection of PFAS concentrations in a variety of marine wildlife, there is little information on the effects of PFAS on marine organisms at these concentrations.

2.5. Soil and vegetation

Contaminated surface or ground waters used for field irrigation and the use of biosolids as fertilizer in agriculture are the main sources of PFAS contamination in soils (Stahl et al., 2009; Wen et al., 2015; Ghisi et al., 2019). It is difficult to identify the sources of PFAS contamination in agricultural soils however, due to the vast range of applications that contain and/or use these substances. For example, soil close to airports and fire-training locations are likely to have higher concentrations of PFAS than sites further away. Moreover, understanding the extent to which PFAS is absorbed by plants is difficult as accumulation can be affected by concentration, chain length, functional group, plant species and variety and soil type/characteristics (Ghisi et al., 2019). Once PFAS have been taken up by the plants, shorter chained compounds can accumulate in the leaves and fruits and longer chained compounds accumulate within the roots (Krippner et al., 2015). The interactions of PFAS in soil and vegetation needs further investigation in order to explain the role of each factor influencing presence, uptake, distribution and accumulation of these compounds (Ghisi et al., 2019).

2.6. Animals and humans

There is increasing reports of substrates and animals containing trace amounts of PFAS, ranging from macro-invertebrates (Ji et al., 2008) to human blood serum and breast milk (So et al., 2006). For example, Moody et al. (2002) detected PFOS in the range of 2.0–72.9 ng/L in fish liver samples following an accidental release of fire-fighting foam into a creek in Etobicoke, Canada, and many other reports of PFAS concentrations at ng/L levels in wildlife are available, identifying the global spread of PFAS exposure (Giesy and Kannan, 2001; Ding and Peijnenburg, 2013; Hamid et al., 2018; Nakayama et al., 2019).

PFAS in humans can occur through eating and drinking contaminated food and water or via job related exposure. Firefighters, who had been exposed through training with firefighting foam, tend to have the highest concentrations of PFAS in their blood. The greatest PFOS and PFOA concentrations in humans were reported at 164 ng/mL from Kentucky, U.S.A and 256 ng/mL from Korea (So et al., 2006). Trace amounts of PFAS have also been reported in human breast milk; PFOS has been reported at 360 ng/L and PFOA at 210 ng/L (So et al., 2006). Sznajder-Katarzy'nska et al. (2019) published a detailed review of possible toxicology of PFAS following a recent report from the US Centres for Disease Control that showed that nearly all Americans had PFAS in their blood (Dong et al., 2017).

2.7. Guideline limits of PFAS

Since PFAS are ubiquitous and resistant to degradation there is justifiable apprehension over their potential health impacts. The detection of PFAS in humans has raised international concern and led to the creation of global restrictions on the use and management of PFAS. Many countries have introduced regulations and/or guidelines to phaseout or limit the use of PFOS, PFOA, and many other PFAS (McCarthy et al., 2017). There is however, limited research about the potential ecological harm these compounds are having especially at the concentrations routinely detected in the environment (So et al., 2006).

Interestingly, the strict guidelines have done little to reduce the amount of PFAS that is being detected. For example, Dong et al. (2017) note that approximately six million citizens of the United States of America are exposed to drinking water exceeding the US EPA PFOS and PFOA guidelines. Similarly, Australia has set PFAS guidelines at the limits of detection in drinking water meaning any samples containing detectable concentrations of PFAS have theoretically breached the guideline limits. A national survey of PFAS concentrations in drinking water in Australia showed PFOS and PFOA concentrations ranged between 0 and 15.6 ng/L and 0–9.6 ng/L, respectively (Table 1) (Thompson et al., 2011).

The Heads of the Environmental Protection Agencies for Australia and New Zealand (HEPA) have set PFAS guideline values with a large margin of protection in the aquatic (0.00023 μg/kg for PFOS; 19 μg/kg for PFOA), and terrestrial (1000 μg/kg for PFOS; 10,000 μg/kg for PFOA) environments. These limits are based on the lowest concentrations using the precautionary principle. The precautionary principle (or precautionary approach) is a strategy for approaching issues that may have environmentally harmful consequences. It states that when extensive scientific knowledge on the matter is lacking, it is better to control that activity now with policies than to wait for potential harm. However, the presence of a substance does not mean it is toxic at that concentration, and there is little to no consistent evidence that supports adverse effects of PFAS at the concentrations detected in ecosystems. This does not mean such effects do not exist of course, just that they have not been observed.

3. Toxic effects

There has been an increase in ecological toxicity studies as researchers try to understand the effects of PFAS on different ecosystems. PFOS and PFOA are the often the predominant PFAS found in the environment so are the most widely studied (Gomis, 2017). A number of challenges remain to determine the toxicity of PFAS however, not least the sheer diversity of compounds that might be found, and the differences between their modes of action in a range

Table 2
Summary of Freshwater PFAS (mg/L) toxicity data in invertebrates, fish and amphibians.

Species	PFOS	PFOA	Length of exposure	Effect	Source
Harlequin fly*(Chironumus riparius)*	0.01	0.01	Multi Gen.[a]	NOAEL	Marziali et al. (2019)
Harlequin fly (Chironumus tentans)	0.02	100	20 day	NOAEL	MacDonald et al. (2004)
Cladoceran (*Daphnia magna)*	25.00	500	24 h	NOAEL	Ji et al. (2008)
Cladoceran*(Moina macrocopa)*	17.95	199.51	48 h	LC50	Ji et al. (2008)
Zebra fish *(Danio rerio)*	0.0006	-	Multi Gen.[a]	LOAEL	Keiter et al. (2012)
Zebra fish *(Danio rerio)*	0.25	-		LOAEL	Wang et al. (2011)
Zebra fish *(Danio rerio)*	22.20	-	96 h	LC50	Sharpe et al. (2010)
Rainbow trout (Oncorhynchus mykiss)	2.50	-	96 h	LC50	Sharpe et al. (2010)
Rainbow trout - Juvenile (Oncorhynchus mykiss)	3.00	-		LOAEL	Oakes et al. (2005)
Rainbow trout (Oncorhynchus mykiss)	12.50	125	24 h	NOAEL	Ji et al. (2008)
Rainbow trout (Oncorhynchus mykiss)	0.50	-		LOAEL	Sharpe et al. (2010)
Bullfrog larvae (*Rana catesbeiana*)	99.00	1038	96 h	LC50	Flynn et al. (2019)
Northern leopard frog (*Rana pipiens)*	3.00	-	2 weeks	LOAEC	Ankley et al. (2004)

LC50, concentration that caused mortality in 50% of the sampled population; NOAEL, No Observed Adverse Effect Level in experimental study; LOAEL, Lowest Observed Adverse Effect Level in experiment.
[a] Multigenerational experiment.

of organisms. The fate and behaviour of PFAS in differing environments and conditions is mostly unknown (Dong et al., 2017) as are the effects of complex mixtures of compounds occurring at low doses but with continuing lifelong exposure. These are areas where substantial further research is needed.

The uptake rates and species transfer of PFAS within the environment is not well understood though it is a growing area of interest. For example, McCarthy et al. (2017) highlight the fact that measuring uptake of PFOS through the food web may not be as important as initially assumed - contradicting Ahrens and Bundschuh (2014). PFOS has been observed to accumulate in higher concentrations than PFOA in aquatic organisms, while the converse is true in plants (McCarthy et al., 2017) therefore determining the toxicity and uptake of these compounds differs between flora and fauna. Increased data on PFAS toxicity and uptake would strengthen our understanding of how PFAS compounds move through a food web. Currently tinformation on this topic is too limited to form firm conclusions.

3.1. Aquatic ecosystems

Where levels of PFOA and PFOS can be directly compared within an individual, PFOS has a higher toxicity than PFOA in fresh water organisms (Ji et al., 2008; Li 2009). For example, using the water flea, *Moina macrocopa* to determine the lethal concentration of PFOA and PFOS, Ji et al. (2008) showed PFOS to be approximately ten times more toxic than PFOA. As shown in Table 2 the LC50 (50% of the lethal concentration) in this study was 17.95 mg/L for PFOS and 199.51 mg/L for PFOA.

A recent study by Marziali et al. (2019) addressed the unknown long term effects of PFAS using a multigenerational test with the non-biting midge, *Chironomus riparius*. The organisms were exposed to perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorobutane sulfonate (PFBS). Larvae were exposed for 10 generations to 10 μg/L nominal concentrations of PFOS, PFOA, and PFBS which are comparable with the maximum levels of these compounds reported in European rivers. All treatments showed reduced growth but no effects on survival, development, and reproduction were observed. Since no effects at the population level (population growth rate) were observed the authors felt the toxicity risk was low in real ecosystems at the concentrations tested (Marziali et al., 2019) (Table 2). Similar results were found in a 20-day life cycle experiment in *Chironomus tentans* following exposure to PFOS (MacDonald et al., 2004). Here a decrease in emergence was observed at a PFOS concentration of less than 2.3 μg/L, with the organisms relatively unresponsive to PFOA.

A short term 48 and 96 hour (h) acute study by Boudreau et al. (2003) exposed a number of aquatic flora and invertebrates to PFOS under laboratory conditions and concluded PFOS is highly toxic to freshwater systems at 100 mg/L. The authors clearly stated that at the known environmental concentrations of PFAS range from ng/L to μg/L, there is seemingly no adverse risk of PFOS to freshwater ecosystems (Boudreau et al., 2003).

Ankley et al. (2004) reported significant mortality to the frog species, *Rana pipiens* at PFOS concentrations of 10 mg/L and increased time to metamorphosis and decreased length of tadpoles at concentrations greater than 3 mg/L. However no effect was seen at concentrations of <3 mg/L (Ankley et al., 2004). Conversely a recent study on the American bullfrog *(Rana catesbeiana)* larvae determined a 96 h LC50 for PFOS to be 99 mg/L and PFOA of 1038 mg/L (Table 2) (Flynn et al., 2019). These levels are far above background PFAS concentrations in most systems.

Interestingly, fish tend to show developmental effects at much lower concentrations than frogs. The difference between fish and frog sensitivities may be due to the ability for frogs to move from water to land, avoiding contamination or because they are more tolerant to contaminants in their early life stages compared to freshwater fish. Decreased larval survival and embryo development was observed when fish embryos and larvae were exposed to PFAS (Oakes et al., 2005; Wang et al., 2011; Keiter et al., 2012) (Table 2). Moreover, Wang et al. (2011) found reduced body size and altered sex ratio in adult zebrafish after a five month chronic PFAS exposure to 50 μg/L. Additionally, Sharpe et al. (2010) reported the 96 h LC50 value of PFOS in adult zebrafish and trout to be 22.2 and 2.5 mg/L, respectively. Therefore, while these studies all indicate possible adverse effects on the reproduction of fish due to PFAS exposure, they all used exposure concentrations far higher than generally found in the environment.

PFAS have been detected in various compartments of marine ecosystems, however, again, the effect of exposure is mostly unknown. An all-inclusive study using early life stages of fauna from multiple trophic levels, such as primary producers, primary consumers, and secondary consumers, to obtain EC50 for PFOS and PFOA was conducted by Mhadhbi et al. (2012) (Table 3). Interestingly, the authors found the responses to PFAS occurred at much higher concentrations than in freshwater organisms (Mhadhbi et al., 2012). Latała et al. (2009) reported PFOA 72-h EC50s for growth inhibition for four different Baltic algae species ranging from 41.6 to 977 mg/L (Table 3). However, the mussel, *Mytilus galloprocincialis* larvae showed sensitivity when exposed to a range of PFOS and PFOA concentrations between 0.01, 0.1, 1, 10, 100,

Table 3
Summary of Marine PFAS (mg/L) toxicity in a range of vegetation and biota.

Species	PFOS	PFOA	Length of exposure	Effect	Source
Haptophyta *(Isochrysis galbana)*	37.5	163.6	72 h	EC50	Mhadhbi et al. (2012)
Sea Urchin *(Paracentrotus lividus)*	20	110	48 h	EC50	Mhadhbi et al. (2012)
Zooplakton *(Siriella armata)*	6.9	15.5	96 h	EC50	Mhadhbi et al. (2012)
Flatfish *(Psettas maxima)*	0.11	11.9	144 h	EC50	Mhadhbi et al. (2012)
Mytilus galloprocincialis embryos	0.0001	0.0001	48 h	LOAEL	Fabbri et al. (2014)
Baltic algae species		977	72 h	EC50	(Latała et al., 2009)

EC50, concentration that results in an effect in 50% of sample population; LOAEL, Lowest Observed Adverse Effect Level in experiment.
[a] Primary producer, primary and secondary consumers.

Table 4
Summary of Soil PFAS (mg/L) toxicity experiments in invertebrates and vegetation.

Species	PFOS	PFOA	Length of exposure	Effect	Source
Earthworms (*Eisenia fetida*)	1	1	21 days	NOAEL	Karnjanapiboonwong et al. (2018)
Earthworms (*Eisenia fetida*)	120	-	42 days	LOAEL	Xu et al. (2013)
Earthworms (*Eisenia fetida*)	1	1	30 days	NOAEL	Zhao et al. (2014)
Earthworms (*Aporrectodea caliginosa)*	1	1	40 days	NOAEL	Zareitalabad et al. (2013)
Chinese Cabbage	51	-	15 days	NOAEL	Zhang et al. (2011)
Three species seeds - Lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), and Pakchoi (*Brassica rapa chinensis*)	>200	>200	5 days	NOAEL	Li (2009)
Wheat biomass	10	-	7 days	LOAEL	Qu et al. (2010)

NOAEL, No Observed Adverse Effect Level in experimental study; LOAEL, Lowest Observed Adverse Effect Level in experiment. *LOAEL on weight.

1000 μg/L. A delay of larvae shell development was seen at levels as low as 0.01 μg/L PFOS and 0.01 μg/L PFOA (Fabbri et al., 2014). Perhaps coincidentally, disrupted/delayed larvae developmental time tends to be the most common effect of exposure to PFAS in a wide range of organisms. However, researchers are beginning to use other endpoints, such as genetic and transcriptomic responses following exposure to PFAS. This is because molecular biology techniques tend to be more sensitive in detecting the overall effects before common endpoints such as growth and mortality can be observed (Jones et al., 2013).

3.2. Terrestrial ecosystems

Earthworms are regarded as one of the most suitable animals for testing the toxicity of compounds in soils, as a result have become model organisms for ecotoxicological testing (Joung et al., 2010; Lankadurai et al., 2012). As they spend their entire lifecycle within the soil, they can provide an indication of the health of the system (Baylay et al., 2012).

Xu et al. (2013) showed reduced growth in *Eisenia fetida* following a 14 and 42-day exposure to 120 mg/kg PFOS-contaminated soil, with no observed effects on growth at 80 mg/kg. Zareitalabad et al. (2013) and Zhao et al. (2014) both reported no toxic effects of PFOS and PFOA at concentrations of 1 mg/kg in the earthworm, *Aporrectodea caliginosa.* Zareitalabad et al. (2013) found survival was less than 40% out of a total of five adult earthworms (*Aporrectodea caliginosa)* when they were exposed to 100 mg/kg of PFOA and PFOS. Furthermore the authors had total mortality occur at the highest exposure concentration of 500 mg/kg of PFOA and PFOS in soil (Table 4) (Zareitalabad et al., 2013). Relatively few studies to date have attempted to uncover the effects of PFAS on ecosystems, at environmentally relevant concentrations. A 21-day exposure, by Karnjanapiboonwong et al. (2018), investigated the effects and bioaccumulation of four PFASs on the earthworm (*Eisenia fetida*) growth, using a concentration between 0.1 and 100,000 μg/kg. They found concentrations up to 1,000 μg/kg resulted in no effect on growth. Indeed, growth was only reduced at concentrations of 100,000 μg/kg (Table 4), several magnitudes higher than ever likely to be found in the environment. As global concentrations of PFAS tend to be measured in ng/kg or μg/kg in soil (unless soil is directly contaminated from landfill sites or WWTPs), it can again be assumed from these data that there is a very low risk of PFOS and PFOA affecting earthworms. However, adverse effects may still occur due to long term (chronic) exposures at environmentally relevant concentrations.

Although it is acknowledged that one species of organism, such as the earthworm, cannot represent the effects of PFAS exposure on an entire community, it is well established that model organisms can provide an indication of the impact. As more data are generated from studies on individual organisms there will be a reduction in the uncertainty of the overall effects of PFAS and provide a clearer understanding of how PFAS can affect and move through a system.

Interestingly, Zhao et al. (2014), and Sunderland et al. (2019) propose that PFAS in soil can bioaccumulate in earthworms and plants. Vegetation is known to respond to contaminants and can be the first point of determining biomagnification or accumulation across trophic levels as it is the primary producer in most systems. An investigation on PFAS compound transfer in spring wheat, oats, potatoes, maize and perennial ryegrass grown in contaminated soils was conducted by Stahl et al. (2009). The range of PFOS and PFOA was 0–50 mg/kg, interestingly all plant species accumulated the compounds, though PFOA concentrations were generally higher then PFOS (Stahl et al., 2009).

Krippner et al. (2015) exposed straw and grains of maize to PFAS-spiked soil (0.25–1 mg/kg) and saw accumulation of these compounds within the plants (Table 4). The authors determined that, as expected, the higher the concentration of PFAS in soil, the more the plants tended to accumulate. Interestingly, Krippner et al. (2015) found the difference in accumulation between PFAS depended on the chain length, with short-chain PFAS had a greater accumulation rate than long chain ones. Growth inhibition of Chinese cabbage (*Brassica rapa*) occurred following exposure to 87 mg/L PFOS but no observed adverse effect on growth and survival were seen at 51 mg/L (Zhang et al., 2011) (Table 4). There was no

Table 5
Reported effects linked to PFAS exposure on human health as described in Khalil et al. (2015), and associated risk.

Human Health	PFAS Exposure Outcome	Risk [a]
Lipids	High exposure increases the risk of incident hypercholesterolemia in adults and low association to lipid profiles.	
Uric Acid	Limited evidence supports an association between hyperuricemia and PFAS.	
Kidney Disease	Limited and inconsistent evidence links chronic kidney disease.	
Heart Disease and Hypertension	No significant evidence with CHD and hypertension.	
Cerebrovascular Disease	Limited evidence with incident and prevalent stroke.	
Diabetes	Unlikely association with Type 2 Diabetes mellitus	
Liver Function and Liver Disease	Inconsistent positive associations with liver function biomarkers. Negative prevalence or incidence of liver diseases including hepatitis or non-alcoholic or alcoholic fatty liver disease.	
Immune Function	No evidence of any increased risk of non-infectious lung disease.	
Autoimmune Disease	Probable link between exposure and ulcerative colitis. No probable link between PFOA and any of the other autoimmune diseases (rheumatoid arthritis, lupus, type1 diabetes, Crohn's disease, or multiple sclerosis).	
Osteoarthritis and Bone Mineral Density	Limited and inconsistent evidence on osteoarthritis.	
Thyroid Function	Limited and inconsistent evidence of thyroid function.	
Thyroid Disease	Inconsistent evidence on thyroid disease.	
Neurological and Neurodegenerative Disorders	Limited evidence on development of cognitive impairment or neurodegenerative diseases.	
Cognitive and Behavioural Disorders in Children	Limited evidence on childhood 23ehavioural and cognitive development.	
Reproductive and Developmental Outcomes	Limited and inconsistent evidence of reproductive and developmental outcomes.	
Overweight and Obesity in Offspring	Limited and inconsistent evidence regarding early life exposure to PFAS with later obesity.	

[a] *Key for risk assessment likelihood*

Increased / Known risk

Inconsistent / Unknown risk

Unlikely / No known risk

inhibition of growth to three species of plants, Lettuce *(Lactuca sativa)*, cucumber *(Cucumis sativus)*, and Pakchoi *(Brassica rapa chinensis)*, at concentrations of 200 mg/L for both PFOS and PFOA shown in a study by Li (2009). Conversely, Qu et al., 2010 saw a significant decrease in wheat biomass exposed to 10 mg/L PFOS (Table 4). Overall, while the tolerance and uptake of PFAS of various chain lengths varies greatly in plants, studies on plant accumulation of and toxicity to PFAS have shown no consistent effects at detectable PFAS concentrations. Any adverse effects observed in the literature have been caused by much higher concentrations of PFAS than is generally detected in ecosystems.

3.3. Human/mammalian toxicity

The general public understandably is most concerned with the possible effects of PFAS on humans and so it is worth discussing this topic here briefly. Human exposure to PFOS and PFOA can occur via multiple pathways, but the main routes are via food and drinking water. A recent report indicated the positive association from food intake, such as fish and crustaceans, with PFAS concentrations in the plasma of women, while intake of soy products and water was associated with lower PFAS concentrations (Zhou et al., 2019). Similarly, Knutsen et al. (2018), determined a considerable proportion of the population, using benchmark modelling of blood serum level of PFAS, exceeds the proposed tolerable weekly intakes (TWI) based on dietary exposures.

In general, there is limited and incomplete information surrounding the occurrence, fate and toxicity of PFAS in humans, making it difficult to determine the potential risk that PFAS contamination poses – although it is generally agreed that there is a risk. Table 5 lists the potential effects of PFAS exposure, with the associated known risk. As there is limited data available on acute toxicity in humans international policies provide information based on dose-response relationships, and effects on human health based on animal toxicity exposures, (CRC CARE, 2016). Direct comparisons are important because the information gained can indicate potential modes of action that the compounds have are different between animal and human effects; they are also difficult since animals may not respond in the same way to the same chemical as humans.

Studies have associated exposure to PFAS with adverse health outcomes in common laboratory mice, rats and primates (Seacat et al., 2003; Stylianou and Maria, 2019; Thibodeaux et al., 2003; Bjork et al., 2008; Chang et al., 2018). Effects linked to PFAS include developmental toxicity (Lau et al., 2004; 2007), neurotoxicity (Mariussen 2012), carcinogenicity, cell membrane disruption, and genetic damage (Gong et al., 2019). Links have been made between exposure to PFOS and an increase in liver weight; liver cell hypertrophy; histopathological changes to lungs; decreased hormone levels; decreased reproductive outcomes; and development delays (Pizzurro et al., 2019). Again however, these concentrations that cause these effects are generally higher than those found in the environment.

PFOA has been linked to increase liver weight; and reduced immunoglobulin (IgM) antibody titres (Khalil, et al. 2015; Pizzurro et al., 2019). Nevertheless, in it is not clear what concentration of PFOS or PFOA cause the adverse effects. Moreover, most of the human health studies in this area are cross-sectional analyses. Cross-sectional studies collect a range of data sets at a point in time and cannot be used to conclude causality, for example if the test organism is deceased and PFAS has been detected, it cannot be assumed PFAS was the cause of death – yet it can be listed to be a potential cause with further evidence. This means that the majority of these results are insufficient to draw any definite conclusions about the adverse effects caused by PFAS (CRC CARE, 2016). Conversely, Grandjean, (2018) insists all effects potentially linked to PFAS exposure should be acknowledged and included into guideline policies for drinking water limits around the world, even if they are inconsistent and difficult to draw solid conclusions from. While this precautionary principle approach is attractive at the policy level it is not fully based on scientific evidence. It is paramount that the concentrations used to cause an effect and the types of studies, e.g. whether they are cross-sectional or otherwise, be taken into consideration when developing global restrictions and limits of PFAS exposure.

3.4. Other toxicity considerations

Ahrens and Bundschuh (2014) were some of the first to discuss the potential bioaccumulation of PFAS changing among species. A consistent view since then is that bioaccumulation depends on the PFAS chain length (Gomis et al., 2018). It has been demonstrated that the chain length can also affect which PFAS are excreted from the organisms (D'eon and Mabury 2011; Whitacre 2015; Kariuki et al., 2017; Land et al., 2018). For example, PFOA (C7 fluorocarbon) has a low bioaccumulation potential (Zhao et al., 2013; Chiesa et al., 2018; Land et al., 2018; Palmer et al., 2019). In contrast PFOS (C8 fluorocarbon) has a high bioaccumulation potential (Ahrens and Bundschuh, 2014).

Bioaccumulation of PFAS can be species-specific, for example, mammals have greater bioaccumulation potential of PFAS compared to invertebrates (Land et al., 2018; Palmer et al., 2019). McCarthy et al. (2017) go as far as to state that studies regarding bioaccumulation and biomagnification of PFAS are variable and not reported in a standardised fashion (e.g. dry weight versus wet weight), which limits their effectiveness. The authors highlight the limited data that has been collected for fish, benthic organisms, terrestrial biota (including reptiles and amphibians) and state toxicology testing across all classes of organisms has not been consistent (McCarthy et al., 2017).

For government and industry to create and follow effective guidelines, consistent evidence to better understand the adverse effect of PFAS on our ecosystems at environmentally relevant concentrations is needed. There is little point setting an environmental quality limit (EQL) so low that it is mostly impossible to meet or basing policy on tests conducted using concentrations far higher than found in the environment or to focus on acute effects and ignore the potential of chronic ones (of course it also not useful to set EQLs so high that nobody would ever fail to meet them). Therefore, although the information acquired to date is useful in understanding routes of PFAS toxicity and potential mode of action, it adds little information on potential adverse effects caused by PFAS at current exposure levels. Further research is not only desirable for single compounds, but is necessary to understand the toxicity of low doses of mixtures of PFAS (Ruan et al., 2015; Hamid et al., 2018; Nakayama et al., 2019). A lack of understanding of how PFAS interact with one another and move through a biological system currently undermines our current/limited toxicological understanding of these compounds.

There is emerging research on the potential of natural elimination of PFAS occurring within organisms following exposure (D'eon and Mabury 2011; Sharpe et al., 2010; Hassell et al., 2019; Zhang et al., 2015; Pizzurro et al., 2019). A recent study found that longer chained compounds bind with proteins and are excreted at a greater rate than shorter chained PFAS following faecal excretion in dogs and cats from the USA (Ma et al., 2020). The authors found the sum concentrations of 13 PFAS varied between 21.6 and 474 (mean: 85.4 ± 94.5) ng/g dry weight for dogs. These concentrations were slightly higher than those found in cats (range: 18.0–165 ng/g dry weight, mean: 54.7 ± 26.9 ng/g dry weight) (Ma et al., 2020). After extensive exposure and uptake, PFOS and PFOA are distributed

mainly in the kidneys and liver, and then are excreted by the kidneys without undergoing biotransformation (Lau et al., 2007). Results by Zhang et al. (2015) and Pizzurro et al. (2019) also indicate that urine is an important elimination pathway for PFOA and PFOS in primates such as humans. This would remove the contamination from systemic circulation and reduce its potential affect.

4. Biomarkers of exposure

Development of molecular (e.g., genomic, proteomic or metabolomic) markers of exposure can, potentially, provide an overview of contaminant exposure on systems before other more commonly used ecotoxicological endpoints, such as growth, reproduction, or mortality become apparent (Jones et al., 2013). This is potentially useful in identifying an early warning sign of exposure. Therefore, biomonitoring programs that include early warning biomarker endpoints, such as genomics, transcriptomics and metabolomics, may be more able to detect impacts of exposure to PFAS than programs without biological testing. Finding the range of doses where PFAS concentrations interrupt the normal biochemistry of biota could enhance our overall understanding of the mode of action, effects and toxicity of PFAS in ecosystems around the world (Jones 2018; Yao et al., 2019).

Some omics studies on PFAS exposure have already been published (Yao et al., 2019). Examination of the *Caenorhabditis elegans* genome (Stylianou and Maria. 2019) showed there was a distinct transcriptional response to different PFOS exposures. Similarly, Lankadurai et al., 2013b used nuclear magnetic resonance (NMR) based metabolomics to look at the effect of sublethal exposure to PFAS on the earthworm *Eisenia fetida*. Earthworms were exposed to a range of PFOS concentrations of 5, 10, 25, 50, 100 or 150 mg/kg, for two, seven and fourteen days. The authors were able to distinguish between the responses of PFOS-exposed and control (unexposed) earthworms even at the lower exposure concentrations (Lankadurai et al., 2013a,b). Annunziato et al. (2019) found subtle morphometric and gene expression changes in larval zebrafish following exposure to PFAS, that would have not been unobserved using the more traditional ecotoxicological endpoints. Though, to date few studies have included and used metabolomics to show the effects of PFAS on the biochemistry of individual organisms (Yu et al., 2016).

Of the omics techniques Metabolomics is the one most commonly used to investigate the complete set of small biological molecules in a system and it is increasingly being used as part of a weight-of-evidence approach for environmental assessments (Sinclair et al., 2019a). This approach has proven to be highly sensitive and can reveal a mechanistic link between an organism and a chemical, enabling a better understanding of early effects of specific pollutants in an ecosystem (Jones et al., 2008). Metabolomics can validate gene expression alterations, reflect the changes in cellular processes and highlight the pathways that were affected. Changes in metabolite profiles can also provide a sensitive and holistic indication of the biochemical response. This approach has been recognized as a growing tool for ecotoxicologists to monitor and detect changes caused by exposure to low environmentally relevant concentrations of a range of chemicals on organisms (Long et al., 2015; Jones, 2018; Sinclair et al., 2019a; 2019b).

Peng et al. (2013) used metabolomics to discover contaminant-specific biomarkers following toxicant exposure and suggested their techniques would be useful for detecting internal, initial effects of PFAS. A recent review by Yao et al. (2019) listed studies that have used metabolomics successfully as potential indicators for PFAS exposure and suggested current methods and future

Table 6
A summary of the information in Yao et al. (2019) on papers that use metabolomics to investigate the biochemical response of exposure to PFAS.

Species	Method	PFOS	PFOA	Length of exposure	Metabolite response	Source
Mice	LC-MS/MS	-	2.5 mg/L	28 Days	10 metabolites were significantly different from control - three metabolites being greater than control and seven being less. These metabolites were identified as potential biomarkers of functional responses of brain to exposure to PFOA.	Yu et al. (2016)
Rats	1H NMR	-	0.5[a] mg/L	110 days	Glycine, proline, succinate, glutathione (GSSG), inosine, nicotinurate, and uracil, as well as choline, phosphorylcholine, and glycerophosphorylcholine were elevated in liver extracts. Additionally, pyruvate, malate, glucose, glycogen, and taurine were clearly depleted.	Ding et al. (2009)
Zebrafish (embryos/larvae)	HPLC	19 μM	-	24 h	Different response at two life stages. 16 significantly affected metabolites	Huang et al. (2016)
Zebrafish (embryos/larvae)	LC-HRMS	0.06–2 μM	-	48–120 h	19 significantly affected metabolites trended down in abundance and 16 had an upward trend in abundance.	Ortiz-Villanueva et al. (2018)
Eisenia fetida (Earthworms)	1H NMR	6.25e50 mg/cm^2	5–150 mg/kg	2–14 days	myo-Inositol, Glutamate and succinate had an upward trend following exposure. Valine, Leucine, Lysine, Maltose had a downward trend.	Lankadurai et al. (2012)
Daphnia magna (Cladoceran)	1H NMR	36 mg/L	-	48 h	Ontogenetic changes. Targeted metabolites decreased in neonates and increased in adults.	Wagner et al. (2017)
Daphnia magna (Cladoceran)	1H NMR	15–60 mg/L	-	48 h	Energy metabolic pathways. Both ATP and uracil increased. Glucose/maltose reserves decreased.	Kariuki et al. (2017)

[a] PFDoA – Perfluorododecanoic acid.

directions for using metabolomics to understand the overall effects of PFAS (Table 6). Indeed, metabolites such as fatty acids, cholesterol and glycogen, are increasingly reported to be affected by PFAS exposures in a variety of organisms indicating a potential mode of action (Bjork et al., 2008; Lv et al., 2013; Wan et al., 2014). Other energy markers such as glycogen, depleted in zebrafish livers following exposure to PFAS, may demonstrate the action of PFAS once taken up (Hagenaars et al., 2008, 2013). Therefore, the use of biochemical markers can assess early changes and chronic effects, thus aiding in the overall understanding of PFAS toxicity at environmentally relevant concentrations. More studies using this approach and following The MEtabolomics standaRds Initiative in Toxicology (MERIT) approach to metabolic toxicology are therefore recommended (Viant et al., 2019).

5. Calculated environmental risk

Hazard Quotients (HQs) can be used to determine the risk of pollutants on ecosystems by using the level measured in an environment and a concentration that causes an observed effect (Sardiña et al., 2019). Given that we have data on environmental occurrence of PFAS and data (albeit limited) showing the exposure levels needed to cause an effect it is possible to calculate a hazard quotient (HQ) for PFAS using the maximum reported measured environmental concentration (MEC). Since there is a lack of environmental measurements, the MEC used in this current study took the reported concentrations of PFAS reported in a secondary treated wastewaters in a review by Arvaniti and Stasinakis (2015) and divided them by 10. This was to mirror the approximately 1:10 dilution ratio of effluent to receiving water used by most WWTP operators and regulators. The calculated MEC was then divided by a predicted no-effect concentration (PNEC). The PNEC can be collected in a number of ways, but in this case the lowest minimum effect concentration listed in Table 3 was used. The aim of the HQ is to represent the risk value of PFAS for multiple species across the world, so the lowest recorded value was used for the PNEC to ensure it would have no observable effects for the predicted majority of organisms. The HQ equation is thus HQ = MEC/PNEC and the data are shown in Table 7.

Figs. 2 and 3 represent the HQ risk associated with PFOS and PFOA collected from wastewater treatment plants around the world. The first threshold represents the quotient value entering a 'mild/moderate risk' level meaning that PFAS has entered waterways and needs to be monitored and addressed before adverse effects are observed in the environment. The second (red) threshold represents 'high risk', indicating the level of PFAS in the waterway is of concern to the continued health of the ecosystem. For the majority of countries PFOS and PFOA HQs are <1, indicating a low risk to an ecosystem, only Singapore had a high HQ risk of >1 for PFOA (Fig. 3). The values were calculated from a WWTP which has 60% industrial wastewater and 40% domestic wastewater (Yu et al., 2009); although, the authors did not disclose which industry the wastewaters were from, it is likely that industry would be the cause of such high values. In contrast, the HQ values from seven WWTPs located in China, including two of the largest plants in Beijing, were calculated to have a low HQ risk (<1) (Pan et al., 2011). The authors of the study the occureance data came from noted that the WWTP with the highest PFOA concentrations received only domestic and commercial wastewaters (Pan et al., 2011).

The concentrations of PFOA and PFOS in industrial wastewater largely depends upon the specific industry or commercial applications that are producing said wastewaters. For example, Kim et al. (2012) state that relatively high PFOS values are often detected near oil/chemical, steel-mill and metal plating, and metal plating/processing industries, whereas relatively high PFOA values are detected near fabric/textile and paper- mill industries. PFOS values from Thailand were in the mild HQ risk level. Kunacheva et al. (2011) believe industrial activities are the major sources of PFAS in this country and that the increase of PFOS could be due to the degradation of larger PFAS in the activated sludge processes. Japan also had a mild HQ for PFOS, this value could be affected by the collection of effluents during the dry weather season (Murakami et al., 2009). Interestingly, the authors found the concentrations were significantly higher in secondary effluents than in influent samples and that this increase of PFAS accross the works are consistent with results from WWTPs located in the USA (Sinclair and Kannan 2006; Loganathan et al., 2007) and with data from Korea (Kim et al., 2012; Murakami et al., 2009).

The HQ risk associated with PFOS and PFOA is an indication of their likely toxicity risk in an environment using known concentrations linked to adverse effects, such as growth. Additionally, when the HQ risk value was derived using the current Australian guideline value of 0.00023 μg/L for the protection of 99% of species, PFOS resulted in a low- risk (Sardiña et al., 2019). As these values have been collected from WWTPs from around the world, it is likely that the HQ calculation will become more reliable as ecotoxicity information increases to similar levels as occurance data.

Table 7
Calculated hazardous quotient for PFOS and PFOA (PNEC = 100 ng/L) (Sardiña et al., 2019).

Region	Country	PFOS (ng/L)	MECPFOS	PFOS HQ	PFOA (ng/L)	MEC PFOA	PFOA HQ	Source
Asia	Japan	635.00	63.50	0.64	68.00	6.80	0.07	Murakami et al. (2009)
	Singapore	461.70	46.17	0.46	1057.00	105.70	1.06	Yu et al. (2009)
	Korea	110.00	11.00	0.11	591.00	59.10	0.59	Kim et al. (2012)
	Hong Kong	28.80	2.88	0.03	4.10	0.41	0.00	(Ma and Shih 2010)
	Taiwan	0.00	0.00	0.00	480.30	48.03	0.48	Lin et al. (2010)
	Thailand	552.80	55.28	0.55	16.90	1.69	0.02	Kunacheva et al. (2011)
	China	7.30	0.73	0.01	26.20	2.62	0.03	Pan et al. (2011)
Aust.	Australia	38.60	3.86	0.04	27.00	2.70	0.03	Thompson et al. (2011)
USA	Iowa	26.00	2.60	0.03	22.00	2.20	0.02	Boulanger et al. (2005)
	New York	68.00	6.80	0.07	398.00	39.80	0.40	Sinclair and Kannan (2006)
	Kentucky	28.00	2.80	0.03	183.00	18.30	0.18	Loganathan et al. (2007)
	Georgia	13.00	1.30	0.01	102.00	10.20	0.10	Loganathan et al. (2007)
	California	190.00	19.00	0.19	180.00	18.00	0.18	Plumlee et al. (2008)
Euro.	Germany	82.20	8.22	0.08	77.60	7.76	0.08	Ahrens et al. (2009)
	Denmark	18.10	1.81	0.02	24.40	2.44	0.02	Bossi et al. (2008)
	Switzerland	303.00	30.30	0.30	35.00	3.50	0.04	Huset et al. (2008)
	Greece	25.30	2.53	0.03	34.00	3.40	0.03	Stasinakis et al. (2013)
	Spain	91.00	9.10	0.09	14.90	1.49	0.01	Campo et al. (2014)

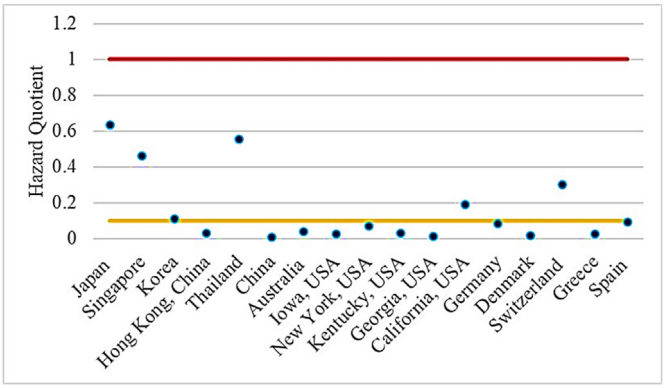


Fig. 2. The risk posed by PFOS to environments around the world, measured using hazard quotients (HQs) from Table 7. Mid Risk of PFOS contamination = >0.1. High risk of PFOS contamination = >1.

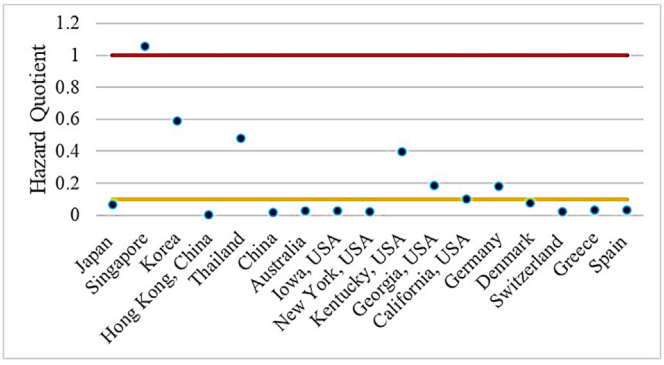


Fig. 3. The risk posed by PFOA to environments around the world, measured using hazard quotients (HQs) = MEC/PNEC from Table 7. Mid Risk of PFOA contamination = >0.1. High risk of PFOA contamination = >1.

6. Future research directions

This review of the literature on PFAS exposure and toxicity has shown that although both humans and the environment are exposed to PFAS on a regular basis the biological consequences are poorly explored particularly in relation to organisms in the environment. Based on current knowledge, despite the public interest in these compounds, the health risks associated with PFAS exposure have only a weak correlation to potential harmful effects in humans and the environment.

PFAS exposure is associated with significant concern around adverse effects (Sunderland et al., 2019). However, many of these findings are inconsistent for a number of reasons including the concentrations and types of PFAS used, and the variety of different endpoints used to assess toxicity. Consequently, there is no consistent evidence that the concentrations of PFAS generally detected in environments are harmful to wildlife (or humans) even in the case of highly exposed individuals, such as firefighters. Moreover, ecological toxicity assessments are not yet providing consistent evidence that can be linked to current environmental conditions. While there is evidence to suggest PFAS can bioaccumulate and is persistent, there are large gaps in the knowledge about their effects on biota and human health that need to be addressed if environmental policy is to be effective. Future research must determine the effect of PFAS at environmentally relevant concentrations and attempt to unravel the mode of action/elimination process for PFAS in a range of organisms.

Due to the large number of PFAS continuously being detected at trace levels in the environment it is difficult to quantify the exposure rates for each individual compound in the field or their effects. For this reason it has been suggested to use established total organofluorine (TOF) measurements (D'Agostino and Mabury 2017) to assess total PFAS exposure and then compare that value to biological changes observed in the same sample (Jones 2018; Yao et al., 2019).

PFAS are continuously introduced into ecosystems and are present in complex mixtures, with increased baseline information, mixture exposures should be investigated. Concern over the occurrence of short-chained (C = 4-7) and ultra-short chained (C = 2-3) compounds replacing the use of long-chained (C > 7) compounds within industry is growing, as there has been mounting evidence indicating persistence and toxicity increases for short-chained PFAS (Krippner et al., 2014; Zhao et al., 2018; Ateia et al., 2019; Ghisi et al., 2019). It is known that there are over four thousand related compoinds that can be classed as PFAS, yet to date monitoring has been limited to a subset of common compounds. Using a single analytical methods Coggan et al., 2019a,Coggan et al., 2019) identified fifty-three additional PFAS that are not currently on regulatory guideline lists or are routinely monitored. Equally, many studies have identified hundreds of previously unreported and unknown PFAS-related substances that have the potential to degrade into other, more well known, PFAS (D'Agostino and Mabury 2014; 2017; Barzen-Hanson et al., 2017). Thus it is vital to identify new PFAS and investigate what the degradation and effects of long, short and ultra-short chain compounds will be if we are to fully understand this issue (Ahrens and Bundschuh 2014).

It is also important to note that other stressors such as heat, disease, the presence other toxicants all potentially affect PFAS toxicity. For example Krippner et al. (2014) showed pH fluctuations in soil determined which PFAS compound was accumulated in maize, with PFAS with longer chain lengths increasing in accumulation with the increasing pH in the soil. Future research therefore needs to explore the additional effects of existing natural stressors, such as temperature, salinity and pH fluctuations on the bioavailability and toxicity of PFAS. The toxicity of PFAS may also vary between an organism's life stage, sex, length of exposure and these all need to be considered for accurate toxicity testing and the setting environmental guidelines.

7. Conclusion

PFAS have been routinely detected in trace amounts around the world since the early 2000s. This has created global concern over their potential adverse effects, which have led to very strict environmental guidelines due to public pressure. The guideline limits are set to ensure ecological protection and have been determined to be technically feasible, both to measure and as a target for treatment facilities, either in WWTP or landfills. The limits do not necessarily mean that at higher concentrations PFAS will cause an adverse effect. Nor do they consider if it is economically efficient for governments to enforce or industry to meet them. In fact, little is known about the effects of PFAS exposure, the interactive toxicity of PFAS mixtures at environmentally relevant concentrations or about the interactions PFAS has with other natural and anthropogenic stressors. HQ analysis indicates that PFAS may not presently be an environmental concern. This may be a misrepresentation of the toxicity of PFAS, as the HQ analysis depends on traditional endpoints such as growth, reproduction and mortality which may not be sensitive indicators of PFAS toxicity. This review identifies that sublethal effects are more likely to result from low level PFAS exposure. More sensitive endpoints using advanced molecular biological approaches will generate more sensitive sublethal information for the improved environmental assessment of PFAS.

CRediT author statement

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Declaration of competing interest

The authors declare no competing interests.

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Towards Better Management of PFAS in Victoria

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A transdisciplinary RMIT research team has developed this policy brief to inform policy stakeholders on PFAS contamination in Victoria and discuss approaches to improve PFAS management across the State.

The Impact of PFAS

Poly and Per-FluoroAlkyl Substances (PFAS) are a family of synthetic chemicals based on carbon-fluorine bonds, which are highly stable. PFAS have been used in various industrial and commercial products, including non-stick cookware, fabric protection products, upholstery and carpets, waterproof clothing, cosmetics, food packaging and firefighting foams. Some PFAS are listed in the Stockholm Convention on Persistent Organic Pollutants, and their use is being restricted or phased out in signatory countries, including Australia. However, their persistence means that even no longer-used compounds are still found in the environment. Restrictions on such 'legacy PFAS' have led to increased use of replacements such as perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs), and fluorotelomer alcohols (FTOHs), the effects of which are not as well understood.

The exact number of PFAS varies depending on the definition used. Estimates of between 8,000 and 7 million chemicals are commonly given, and the US EPA toxicity database lists 14,735 unique PFAS. Those that are of environmental concern are resistant to degradation and highly persistent in the environment. These properties have led PFAS to be dubbed "forever chemicals" (although this term is a misnomer[1]). PFAS have been found in the environment, including in drinking water supplies, globally. They are of substantial public concern due to their reported links with various health effects, including immune system suppression, endocrine disruption, metabolic disorders, and cancer. Much public concern about PFAS comes from films like 'Dark Waters' and documentaries like 'How to Poison a Planet'. This has led some communities exposed to PFAS to launch class actions against chemical companies. PFAS have featured in the Australian media due to reports of their presence (albeit at very low, ng/L, concentrations) in drinking water catchments in New South Wales reported by the Age and Sydney Morning Herald newspapers, and the recent (October 2024) release of draft National Health and Medical Research Council (NHMRC) guidelines for PFAS in drinking water. However, public perception of the risk associated with PFAS does not always align with our evolving scientific understanding and data on the subject. Discussion about toxicity is futile without considering dose and context; this is often missing from public debate on PFAS, as is the fact that we could never be sure that the concentration of any chemical was zero, just that it was lower than the minimum amount we could measure. Context, in other words, is essential.

Sources of PFAS Contamination

Although not produced in Australia, PFAS have been widely used here. This country's primary source of PFAS was Aqueous Film-Forming Foams (AFFFs), used to suppress flammable liquid fires, particularly those from jet fuel. While the use of these products has ceased, there has been substantial contamination of both land and water where these products were used in large amounts, including defence land/sites, airports, and firefighting training sites. Contamination of these areas has led to concerns about the potential health impacts on local communities, particularly those using groundwater as a drinking water source if the pollution moves offsite. While they have been reported in some drinking water catchments, there is a lack of widespread monitoring of PFAS in drinking water. However, the recent reports of PFAS in drinking water catchments and the release of the aforementioned draft NHRMC guidelines for PFAS in drinking water have focused the issue in the public consciousness. The proposed NHRMC guidelines are more conservative than current Australian guidelines and those of most other jurisdictions.

[1] *The name is also a play on words; the F in forever and the C in chemicals can also stand for Fluorine and Carbon, respectively*.

The exception is the Biden Administration in the US, which recently issued the first national, legally enforceable drinking water standards on PFAS as part of the US EPA's PFAS Strategic Roadmap. Those guidelines are effectively the limit of detection for (4 ng/L) for most PFAS but are only due to come into effect in 2029. The concentrations of PFAS reported in drinking water are generally below the proposed NHRMC guidelines, so the risk here is low, especially because drinking water is not considered the major route for PFAS exposure for most people.

PFAS continue to be used in other processes and products, culminating in PFAS contamination at waste disposal sites such as landfills and wastewater treatment plants. More information is needed on the historical and ongoing use of PFAS and which PFAS have been used in which locations. This information is required to build reliable PFAS records, assess environmental risk, and reassure the public that concentrations of PFAS are not high enough, in most cases, to be a significant health risk.

We recommend the creation of a detailed registry of PFAS use in Victoria, with industry users mandated to report what PFAS products they are using, in what amounts, and how they are stored.

Current PFAS Contamination in Victoria

Little is known about the distribution and behaviour of PFAS in the Victorian environment outside the major Melbourne metropolitan area. Here in Australia, 'Hotspots' have been identified around defence sites (especially RAAF Base East Sale), fire-fighting training facilities (e.g. Fiskville) and heavily industrialised areas in Western Melbourne (see map of Victorian PFAS concentrations in Figure 1). Generally, however, data on the concentration of PFAS in the environment – including in soil, water, plants and animals – is lacking across the state. Even where data exist, they are often inconsistent and not readily comparable due to the different analytical methods, type of measurement(s) used, and quality control measures reported in various studies. This contrasts with other countries, such as the United Kingdom, which have extensive, publicly available mapping data of PFAS. Better information is essential to understand the fate and behaviour of PFAS in the environment and assess potential exposures and health risks to Victoria's environment and human population. This is particularly relevant due to Victoria's General Environmental duty regulations, which require all Victorians to identify and manage environmental risk proactively.

It is recommended that a more detailed assessment of PFAS location and concentrations be undertaken and made publicly available so we can better understand the volumes of PFAS in Victoria. We recommend that this includes testing of tap water at selected locations.

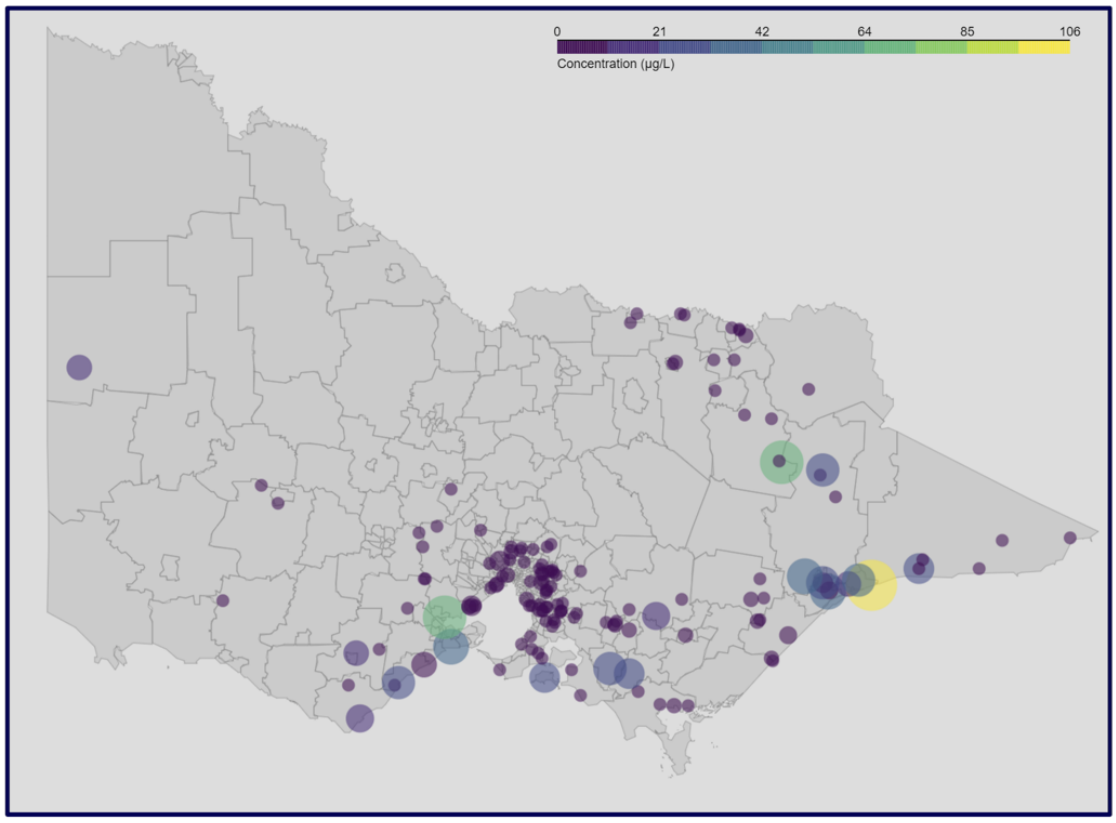

Figure 1: Bubble map of PFAS concentrations around Victoria using data from the scientific literature. The RAAF base in East Sale is responsible for the highest levels (yellow circle on the right-hand side of the image)

Understanding the effects of PFAS

Although PFAS have been associated with a range of health effects in humans, the concentrations of PFAS needed to cause such effects are much higher than those found in the environment (except for highly contaminated sites). There is a lot of misinformation and misunderstanding of the toxicology and pharmacology of PFAS, which, in some cases, has led to undue public concern. This could be alleviated with greater public education. We often overlook the fact that the mere presence of something does not mean it will automatically cause harm. For example, we know we can get skin cancer from UV light, but that does not mean we will get cancer as soon as we go outside. Levels of PFAS in drinking water are generally in the nanogram per litre (ng/L) range. One nanogram per litre is 1 part per trillion. This is equivalent to 1 second in 31.7 thousand years. There is a difference between someone drinking one ng/L of PFAS in their drinking water for life and someone who is exposed to much higher levels through working with firefighting foams. This is further complicated by the fact that the literature on PFAS (eco)toxicity is inconsistent for several reasons, including the concentrations and types of PFAS studied and the variety of tests used to assess the effects. The NHMRC relied on laboratory toxicology data when setting recent draft water quality guidelines, considering the existing human evidence insufficiently robust for the task. That said, some PFAS do bioaccumulate, meaning concentrations within an organism's body can be much higher than in the surrounding environment and, thus, potentially high enough to cause an effect. Dolphins in Victorian waters, for example, have been found to have the highest concentrations of PFAS in dolphins reported anywhere in the world.

It is recommended that a detailed review of the literature on the impact of PFAS be undertaken, with a focus on environmentally relevant concentrations and acceptable daily intakes for lifelong exposure. The recent NHRMC report from SLR Consulting could be used as a basis to avoid data duplication.

Improving Victoria's Management of PFAS Contamination

A consistent national approach to managing PFAS contamination has been promoted since 2020 by the PFAS National Environmental Plan, which provides guidance and supports collaborative action across all layers of government. Effective management of PFAS contamination in the environment requires a robust regulatory approach. Management of PFAS contamination is, however, complex. This is because it spans jurisdictions (the most affected areas are defence sites and airports located on Commonwealth land, which are outside the control of state government) and because it can be unclear who holds ultimate responsibility for PFAS pollution. Water utilities are responsible for wastewater discharge to the environment, for example, but PFAS in wastewater generally comes from industries within their catchment, not the water utility itself. Resolution of 'legacy' pollution issues can also be complicated, e.g. if the original polluter is no longer present. In the case of drinking water, the issue of how any necessary treatment upgrades are funded must also be addressed. If more advanced water treatment processes are needed, the cost of these will likely be borne by consumers (this will hit smaller and regional communities hardest). This is the opposite of the 'polluter pays' principle, in which the polluter bears the clean-up cost.

It is suggested that a Victorian PFAS action group be formed involving government, academia, and relevant industry and community stakeholders. The group should focus on improving PFAS monitoring, advising on new policies, and contributing to developing and implementing a Victoria state action plan to reduce and manage the risks of PFAS to the Victorian population and environment.

We also recommend the government invite traditional owners of Victoria's lands and waters to discuss how they would like to be involved with this issue.

Contact: To discuss any of the issues raised, please contact Professor Oliver Jones (oliver.jones@rmit.edu.au).

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Range of the perfluorooctanoate (PFOA) safe dose for human health: An international collaboration

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ABSTRACT

Many government agencies and expert groups have estimated a dose-rate of perfluorooctanoate (PFOA) that would protect human health. Most of these evaluations are based on the same studies (whether of humans, laboratory animals, or both), and all note various uncertainties in our existing knowledge. Nonetheless, the values of these various, estimated, safe-doses vary widely, with some being more than 100,000 fold different. This sort of discrepancy invites scrutiny and explanation. Otherwise what is the lay public to make of this disparity?

The Steering Committee of the Alliance for Risk Assessment (2022) called for scientists interested in attempting to understand and narrow these disparities. An advisory committee of nine scientists from four countries was selected from nominations received, and a subsequent invitation to scientists internationally led to the formation of three technical teams (for a total of 24 scientists from 8 countries). The teams reviewed relevant information and independently developed ranges for estimated PFOA safe doses. All three teams determined that the available epidemiologic information could not form a reliable basis for a PFOA safe dose-assessment in the absence of mechanistic data that are relevant for humans at serum concentrations seen in the general population. Based instead on dose-response data from five studies of PFOA-exposed laboratory animals, we estimated that PFOA dose-rates 10–70 ng/kg-day are protective of human health.

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1. Introduction

The development of a safe, or subthreshold,[2] dose for perfluorooctanoate (PFOA) has been ongoing for several years. In 2002, a suggested value of 4 μg/kg-day was developed by a team of scientists for the State of West Virginia (2002). This assessment was subsequently relied on, in part, by the U.S. Environmental Protection Agency (EPA, 2005) in a draft assessment for EPA's Office of Toxic Substances. Later, EPA (2009) estimated a safe dose of 0.2 μg/kg-day draft assessment for its Office of Water on more recently available dose-response data.

Outside the U.S., other groups were also estimating safe doses for PFOA, including the European Food Safety Authority (EFSA, 2008) and the United Kingdom (COT, 2009), with both estimating a value of 1.5 μg/kg-day.

EPA (2016) revised its assessment by using a 10-fold lower safe dose (thus estimating 0.02 μg/kg-day), and several years later, revised the value again, this time lowering it quite substantially, to 0.0000015 μg/kg-day (EPA, 2022).

Other authorities, such as the Drinking Water Inspectorate (2021), Health Canada (2018), the European Food Safety Authority (2018), Food Standards of Australian and New Zealand (FSANZ, 2017) and the Agency for Toxic Substances and Disease Registry (ATSDR, 2021) also have developed or revised their safe doses. These various values have been described previously (e.g., Mikkonen et al., 2021). The World Health Organization (2022) has also recently reviewed this information.

Table 1 lists some of these currently estimated safe doses for PFOA. The wide range in estimated values is striking. These values range between 0.0000015 μg/kg-day and 0.16 μg/kg-day. This disagreement among expert groups was noted by the Steering Committee of the Alliance for Risk Assessment (2022)[3] as an issue that might be addressed via collaboration of interested and expert scientists.

It was not the intention of this collaboration to conduct a systematic review and evidence integration or otherwise exhaustively review the literature on PFOA, since many authorities have already adequately done this. Nor was it the intention of this work to critique any individual authority's approach, although presumably not all approaches can be "correct," insofar as they disagree by orders of magnitude. Of course, there is still much to learn about the underlying mechanisms of PFOA toxicity before we can arrive at maximally informed estimates of a truly safe dose of PFOA to protect human health. The intent of this work is to estimate a plausible range for such a dose now, anticipating that results of future research will refine and improve on current estimates.

2. Methods

The Steering Committee of the Alliance for Risk Assessment (2022) solicited nominations from interested scientists and managers in the early fall of 2022 to form an advisory committee that would shepherd the project entitled "The Perfluorooctanoate (PFOA) Safe Dose".[4] After reviewing nominations, an Advisory Committee was selected from nominations received as shown in Appendix 1.

The Advisory Committee assembled a list of relevant publications on PFOA safe dose and opened a call for interested scientists in the late fall of 2022 to participate in an international collaboration to investigate this issue. After nominations from scientists interested in this collaboration were reviewed by the Advisory Committee, three independent teams of scientists were selected as also shown in Appendix 1, assuring that various scientific experts were represented in each team.

The overall objective of each team was to review relevant information and various agency positions on PFOA in order to determine their safe dose ranges. The teams considered the following criteria in their evaluation: known or suspected mode of action (MOA), overall consistency in response among studies, coherence between experimental animal and epidemiology data, and robustness of the overall dose response. The science teams were directed to review and discuss relevant literature and positions independently of each other and in the following manner:

- First, focus on evaluating the evidence regarding potential MOAs for PFOA's reported effects and determining whether the available MOA information would support the consideration of the endpoint as a critical effect,
- Then focus on determination of the critical studies for one or more of its critical effect(s),
- Finally, focus on the choice of extrapolation method including the choice of uncertainty factors.

The initial focus on PFOA's MOAs for toxicity was considered a critical part of the problem formulation step for this project (i.e., to identify the range of a safe dose for PFOA). This problem formulation acknowledges that better characterization of hazards (and not merely hazard identification) includes consideration of weight of evidence for the MOA and its impact on dose-response patterns (NRC, 2009 Science and Decisions, Meek et al., 2014). The sequence of work was interspersed with periodic conference calls in which the teams shared and discussed their independently developed results and attempted consensus around the various focus topics. Most of our conference calls and team discussions occurred between December 2022 and March 2023.

3. Results

The results provided below are summarized by the charges given to the three teams. Teams worked independently on each charge and then shared results prior to the periodic international meetings.

The teams reviewed assessments (some of which were draft assessments, indicating ongoing development of standards or policy) by national authorities and other authoritative sources, specifically, the Agency for Toxic Substances and Disease Registry (ATSDR, 2021), the European Food and Safety Authority (EFSA 2018, 2020), the US Environmental Protection Agency (USEPA 2021, 2022), the World Health Organization (WHO, 2022), Food Standards Australia New Zealand (FSANZ, 2017), Health Canada (2018) and the United Kingdom Committee on Toxicity (COT, 2022) After our deliberations had concluded and before the publication of this article, new draft documents were issued by the USEPA (2023) and Health Canada (2023). The draft evaluation by USEPA raised its PFOA safe dose by 20-fold. The Health Canada draft appeared to maintain its current PFOA safe dose but considered a lower water concentration based on the addition of other PFAS chemicals.

3.1. Consideration of mode of action and epidemiological evidence

Because international authorities have selected a variety of critical effects in the determination of the PFOA safe dose, the collaboration first considered an investigation of likely MOAs as part of its problem formulation. Unfortunately, each of the teams found it difficult to identify potential MOAs for the various effects of PFOA because little

[2] The term "safe" dose is used throughout this text and is intended to represent a dose just below the population threshold. This population threshold is a point in the dose scale where the first adverse effect, that is the critical effect, is anticipated in a sensitive group of humans. The safe dose concept is used variously by health organization world wide with slightly different definitions. It is more formally defined here as an estimate (with imprecision spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of adverse effects during a lifetime.

[3] See: https://tera.org/Alliance%20for%20Risk/ARA_Steering_Committee.htm.

[4] See: https://www.tera.org/Alliance%20for%20Risk/Projects/pfoatwo.html.

Table 1
Safe doses of PFOA and PFOS from international authorities.

Authority	Safe Dose ug/kg-day	Point of Departure (POD_{HED})	Uncertainty Factors
Alliance for Risk Assessment (this paper)	0.01–0.07	Various (see text): 4.35 to 23 μg/ml of serum	Animal-human kinetic factor = 1 [a] Animal-human dynamic factor = 3 [b] Human toxicodynamic factor = 3[c] Human toxicokinetic factor = 8.4[d] Database uncertainty factor = 1[e] Human clearance = 0.23 ml/day-kg[f]
European Food Safety Authority (EFSA, 2020)	0.00063[g]	17.5 ng/mL ($BMDL_{10}$) Decreased anti-tetanus and anti-diphtheria antibody concentration	• None applied • BMD derived in sensitive population (infants) and response is risk factor for disease rather than a disease.
Food Standards Australia/ New Zealand (2017)	0.16	4.9 μg/kg-day	Within human variability = 10 Animal to human extrapolation = 3
Health Canada (2018)	0.02	0.52 μg/kg-day	Within human variability = 10 Animal to human extrapolation = 2.5
US Environmental Protection Agency (2022)	0.0000015	0.0000149 μg/kg-day decreased anti-tetanus antibody concentration	Within human variability = 10
US Environmental Protection Agency (2023 DRAFT)	0.00003[h]	Various (human): 0.000305 μg/kg-day (decreased anti-tetanus and anti-diphtheria antibody concentration), 0.000275 μg/kg-day (increased serum cholesterol) 0.000292 μg/kg-day (decreased birth weight)	Within human variability = 10
World Health Organization (2022)	0.02	Estimated based on PFOA water level of 100 ng/L	• WHO made a risk management call of 100 ng/L • This value can be used to estimate the comparable safe dose of 0.02 μg/kg-day using 2 L of water consumption per day, a 60 kg body weight and a 20% relative source contribution.

[a] Factor is not needed since PODs are based on serum concentrations.
[b] The use of a 3 is the US EPA default position (U.S. Environmental Protection Agency EPA, 2014); the IPCS (2005) default is 2.5.
[c] The use of a 3 is both the US EPA and IPCS default positions.
[d] This value of 8.4 is derived by dividing the value of 0.79 ml/day/kg, which is the arithmetic mean clearance of average group from Zhang et al. (2013, Table 2) by a value of 0.094 ml/day/kg, which is the arithmetic 95% lower bound clearance of sensitive group from Zhang et al. (2013, Table 2).
[e] Data base factor of 1 was considered appropriate for most PODs.
[f] This value of 0.23 ml/day/kg is the geometric mean clearance from Zhang et al. (2013, Table 2) assuming steady state.
[g] Sum of four PFAS: PFOA, PFNA, PFHxS, and PFOS.
[h] USEPA, 2023 is DRAFT RfD in response to SAB comments that EPA consider multiple studies of different endpoints in different populations to derive an RfD.

mechanistic evidence could be found apart from studies related to the disruption of lipid and fatty acid processing in the liver, which has been suggested to be responsible for many of the liver effects of PFOA observed in rodents (Andersen et al., 2021). These liver effects of PFOA have been shown to involve activation of multiple, related nuclear receptors including PPARα, PPARγ, CAR, FXR, LXR, and PXR (Andersen et al., 2021).

However, humans and rodents have been shown to have strikingly different responses, both quantitatively and qualitatively, to lipid-related receptor activation. In both species, there is a core response leading to upregulation of a family of genes controlling fatty acid processing; but, in the rat, there is a secondary pathway controlled by PPARα that makes the cells more responsive to proliferation (McMullen et al., 2020). Therefore, the relevance of rodent data for the development of a safe dose range for PFOA is somewhat uncertain. Each of the teams concluded that answers to questions regarding the relevance of animal findings and their associated MOAs to humans were most likely to come from additional *in vitro* dose-response studies with both rodent and human cells, or in experimental animal models that more closely resemble humans.

There was general agreement that the most likely MOAs for PFOA involved fatty acid mimicry. Fatty acids serve several functions in multiple systems of the body including the ability of the cell to maintain normal fatty acid homeostasis. Membrane fluid dynamics due to the insertion of PFOA into plasma membranes was raised as a possible MOA that could possibly be effective at concentrations below those associated with receptor activation. Such fluidity might be expected due to PFOA's chemical similarity to plasma lipids and limited volume of distribution from the sole clinical study in humans (suggesting quick sequestration). Insertions of PFOA molecules into the membrane without associated hydrogen bonding might make such membranes less efficient, and if given sufficient dose, might be expected to cause a host of effects. While this was considered a plausible hypothesis there is not yet adequate data supporting it. However, a recent study (Kasten-Jolly and Lawrence 2022) that examined the effects of *in vitro* exposure of human peripheral blood mononuclear cells to 1, 10, or 100 μM PFOA only observed clear effects on immune cells at the highest concentration (41 μg/mL).

Discussion then segued into the widely different choices of critical effect[5] and their tentative MOA evidence among national authorities. The critical effects identified by national and state authorities included liver effects, developmental effects (decreased body weight, delayed ossification), and impaired T-cell dependent antibody response (TDAR). Until recent years, most critical studies were animal toxicological studies. In 2020, EFSA chose a study of vaccine response to tetanus and diphtheria in one year old infants (Abraham et al., 2020) to derive a toxicity value of 0.0006 μg/kg-day based on a tolerable weekly intake (TWI) of 4.4 ng/kg-bw for four PFAS, including PFOA (EFSA, 2020). Most recently, the USEPA (2023) used epidemiological data for quantitative dose-response assessment when deriving the RfD of 0.00003 μg/kg-day for PFOA as part of recent rulemaking for National Primary Drinking Water Regulations for PFAS. The endpoints and studies

[5] Critical effect is defined here as the first adverse effect, or its known and immediate precursor, that occurs as dose is increased. It is recognized that multiple effects may be critical (occurring at or around the same dose), and that critical effects in experimental animals may not reflect these same effects found or expected in humans. However, if the critical effect is prevented, then it is assumed that all subsequent adverse effects are prevented.

described as co-critical effects included decreased antibody response to tetanus and diphtheria vaccine boosters in children (Budtz-Jorgensen and Grandjean, 2018), decreased birth weight in infants (Wikström et al., 2020), and increased total cholesterol in the general population (Dong et al., 2019) (Table 1).

Budtz-Jorgensen and Grandjean (2018) conducted benchmark dose modeling based on a birth cohort epidemiological study of PFAS and vaccine response in the Faroe Islands. The birth cohort analyzed included 401 children born during 1997–2000 (Grandjean et al., 2012). This study reported a 23% decrease in vaccine antibody titer (VAT) counts for serum anti-diphtheria at age seven per two-fold increase in PFOA and a 28% decrease in VAT counts for serum anti-tetanus at age seven per two-fold increase in PFOA at age five years and after adjusting for age, sex, booster type, and the child's specific antibody concentration at age five years (Grandjean et al., 2012). The geometric mean concentration of PFOA at age 5 years (2002–2005) was 4.1 ng/ml (interquartile range, 3.3–5 ng/ml) indicating low variability in exposure. Both the Food Standards Australia New Zealand (FSANZ, 2021) and a science panel convened to evaluate immunotoxicity of PFOA (Garvey et al., 2023) reviewed the Faroe Island data in the context of the broader toxicology and epidemiology literature, and concluded that while VAT may be a biomarker of immunomodulation, it is not suitable to establish immune suppression as a critical endpoint for quantitative risk assessment due to the complexity of accounting for a wider range of potential confounders. Currently, the animal and human evidence for associations between PFAS exposure and incidences of infectious diseases is mixed and inconclusive (Antoniou et al., 2022).

Dong et al. (2019) found an approximate 1.5 mg/dL increase in total cholesterol per ng/mL increase in PFOA in cross-sectional studies of NHANES participants from 2003 to 2017. Wikström et al. (2020) found birth weight in 1533 infants born during 2007–2010 was decreased by approximately 68 g per ln-unit of PFOA in maternal blood serum. Maternal blood was sampled early in pregnancy and the association between maternal serum PFOA and decreased birth weight in statistically significant in girls, but not boys. Other agencies reviewed epidemiological studies and found consistent associations consistent associations between PFOA in blood and increases in total cholesterol, decreases in birth weight, and decreases in antibody response to vaccine (ATSDR, 2021; EFSA, 2018, 2020). However, many of the epidemiological studies were cross-sectional designs and there remains the possibility that the associations are confounded by physiological determinants of both biomarkers of exposure and effect or that reverse causation explains the observed associations. For example, EFSA had initially derived a provisional TDI for PFOA and PFOS based on increased cholesterol as the critical effect (EFSA 2018). In the final assessment, EFSA (2020) stated that uncertainty had increased regarding a causal association between PFAS and increased cholesterol because of potential confounding by physiological determinants of PFOA serum concentrations and cholesterol via enterohepatic cycling of bile acids. This hypothesis was one of several discussed in a workshop report of potential mechanisms of increased cholesterol in relation to PFAS that included many recommendations for elucidating mechanisms (Anderson et al., 2021).

Similarly, the association between PFOA (and other PFAS) and decreases in birth weight may be confounded by pregnancy hemodynamics. Both plasma volume expansion and an increased glomerular filtration rate in pregnancy lead to increased elimination of PFOA (Verner et al., 2015). Separately, pregnant women with an impaired glomerular filtration rate are more likely to give birth to babies of lower birth weights while also having increased concentrations of PFOA due to impaired kidney filtration. Meta-analyses of birth weight and PFOA (Steenland et al., 2018) reported small summary decreases in birth weight (average of −10.5 g per ng/ml PFOA, or approximately 0.35 ounces). In sensitivity analyses to evaluate potential bias associated with timing of maternal blood sampling, Steenland et al. (2018) reported no effect on birth weight when maternal blood was sampled early in pregnancy while a larger effect on birth weight was seen when maternal blood was sampled later in pregnancy.

At a population-level, PFOA blood concentrations have decreased substantially over the past 20 years, from median concentrations of 5.2 ng/ml PFOA (95th percentile, 11.9 ng/ml) in the 1999–2000 cycle of the National Health and Nutrition Examination Survey (NHANES) to 1.47 ng/ml PFOA (95th percentile, 3.77 ng/ml) in the 2017–2018 cycle of NHANES (CDC, 2022). This suggests that there is little variation between individuals in what might be considered "background" exposure to PFOA and these small differences in concentration partially reflect differences between individuals in the underlying physiological processes that influence uptake, distribution, metabolism, and excretion as well as actual differences in environmental exposure.

Other recent research is also relevant: Crawford et al. (2023) reported a summary estimate of an approximate 12% decrease in anti-diphtheria (95% CI -23%–0%) and an approximate 12% decrease in anti-tetanus (95% CI -24%–0%) antibodies per two-fold increase in PFOA in children, a smaller effect than that reported by others (Budtz-Jorgensen and Grandjean, 2018; Grandjean et al., 2012). Porter et al. (2022) and Bailey et al. (2023) each found that PFOA was not associated with decreased response to COVID-19 vaccinations when using statistical methods that allowed for the analysis of repeated measures of serum antibody concentrations and in populations that had larger variability in PFOA blood concentrations than Abraham et al. (2020) or Budtz-Jorgensen and Grandjean (2018). Bailey et al. (2023) studied members of two communities in western Michigan where PFAS had contaminated drinking water (geometric mean 10.3 ng/mL PFOA in one community and 1.62 ng/mL PFOA in the second community). Porter et al. (2022) studied current and retired workers of one facility that manufactured POSF median PFOA concentration was 1.63 ng/ml (75th percentile, 4.54 ng/ml; 95th percentile 31.70 ng/ml. At a population level, epidemiological studies have reported inconsistent associations between PFOA blood concentrations and risk of infections, infectious diseases (including hospitalizations) with some studies reporting positive associations (e.g., Dalsager et al., 2021, Timmerman et al., 2020), most studies reporting null associations (e.g., Ait-Bamai et al., 2020; Huang et al., 2020; Manzano-Salgado et al., 2019; Grandjean et al., 2020) and one study reporting a negative association in boys and a positive association in girls (e.g., Fei et al., 2010; Goudarzi et al., 2017) while other studies reported mixed evidence (Bulka et al., 2021).

As a result, not all critical effects were thought relevant to risk assessment intended to protect human health, especially in the absence of a postulated mode of action linking early necessary key events to late key events. While observed associations between PFOA blood concentrations in populations and diminished levels of serum antibodies following immunization to one or more specific types of vaccines might prompt additional investigation of immunosuppressive effects, the current serum concentration/antibody level data were not deemed suitable for developing a safe dose since the assessments were based upon secondary immune response (response to diphtheria and tetanus boosters), rather than primary, which contradicts the WHO immunotoxicology guidelines (derived from Van Loveren et al., 1999), as a reliable quantitative measure of immune function. Moreover, as several team members noted, it was unclear whether small decreases in antibody response to vaccines are clinically significant because vast inter- and intra-individual human variability in natural vaccine response exists. This variability precludes any definitive statement in the choice of this endpoint as the critical effect. Recently, a SciPinion panel (2023, also published as Garvey et al., 2023) on immunotoxicity of PFOA suggests that the vaccine threshold of 0.1 IU/ml was not helpful for risk assessment since it is a surrogate of protection and basic immunity is presumed at even lower antibody concentrations (WHO, 2009), most recently 0.01 IU/ml.

Clinical effects in many of the other human observational studies, such as increases in cholesterol and decreases in birth weight, were also of small magnitude or imprecisely estimated. Investigators generally

Table 2
International collaboration consensus statements.

Consensus on Mode of Action	Several MOAs could be envisioned but not enough evidence exists to establish any one of these MOAs with certainty. Certain effects appear to be irrelevant for the determination of a safe dose in the absence of mode of action information relevant to humans, specifically differences in cholesterol & vaccine response. Studying inflection points or perhaps hormesis might help resolve why we have 100,000-fold differences in the PFOA safe dose internationally.
Consensus on Critical Effect	Existing human observational studies cannot be used reliably for developing the critical effect in the absence of mechanistic data relevant to humans at serum concentrations seen in the general public. Existing human observational vaccine findings are not primary immune responses and not of clinical relevance. Epidemiological studies of risk of infectious diseases have been mixed. In populations with higher PFOA blood concentrations, there was no association with antibody response to MRNA vaccines against COVID-19. The overall uncertainty in the database is sufficient to give pause to the development of a credible critical effect for PFOA. However, in recognition of the importance of managing PFOA potential health risks, a provisional approach could be developed based on several experimental animal studies.
Consensus on Extrapolation Method	The various positions of the three science teams overlap, so developing a provisional range in the PFOA safe dose, based on differing experimental animal studies, seemed reasonable. Human data are not an acceptable basis of the safe dose. PFOA has an enormous database, but still has some uncertainty, suggesting that a 3-fold factor may be reasonable. A clearance value from the Zhang et al. (2013) should be used with any of the experimental animal points of departure and can be used for a data-derived value for human toxicokinetics.

reported that these differences were within normal laboratory reference ranges in relation to PFOA blood concentrations and thus might reflect pharmacokinetic bias or reverse causality due to the fatty acid mimicry based on PFOA's chemical structure (Andersen et al., 2021). Although cholesterol changes did not appear definitive and were deemed not likely to be the critical effect, studying other inflection points or hormetic responses seemed worthwhile. Reverse causality or confounding by physiological determinants of exposure and effect biomarkers may apply to more than one effect.

An argument can be made that small differences in clinical chemistry biomarkers or clinical effects, such as decreases in antibody concentrations or increases in cholesterol associated with PFOA blood concentrations, can lead to a shift in the population distribution of these clinical parameters, and potentially result in a higher proportion of individuals that experience increased risk of clinical disease. The basis of this argument is an assumption that a causal relationship exists between PFOA and clinical disease in the population. However, increases in frequency and occurrence of infectious disease have only been inconsistently associated with PFOA. For example, some studies have reported an increased risk of hypercholesterolemia (cholesterol level of $\geq$240 mg/dL) with PFOA (Steenland et al., 2009; Winquist and Steenland 2014; Lin et al., 2019) while cardiovascular disease has not been increased with PFOA. In general, studies have not found an increased risk of low birth weight (<2500 g) or long-term developmental outcomes associated with decreased birth weight. There is currently insufficient evidence of these adverse effects at the population-level. *In vitro* studies with human cells/tissues over a range of relevant concentrations, similar in design to Kasten-Jolly and Lawrence (2022), are critically needed to elucidate potential MOAs for effects reported in epidemiological studies in order to support any reliable assessment of causality.

A final discussion ensued over whether the dose response information was adequate to develop a safe dose range. This question led to discussion of inflection points or potentially hormetic responses that might yield useful information, such as human observational studies showing an increase in cholesterol at mean or median blood concentrations of 1000 ng/ml or less (Sakr et al., 2007a, 2007b; Steenland et al., 2009; Eriksen et al., 2013; Dong et al., 2019) but the sole human clinical study on PFOA showed decreases at blood concentrations of 175,000–230,000 ng/ml (Convertino et al., 2018).

After presentations, clarifying questions and discussion, the consensus positions summarized in Table 2 and shown below were developed:

1. Several MOAs could be envisioned but not enough evidence exists to establish any one of these MOAs with certainty.
2. Some effects appear irrelevant for the determination of a safe dose from current epidemiology data, specifically cholesterol changes and vaccine status.
3. Studying inflection points or perhaps hormesis might help resolve why we have 100,000-fold differences in estimated PFOA safe doses internationally. While differences among such groups can often span a range of 3-fold due to differing times of analyses and methods, this large difference in PFOA is clearly not acceptable for informing confident decision-making, nor can all groups be correct.

3.2. *Determination of studies for PFOA's critical Effect(s)*

After reviewing the plethora of relevant information, none of the teams independently considered the epidemiology data, composed primarily of observational studies, to be sufficient to determine a critical effect considering the lack of information regarding the mode of action (s). The results from these studies were considered not only potentially confounded, with confounding that was not readily quantified, but also to have serum concentrations from unidentified sources of exposure to PFOA that were not significantly different from background in most studies, making it difficult or impossible to assign a clear exposure-response association, much less causation.

Because of these multiple and significant concerns regarding human observational data, all three teams focused on experimental animals for consideration of the critical effect. However, each team independently reached a different conclusion about the critical effect. One team considered monkey studies as most relevant due to the closeness to humans with PPARα activation for potential liver effects and general physiology, and the difficulty in interpretation of rodent developmental effects. Non-adverse liver effects were seen at all the doses tested in monkeys (3, 10, 20, and 30 mg/kg-day). These effects correlated roughly with non-adverse liver effects seen in the human observational studies and was consistent with the sole human clinical study by Elcombe et al. (2013).[6] Although these liver effects were not considered adverse in monkeys, mortality was also observed in monkeys at the higher doses leading to a clear No Observed Adverse Effect Level/Low Observed Adverse Effect Level (NOAEL/LOAEL) boundary. One member from this team reached out to the investigators of the monkey studies to ask for any additional data but none were available.

Another team selected rodent developmental studies rather than liver changes, and specifically Lau et al. (2006) as most relevant due to the consistency in response of several rodent species considering that the

[6] The human clinical study of Elcombe et al. (2013) is in the same range and showed no overt effects (50–1200 mg/week ÷ 7 days ÷ 70 kg ~ 0.1–2.4 mg/kg-day).

likely MOA involved fatty acid mimicry. Specifically, PFOA has access to mid-chain fatty acid transport, and biliary and renal excretion and resorption. And while such mimicry might be readily handled by organs such as the liver, it might more readily disturb fatty acid homeostasis in the developing organism, thus supporting the selection of developmental effects as the critical, or perhaps co-critical effect. Moreover, PPARα induced liver effects occurred in rodents at about a 10-fold higher dose than those evoking developmental toxicity.

The third team did not judge that the liver effects seen in monkeys, or perhaps other species, were appropriate, since the effects seen were not adverse. Nor did this team consider the developmental effects by Lau et al. (2006) appropriate due to statistical issues associated with the study. Rather, this team was of the general opinion that the overall database was insufficient at this time to make a reliable judgment of critical effect and supported this position with the observation that different health agencies around the world have come to very different decisions. While these differences may not be direct evidence for the overall weakness in the database, the WHO (2022) came to the same conclusion. Specifically, the overall database was considered too uncertain to determine a scientifically based judgment of critical effect. Instead, WHO (2022) made a risk management recommendation.

Finally, all three teams did not rely on several potentially relevant studies of PFOA, and after discussion, agreed that the two-generation study by Macon et al. (2011) was not considered reliable for development of a safe dose range because the statistics in this study appeared to be based on pups and not their mothers. Using pups as the basis of the assessment is not in accordance with US EPA (1991) guidelines. In addition, neither Onischenko et al. (2011) nor Koskela et al. (2017) were used because of too few animals and limited doses used in these studies to generate a confident estimate of the NOAEL/LOAEL interface, and furthermore, it was not certain that the statistics were based on the maternal experimental animals.

After these presentations, clarifying questions and discussion, the following consensus positions were developed as summarized in Table 2 and shown below:

1. Should human studies be used for the development of the critical effect?

No, existing human observational studies cannot be used reliably for this purpose. For example, changes in cholesterol appear to have only a small effect at low doses and an opposite effect at higher doses. These studies may support the choice of critical effect with some of the experimental animal work, however.

2. Should vaccine responses be used for the development of the critical effect?

No, existing human observational vaccine findings are not primary immune responses and of questionable clinical relevance. Based on epidemiological study results, it is premature to assume that a population shift in the distribution of antibody concentrations – if one exists – results in increased risk of susceptibility to diseases. Moreover, higher dose worker exposures do not suggest immune responses.[7]

3. Should experimental animal studies be used for the development of the critical effect?

The overall uncertainty in the database, both epidemiology and experimental animal, is sufficient to give pause to the development of a credible critical effect for PFOA. This conclusion is similar to what WHO (2022) found and for the same or similar reasons.

[7] Experimental animal work indicates some immune toxicity but only at doses higher than those suggested in human observational studies.

4. However, in recognition of the importance of managing PFOA potential health risk, and despite the overall difficulties in the experimental animal studies, a provisional approach was explored as follows:
   - o Frank toxicity in both monkeys and rats has been observed in a dose related manner. We might be able to tie these effects into other liver and or developmental endpoints. One member volunteered to conduct a Benchmark Dose (BMD) approach on the relevant monkey and rodent studies and send this to all three teams for consideration (information available upon request).
   - o One team member asked participants to critique and improve upon Green and Crouch (2019) who reviewed the basis of Massachusetts Department of Environmental Protection's Groundwater and Soil Standards for PFOA and PFOS and suggested an alternate animal test model and target endpoint (i.e., monkey liver toxicity) using a BMD approach.
   - o PFOA is the fluorinated version of the naturally occurring caprylic acid. A big difference between these two chemicals is their half-lives in the human body. Considering whether potential long-term toxicity from caprylic acid matches any of the findings with PFOA may prove useful.

3.3. Choice of extrapolation method

All teams developed a range in the PFOA safe dose. One team decided to build a range in the safe dose based on several studies of developmental effects in mice. The first study was Lau et al. (2006) with a NOAEL of 23 μg/ml for dose dependent growth deficits in offspring. Other studies considered were Onishchenko et al. (2011), Koskela et al. (2017), Loveless et al. (2006), and Macon et al. (2011). The resulting safe dose range from this collection of studies was 0.011–0.27 μg/kg-day.

A second team remained of the opinion that the overall database was insufficient at this time to make a reliable judgment of critical effect. Nevertheless, in order to develop a provisional range, this team focused on two mouse studies, specifically the developmental/reproduction study of Abbott et al. (2007) and the immunotoxicity study of DeWitt et al. (2016), with a range in the NOAELs from 0.3 to 0.94 mg/kg-day. The resulting safe dose range was 3–9.4 μg/kg-day from these two values. This team also developed a separate range by adjusting the kinetic comparison between mice and humans based on the work of Zhang et al. (2013) to develop a range of 0.3–515 μg/kg-day.

The last team considered liver effect as best meeting the criteria laid out initially and that the results in monkey were most relevant due to comparability of PPARα activation for potential liver effects and general physiology with humans, despite the small numbers of animals and some inconsistency with the reported observations. Butenhoff et al. (2002) showed liver weight increases in monkeys and Green and Crouch (2019) developed a benchmark concentration from these data of 19 μg/ml based on data from this study.

Discussion around these various ranges centered on whether the use of a clearance value from human study Zhang et al. (2013), as describe by Campbell et al. (2022), would be a better choice than clearance values from human observational studies described by Lorber and Egeghy (2011). Also discussed was whether the use of a database uncertainty factor would be reasonable, given the large uncertainty in the overall database. Some concern was also expressed over the use of Onishchenko et al. (2011) and Koskela et al. (2017) due to the small number of experimental animals and potential use of pup-based statistics. Lastly, the large range in the second team's calculation appeared to be due to conflating the mouse to human uncertainty factor for toxicokinetic variability with the within human uncertainty factor for toxicokinetic variability. Separating these two seemed reasonable to all participants.

The following consensus positions were developed as summarized in Table 2 and shown below:

Table 3
Experimental animal studies as the basis of the provisional safe PFOA dose.[a]

Reference	Safe Dose ug/kg-day	Point of Departure (POD)
Butenhoff et al. (2002).	0.06	**Monkey**: Point of Departure = 19 μg/ml from Green and Crouch (2019) based on a serum PFOA benchmark concentration (BMC) for *increased liver weight*
Lau et al., 2006	0.07	**Mouse**: Point of Departure = 1 mg/kg-day or 23 μg/ml No Observed Adverse Effect Level (NOAEL) for *dose-dependent growth deficits* for gestation days 1–17
Loveless et al. (2006)	0.01	**Mouse**: Point of Departure = 4.35 μg/ml based on a serum PFOA benchmark concentration by New Jersey/New Hampshire (Post, 2021) for *lipid parameters/relative liver weight* in male mice
Abbott et al. (2007)	0.03	**Mouse**: Point of Departure = 0.3 mg/kg-day (10.4 μg/ml) NOAEL for *neonatal survival*
DeWitt et al. (2016)	0.07	**Mouse**: Point of Departure = 0.94 mg/kg-day (no serum values available) NOAEL for *immune suppression*

[a] See Appendix 2 for details of the various calculations.

1. The various positions of the three science teams appear to overlap, so that developing a provisional range in the PFOA safe dose, based on differing experimental animal studies, seemed reasonable. After discussion by all three teams, there was an agreement to develop a range of the safe dose based on liver effects in monkeys and developmental and immunological effects in mice.
2. The use of human data for this exercise was not entertained, consistent with the earlier consensus of all three science teams that the existing human data were not adequate for identifying safe doses.
3. PFOA has an enormous database, but still has some uncertainty, especially in choosing the critical effect largely due to the relevance to humans of mode(s) of action in animals. A factor of 3-fold for this area of uncertainty should be considered.[8]
4. The use of the average clearance value (either mean, median, mode or geometric versions of these) from the Zhang et al. (2013) human study should be used with any of the experimental animal points of departure if in ug/ml of serum, or by comparison with kinetic information from the relevant species if the points of departure are in units of dose. Moreover, the Zhang et al. (2013) also shows human variability that can be used to develop a data-derived value for within human toxicokinetics. A preliminary analysis by Team 1 gave this a value of ~9-fold.

3.4. Development of a provisional safe dose range

A specific provisional range in the PFOA safe dose was subsequently developed based on information from the various consensus calls regarding PFOA's underlying MOA for various effects, its likely critical effect(s), and the extrapolation of experimental or human data to the presumed sensitive subgroup. The range of the PFOA safe dose is provisionally estimated to be 0.01 to 0.07 μg/kg body weight-day (10–70 ng/kg body weight-day) based on points of departure in Table 3and uncertainty factors from the studies described in Appendix 2.

4. Discussion

PFAS in general, and PFOA in particular, differ from many other chemicals and mixtures for which safe doses have been estimated. Exposure-response data for the two populations that have been most highly exposed to PFOA are limited in scope. These two PFOA-exposed groups were (i) workers who manufactured PFOA, and/or were otherwise occupationally highly exposed and (ii) a small group of end-stage cancer patients who were administered large doses of PFOA as a cancer chemotherapeutic drug (Elcombe et al., 2013; Convertino et al., 2018). Notably, though, observations in both such groups fail to indicate that PFOA presents a significant risk of toxicity.

As noted above, the observational epidemiologic data that associate PFOA body burdens *in the general public* with various biological endpoints cannot, in our judgment, serve as reliable basis for safe dose-assessment. These studies were considered not only unquantifiable and confounded but also to have exposures that were not significantly different from background, which makes the interpretation of any association problematic. We recommend that the reliability of the results from these epidemiological studies are reconsidered after mechanistic data become available that supports (or argues against) the hypothesized MOAs; however, in the absence of mechanistic data relevant to humans at serum concentrations seen in the general population, the uncertainties of the reliability of the human data that show small differences in clinical biomarkers are substantial.

At present, the best that can be done, we believe, is to rely on dose-response data from PFOA-exposed laboratory animals. Mice and rats tend to be good models for humans for most chemicals; but for PFOA, mice and rats are rather less reliable human-models. Monkeys are much better models; but, of course, the numbers of monkeys that have been PFOA-exposed are small; and the endpoints that have been examined remain limited. Future research using non-human primates might well yield useful information for purposes of human health risk assessment.

With regard to the potential carcinogenicity of PFOA, there was general agreement that the EPA's proposed change in the categorization of PFOA from "suggestive evidence" to "likely carcinogen" is not justified. The EPA's determination was based primarily on clear evidence of PFOA-induced liver tumors in rodents and variously published associations between PFOA concentrations and kidney cancer in humans (Barry et al., 2013; Vieira et al., 2013; Steenland and Woskie, 2012; Shearer et al., 2021), and the EPA identified a case-control study of renal cell carcinoma (RCC) nested within the screening arm of Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial study as particularly influential (Shearer et al., 2021).

However, as is well known, rodent liver tumors are observed only at doses associated with peroxisomal proliferation, a response of limited relevance to human exposures. And, on our opinion, the relevant epidemiological studies have not adequately considered the potential for confounding by impaired renal function, which is associated with both PFOA clearance and kidney cancer.

With regard to kidney cancer, we note that if PFOA were a genuine cause of this cancer-type in humans, then one might expect that the massive doses of PFOA used in the rodent (and monkey) bioassays would have also induced kidney tumors. Yet, they did not.

Kidney cancer is frequently associated with impaired renal function and alterations in renal function that resulted in decreased PFOA excretion would result in a consequent increased PFOA concentration in serum. Cross-sectional analyses of adults exposed at background levels (Shankar et al., 2011) and of children exposed at high levels (Watkins et al., 2013) found a positive association between lower kidney function and higher measured serum PFOA. Dhingra et al. (2017), performed an analysis of cross-sectional studies reporting associations between PFOA and renal function, and concluded that pharmacokinetic confounding

[8] After the meeting several members pointed out that a comprehensive two-generation reproductive toxicity study was conducted in Sprague-Dawley Rats by Butenhoff et al. (2004). EPA used this study to help justify a database UF of 1. See Dourson et al. (1992) for USEPA's justification of minimum database and the use of a related uncertainty factor.

led to the observed associations. While Shearer et al. (2021) adjusted their results for estimated glomerular filtration rate (eGFR), adjusting for eGFR alone would not adequately control for this potential confounding due to the extensive role of renal transporters in the clearance of PFOA.

The international process described in this brief communication has several advantages. Many of the scientists who volunteered for this task are well published in the area of PFOA, or in one or more of PFOA's designated critical effects, or in one or more of the extrapolation methods used to determine the provisional range of its safe dose. Many of these scientists are also intimately familiar with one or more of the agency positions on PFOA. Despite these credentials and familiarity, or perhaps because of them, uniformity of thought was not present, at least initially, and the call meetings were often lively but respectful. Therefore, the eventual consensus of 27 scientists from 8 countries over 6 months can perhaps be afforded a higher degree of trust than position developed with fewer or less diverse viewpoints.

This process, however, also has its drawbacks. First, it depended on group or self-nominations and from individuals from groups that may or may not appreciate a particular agency position. This concern was addressed in two ways. First, nominations to the Advisory Committee were solicited by the Steering Committee from known experts in the field along with an open nomination process. Members were then selected by the Alliance for Risk Assessment (2022) Steering Committee after a review of credentials. This Steering Committee is composed of 5 scientists, 3 from governments, one from a university and one from an environmental science non-government organization. In turn, members of the 3 science teams were selected by the Advisory Committee after an open nomination process and review of proffered biographical sketches/resumes. Balances were maintained among affiliations within each science team. A second drawback is that no funding was received for this work, making it difficult to follow-up on nuances of data that needed additional consideration.

The suggested provisional safe dose range of this international collaboration is 0.01–0.07 μg/kg-day. This range encompasses the single value of Health Canada (2018) and the projected range of values for the WHO (2022) and lies slightly below the value of Food Standards of Australia and New Zealand (FSANZ, 2017; Australian Government, 2022). However, this range is well above the single values of both EFSA (2020) and EPA (2023). The principal reasons for the larger disparity between this provisional range with these latter two single values is the unanimous judgment of the international collaboration that the existing human cancer and noncancer data are not sufficiently credible as a basis of the PFOA safe dose in the absence of mechanistic data that are relevant to humans at serum concentrations seen in the general population. In this regard, Health Canada, the WHO and Food Standards of Australia and New Zealand are in agreement with the Collaboration—the use of human data is not sufficiently credible as the basis for the PFOA safe dose.

Additional thoughts from other colleagues are welcome. We continue to believe that,

> … *It is the mark of an instructed mind to rest satisfied with the degree of precision which the nature of the subject permits and not to seek an exactness where only an approximation of the truth is possible.* Aristotle

Disclaimers

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government. JS is an employee of the U.S. Government. This work was prepared as part of his official duties. Title 17, U.S.C., §105 provides that copyright protection under this title is not available for any work of the U.S. Government. Title 17, U.S.C., §101 defines a U.S. Government work as a work prepared by a military Service member or employee of the U.S. Government as part of that person's official duties.

HC and LD are salaried employees of Ramboll US Consulting, Inc., a consulting firm that provides scientific and technical support to a variety of clients in private and public sectors. Participation in the project and the preparation of the manuscript reflects the professional work of the authors and may not necessarily reflect the views of Ramboll US Consulting, Inc. or its parent company, Ramboll Group A/S. HC and LD did not receive outside funding to participate in this project or prepare the manuscript; the manuscript was prepared on their own time or supported by Ramboll as part of their usual employment responsibilities. Prior to preparing the manuscript, LD has been retained as an expert witness on behalf of defendants in litigation matters pertaining to certain PFAS.

MD and BG are employees of Toxicology Excellence for Risk Assessment (TERA), which has worked over a number of years for governmental and nongovernmental sponsors on PFAS issues. However, no outside funding was accepted to prepare this manuscript nor to do the analyses underlying it.

THK is an employee of GHD, Inc., a consulting firm, serving a variety of clients in the private and public sector. The time spent on this manuscript was performed on the author's own time and was not supported financially by any entity. The views expressed in this article are those of the author and do not necessarily reflect the position or policy of GHD.

FP is an employee of RHP Risk Management, a consulting firm, serving a variety of clients in the private and public sector. RHP has performed consulting and testifying work on various matters including PFAS. Neither FP nor RHP has shared this work with any RHP client nor elicited input into the design, preparation, or review of this work prior to publication. The time spent on this manuscript was either supported by RHP or was performed on the author's own time.

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the New Zealand Environmental Protection Authority.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Several of the authors have worked over a number of years for various sponsors on PFAS issues as shown in part below. However, no outside funding was accepted to do this work by the Alliance for Risk Assessment.

Data availability

No data was used for the research described in the article.

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Appendix 2

Monkey: Point of Departure = 19 μg/ml from Green and Crouch (2019) based on a serum PFOA benchmark concentration (BMC) for *increased liver weight* in Butenhoff et al. (2002).

- Monkey to human toxicokinetic factor = 1 [Factor is not needed since BMD is based on serum concentration]
- Monkey to human toxicodynamic factor = 2.5 [IPCS (2005) default or 3 U.S. Environmental Protection Agency EPA (2014) default]
- Human toxicodynamic factor = 3 [default of IPCS (2005) and U.S. Environmental Protection Agency EPA (2014)]
- Human toxicokinetic factor = 8.4 [0.79 ml/day/kg arithmetic mean clearance of average group from Zhang et al. (2013, Table 2) ÷ 0.094 ml/day/kg arithmetic 95% lower bound clearance of sensitive group from Zhang et al. (2013, Table 2)]
- Database uncertainty factor = 1 (Although it could be argued that the small number of animals in the study justifies an additional uncertainty factor; the counter-argument is that these are primates. See also footnote 7.)
- RfD serum concentration = 0.25 μg/ml [19 μg/ml ÷ (1 × 3 x 3 × 8.4 x 1) = 0.25]
- RfD = 0.06 μg/kg-day [0.25 μg/ml x 0.23 ml/day/kg [geometric mean clearance from Zhang et al. (2013, Table 2) assuming steady state]

Mouse: Point of Departure = 1 mg/kg-day or 23 μg/ml No Observed Adverse Effect Level (NOAEL) for *dose-dependent growth deficits* in the Lau et al., 2006 for gestation days 1–17

- Mouse to human toxicokinetic factor = 1 (Factor is not needed since BMD is based on serum concentration)
- Mouse to human toxicodynamic factor = 2.5 [IPCS (2005) default or 3 U.S. Environmental Protection Agency EPA (2014) default]
- Human toxicodynamic factor = 3 [default of IPCS (2005) and U.S. Environmental Protection Agency EPA (2014)]
- Human toxicokinetic factor = 8.4 [0.79 ml/day/kg arithmetic mean clearance of average group from Zhang et al. (2013, Table 2) ÷ 0.094 ml/day/kg arithmetic 95% lower bound clearance of sensitive group from Zhang et al. (2013, Table 2)]
- Database uncertainty factor = 1 (Although it has been argued that problems with this study might justify an additional uncertainty factor; the counter-argument is that US EPA uses a value of 1. See also footnote 7.)
- RfD serum concentration = 0.30 μg/ml [23 μg/ml ÷ (1 × 3 x 3 × 8.4 x 1) = 0.30]
- RfD = 0.07 μg/kg-day [0.30 μg/ml x 0.23 ml/day/kg [geometric mean clearance from Zhang et al. (2013, Table 2) assuming steady state]

Notes:

- It could be argued that the fetal toxicity is secondary to disruption of lipid metabolism in the dam, as evidenced by the increased maternal liver weight at all doses.
- Several authorities consider the 1 mg/kg/d dose to be a LOAEL, but effects at the lowest dose were only observed in dams. Resulting US State RfDs range from 0.005 to 0.020 μg/kg-day (Post, 2021).

Mouse: Point of Departure = 4.35 μg/ml based on a serum PFOA benchmark concentration by New Jersey/New Hampshire (Post, 2021) for *lipid parameters/relative liver weight* in male mice from Loveless et al. (2006).

- Mouse to human toxicokinetic factor = 1 (Factor is not needed since BMD is based on serum concentration)
- Mouse to human toxicodynamic factor = 2.5 [IPCS (2005) default or 3 U.S. Environmental Protection Agency EPA (2014) default]
- Human toxicodynamic factor = 3 [default of IPCS (2005) and U.S. Environmental Protection Agency EPA (2014)].
- Human toxicokinetic factor = 8.4 [0.79 ml/day/kg arithmetic mean clearance of average group from Zhang et al. (2013, Table 2) ÷ 0.094 ml/day/kg arithmetic 95% lower bound clearance of sensitive group from Zhang et al. (2013, Table 2)]
- Database uncertainty factor = 1 (See footnote 7.)
- RfD serum concentration = 0.058 μg/ml [4.35 μg/ml ÷ (1 × 3 x 3 × 8.4 x 1) = 0.058]
- RfD = 0.01 μg/kg-day [0.058 μg/ml x 0.23 ml/day/kg [geometric mean clearance from Zhang et al. (2013, Table 2) assuming steady state]

Notes:

- It could be argued that a toxicodynamic UF of 0.1 could be applied for rodent to human differences in response to PPAR activation.

Mouse: *Point of Departure = 0.3 mg/kg-day (10.4 μg/ml) NOAEL for neonatal survival found in Abbott et al. (2007)*

- Mouse to human toxicokinetic factor = 1 (Factor is not needed since BMD is based on serum concentration)
- Mouse to human toxicodynamic factor = 2.5 [IPCS (2005) default or 3 EPA (2014) default]
- Human toxicodynamic factor = 3 [default of IPCS (2005) and EPA (2014)].
- Human toxicokinetic factor = 8.4 [0.79 ml/day/kg arithmetic mean clearance of average group from Zhang et al. (2013, Table 2) ÷ 0.094 ml/day/kg arithmetic 95% lower bound clearance of sensitive group from Zhang et al. (2013, Table 2)]
- Database uncertainty factor = 1 (See footnote 7)
- RfD serum concentration = 0.14 μg/ml [10.4 μg/ml ÷ (1 × 3 x 3 × 8.4 x 1) = 0.14]
- RfD = 0.03 μg/kg-day [0.14 μg/ml x 0.23 ml/day/kg [geometric mean clearance from Zhang et al. (2013, Table 2) assuming steady state]

Mouse: Point of Departure = 0.94 mg/kg-day (no serum values available) NOAEL for *immune suppression* found in DeWitt et al. (2016).

Based on Lau et al., (2006), the serum level associated with in the mouse repeated dosing at 1 mg/kg-day is 23 μg/ml. Therefore, dosing at 0.94 mg/kg/d is estimated to be associated with a serum level of 22 μg/ml.

- Mouse to human toxicokinetic factor = 1 (Factor is not needed since BMD is based on serum concentration)
- Mouse to human toxicodynamic factor = 2.5 [IPCS (2005) default or 3 EPA (2014) default]
- Human toxicodynamic factor = 3 [default of IPCS (2005) and EPA (2014)].
- Human toxicokinetic factor = 8.4 [0.79 ml/day/kg arithmetic mean clearance of average group from Zhang et al. (2013, Table 2) ÷ 0.094 ml/day/kg arithmetic 95% lower bound clearance of sensitive group from Zhang et al. (2013, Table 2)]
- Database uncertainty factor = 1 (See footnote 7.)
- RfD serum concentration = 0.29 μg/ml [22 μg/ml ÷ (1 × 3 x 3 × 8.4 x 1) = 0.29]
- RfD = 0.07 μg/kg-day [0.29 μg/ml x 0.23 ml/day/kg [geometric mean clearance from Zhang et al. (2013, Table 2) assuming steady state]

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REVIEW ARTICLE

United States Environmental Protection Agency's Perfluorooctanoic Acid, Perfluorooctane Sulfonic Acid, and Related Per- and Polyfluoroalkyl Substances 2024 Drinking Water Maximum Contaminant Level: Part 2 – Fifteen Misconceptions About the Health Hazards

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ABSTRACT

This paper examines widely held beliefs about the six per- and polyfluoroalkyl substances (PFAS) addressed in the final U.S. Environmental Protection Agency's (EPA) rule on PFAS in drinking water (e.g., the Maximum Contaminant Levels - MCLs). Based on our understanding of the scientific literature and the comments submitted by stakeholders regarding the EPA's regulation that was promulgated in April 2024, we identified 15 misconceptions that had a weak scientific foundation. These are now memoralized in the MCLs for the six PFAS but remain debated due to ongoing ambiguous research findings. Many critics of the MCLs found the EPA's systematic review of the published relevant information, particularly the toxicology of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), to be inadequate. The following seven views are among the most important. First, the EPA asserted that the toxicology of these six chemicals was poorly understood and lacked sufficient data to determine a safe daily intake level for chronic health effects; nonetheless, they promulgated what may be the costliest environmental regulation to date. Notably, adverse effects remain difficult to demonstrate in occupationally exposed individuals even at blood concentrations 50-100 times higher than current background PFAS levels. Second, the Agency indicated that the epidemiology data showed that exposure to PFOA and PFOS caused kidney and potentially other cancers, yet the data were equivocal and do not support that assertion. Third, it was stated that specific non-cancer effects, such as heart disease, would be prevented under the promulgated rule; however, the studies that they relied upon do not show an increased incidence of heart disease even in highly exposed populations. Fourth, the Agency relied on animal data to support its views on the likely toxic effects in humans, despite ample toxicology data that animals, particularly rodents, are poor predictors of the human response to PFAS exposures. Fifth, the EPA predicted a reduction in healthcare expenditures that would offset much of the cost of complying with the MCL, but, they did not have adequate data to support this prediction. Sixth, the EPA suggested that these six PFAS act through a shared mechanism of action (i.e., PPARα pathway induction); however, data indicate that PPARα induction in humans may be 80% less than what is observed in rodents. Also, induction of the PPARα pathway is not a cause of systemic disease. Seventh, the Agency failed to disclose that achieving the new MCL would yield negligible reductions in blood PFAS levels even among highly exposed populations, given drinking water accounts for only 20% or less of total PFAS exposure. The survey that could answer that question, the EPA's fifth Unregulated Contaminant Monitoring Rule, was only 25% complete at the time the MCL was promulgated. Overall, our analysis concluded that while the EPA's intent to regulate these chemicals due to their environmental presence was necessary, the derivation of the MCLs and the alleged health effects was based on the application of the precautionary principle rather than robust scientific evidence.

Abbreviations: ACC: American Chemistry Council; ADME: Absorption, Distribution, Metabolism, and Elimination; AFFFs: Aqueous Film-Forming Foams; ALB: Albumin; ALT: Alanine Aminotransferase; AMWA: Association of Metropolitan Water Agencies; AOPs: Adverse Outcome Pathways; APFO: Ammonium Perfluorooctanoate; ART-PFAS: Accelerated Remediation Technologies-PFAS; AST: Aspartate aminotransferase; ATSDR: Agency for Toxic Substances and Disease Registry; AWWA: American Water Works Association; BIL: Bipartisan Infrastructure Law; BMD: Benchmark Dose; BMR: Benchmark Response

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Level; CDC: U.S. Center for Disease Control and Prevention; CERCLA: Comprehensive Environmental Response, Compensation, and Liability Act; ChE: Cholinesterase; CONTAM Panel: European Food Safety's Authority's Panel on Contaminants in the Food Chain; CSF: Cancer Slope Factor; CVD: Cardiovascular Disease; DBPs: Disinfection Byproducts; DDT: Dichloro-diphenyl-trichloroethane; DOD: U.S. Department of Defense; DWSRF: Drinking Water State Revolving Fund; ECHA: European Chemicals Agency; eGFR: Estimated Glomerular Filtration Rate; EFSA: European Food Safety Authority; EPA: U.S. Environmental Protection Agency; FDA: U.S. Food and Drug Administration; FSANZ: Food Standards Australia New Zealand; FFY: Federal Fiscal Year; GAC: Granulated Activated Carbon; GGT: Gamma-Glutamyl Transferase; HALs: Health Advisory Levels; HBGVs: Health-Based Guidance Values; HFPO–DA: Hexafluoropropylene Oxide Dimer Acid; HI: Hazard Index; HDL: High-Density Lipoprotein; IARC: International Agency for Research on Cancer; IRIS: Integrated Risk Information System; LCTs: Leydig cell tumors; LHA: Lifetime Health Advisory; LDL: Low-density Lipoprotein; MCL: Maximum Contaminant Level; MCLGs: Maximum Contaminant Level Goals; MCSS: Minnesota Cancer Surveillance System; MIE: Molecular Initiating Event; MRL: Method Reporting Limit; MOA: Mechanism of Action; NAS: National Academy of Sciences; NASEM: National Academies of Sciences Engineering and Medicine; NIH: National Institutes of Health; NHANES: National Health and Nutrition Examination Survey; NPDWR: National Primary Drinking Water Regulations; NRWA: National Rural Water Association; OECD: Organization for Economic Co-Operation and Development; OMB: Office of Management and Budget; PA: Prealbumin (Transthyretin); PCBs: Polychlorinated Biphenyls; PECO: Population, Exposure, Comparator, and Outcomes criteria; PFAAS: perfluoroalkyl acids; PFAS: Per- and Polyfluoroalkyl Substances; PFBS: Perfluorobutane Sulfonic Acid; PFDA: Perfluorodecanoic acid; PFOA: Perfluorooctanoic Acid; PFOS: Perfluorooctane Sulfonate; PFHxS: Perfluorohexane Sulfonic Acid; PFNA: Perfluorononanoic Acid; PLCO: Prostate, Lung, Colorectal, and Ovarian Screening Trial; POD: Point of Departure; PPAR: Peroxisome Proliferator-Activated Receptor; PWSs: Public Water Systems; RfD: Reference Dose; RCC: Renal Cell Carcinoma; RIA: Regulatory Impact Assessment; RIVM: National Institute for Public Health and the Environment in the Netherlands; RSC: Relative Source Contribution; SAB: Scientific Advisory Board; SDH: Sorbitol Dehydrogenase; SDWA: Safe Drinking Water Act; SGOT: Serum Glutamic-Oxaloacetic Transaminase; SPGT: Serum Glutamic Pyruvic Transaminase; TB: Total Bilirubin; TC: Total Cholesterol; TFE: Tetrafluoroethylene; THM: Trihalomethanes; TEQs: Toxic Equivalents; TP: Total Protein; UCMR3: Unregulated Contaminant Monitoring Rule 3; UCMR5: Unregulated Contaminant Monitoring Rule 5; U.S: United States; WCRS: Wisconsin Cancer Reporting System; WEF: Water Environment Federation; WHO: World Health Organization; WSSC: Washington Suburban Sanitary

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Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of over 4,700 synthetic chemicals that have been commercially produced since the 1940s (Glüge et al. 2020). There is not a universally defined set of criteria for which chemicals are classed as PFAS (Buck et al. 2011); nonetheless, in this paper, we utilize the 2021 OECD definition of what constitutes PFAS (Organisation for Economic Co-operation and Development (OECD) 2021). PFAS can also be subcategorized by chain-length (i.e., short chain versus long chain), degree of carbon chain fluorination (i.e., perfluorinated or polyfluorinated), functional groups, chemical characteristics, how they were manufactured, and whether they are original products or degradation products. (Organisation for Economic Co-operation and Development (OECD) 2018; Glüge et al. 2020; Interstate Technology and Regulatory Council (ITRC) 2023c).

Because they possess both hydrophilic and hydrophobic regions, chemical stability, and biological stability, PFAS are used in upwards of a million unique products worldwide, including in consumer and commercial products (e.g., non-stick cookware, water-proof and stain-resistant fabrics, food packaging, cosmetics, electronics, cleaning products, paints, varnishes and sealants, semi-conductors), aqueous film-forming foam (AFFFs), and medical devices (e.g., catheters, pacemakers, radiological machinery). Many PFAS are also critical to the national defense of the United States and are used across the U. S. Department of Defense (DOD) in weapon platforms (e.g., fixed-wing aircraft, rotary-wing aircraft, surface ships, submarines, missiles, torpedo systems, radar systems, battle tanks, assault vehicles, and infantry carriers) and as components of plastics, O-rings, gaskets, lubricants, coolants, and fabrics (Department of Defense 2023). PFAS are also necessary for producing semiconductors, and other essential electronics (Dourson et al. 2024).

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were the two most historically produced and used PFAS and their toxicology has been thoroughly studied (Dourson et al. 2024). In 2006, the EPA and eight PFAS manufacturers voluntarily initiated a global stewardship program to end the manufacturing, use, and release of long-chain PFAS, including PFOA, PFOS, and their precursors, by the end of 2015 (EPA 2024e).

In March 2021, the EPA announced a decision to regulate PFOA and PFOS in drinking water under the Safe Drinking Water Act (SDWA). In July 2022, the EPA published interim Health Advisories for PFOA and PFOS at 0.004 and 0.020 ppt, respectively, a sharp reduction from the previous value of 70 ppt. As discussed by Cotruvo et al. (2023), the scientific basis for the EPA's reduction in HA values was unclear and not aligned with the assessments of other international agencies. On March 29, 2023, the EPA announced that it would also regulate perfluorohexane sulfonic acid (PFHxS), hexafluoropropylene oxide dimer acid (HFPO–DA), and its ammonium salt (also known as GenX chemicals), perfluorononanoic acid (PFNA), and perfluorobutane sulfonic acid (PFBS), and mixtures of these PFAS as contaminants under the SDWA (EPA 2023e).

On April 26, 2024, the EPA issued a final rule for six PFAS under the SDWA that set the Maximum Contaminant Levels (MCLs) for PFOA and PFOS at 4.0 nanograms per liter (ng/L) each (EPA 2024i). The EPA also finalized individual MCLs for PFHxS, PFNA, and HFPO–DA at 10 ng/L and a Hazard Index (HI) of 1 (unitless) as the Maximum Contaminant Level Goal (MCLG) and MCL for any mixture containing two or more of PFHxS, PFNA, HFPO–DA, and PFBS (EPA 2024i).

In its deliberations, the EPA derived multiple candidate RfDs for PFOA and PFOS based on 11 studies that examined non-cancer health effect endpoints (e.g., immune, cardiovascular, hepatic, and developmental) which were identified to have the strongest weight of evidence. The EPA calculated candidate RfD values for each of the four prioritized health endpoints to arrive at a health outcome-specific RfD for each of them. After the Agency considered the four-health outcome-specific RfDs, it decided on an overall RfD that was designated to be protective of all adverse endpoints. This process was followed for both PFOA and PFOS.

The Agency decided that PFOA shared the same RfD (3×10^{-8} mg/kg/day) for three (immune, developmental, cardiovascular) of the four endpoints (EPA 2024k). Therefore, this candidate RfD was selected as the overall RfD for PFOA. For PFOS, the EPA selected an overall RfD of 1×10^{-7} mg/kg/day (EPA 2024j). The RfDs informed the MCL development process, but the Agency ultimately chose the lowest reliable limit of detection of 4 parts per trillion (ppt) as the regulatory limit.

While multiple studies claim to report adverse health effects of PFAS exposure at some doses, establishing definitive human exposure thresholds that unambiguously correlate with health outcomes remains challenging. It was stated in the PFAS National Primary Drinking Water Regulation (NPDWR) that there was adequate evidence for concern, at some doses, for adverse health effects, including thyroid effects (Wen et al. 2013), cardiovascular effects (Steenland et al. 2009; Dong et al. 2019), reproductive effects (Rickard et al. 2022), reduced vaccine titers (Budtz-Jørgensen and Grandjean 2018; Timmermann et al. 2022), developmental effects (Sagiv et al. 2018; Wikström et al. 2020), hepatotoxicity (Gallo et al. 2012; Darrow et al. 2016; Nian et al. 2019), and cancer (Shearer et al. 2021; IARC 2023). It is worth reiterating that the PFAS class is broadly defined and has thousands of members. These chemicals vary greatly in their chemical, physical, and toxicological properties and should not be grouped together for regulatory purposes (Buck et al. 2011; Henry et al. 2018).

No unifying modes of action (MOA) have been identified that can explain how these chemicals cause this diverse set of alleged adverse effects (Corton et al. 2018; Felter et al. 2018; Chappell et al. 2020; Heintz et al. 2023; Clewell 2024; Li et al. 2024). Indeed, it is highly unusual that a chemical does not have a target organ or that it exerting adverse effects without preceding subtoxic responses are observed.

Researchers have yet to identify a particular target organ for PFOA, PFOS, or the four other PFAS regulated through the PFAS NPDWR. For example, in the case of PFOA exposure (and likely other PFAS), pharmacokinetic bias is likely confounding the observed associations with certain adverse health effects (Verner et al. 2015; Dhingra et al. 2017; Steenland et al. 2018a; Andersen et al. 2021b; Clewell 2024). Although it has been stated that PFAS are a fatty acid mimic (Luebker et al. 2002; Clewell 2024), a disturbance in fatty acid metabolism seems an unlikely, if not implausible, unifying MOA for the endpoints selected in the MCL. The key non-cancer effects cited by the EPA (i.e., immunotoxicity, developmental effects, cardiovascular effects, and hepatotoxicity) are unlikely to all manifest from a disturbance in fatty acid metabolism, even at serum concentrations many folds higher than those that are observed in the general population.

Decades of PFAS exposure research have yet to connect human-relevant molecular initiating events to disease associations in epidemiological studies (Fenton et al. 2021; George and Birnbaum 2024). As succinctly described in Clewell (2024), "(1) there is no MOA information/mechanistic data to support the reported epidemiological associations of blood concentrations of PFOA as low as 10 ng/mL with health effects, and (2) it is more likely that the reported associations may be due to reverse causality or pharmacokinetic confounding" (Clewell 2024, p. 5). For PFOA, its structure (an 8-chain carbon terminated by a carboxyl group), is similar to the medium-chain fatty acid octanoic acid; however, due to its fluorine bonds, it is resistant to normal fatty acid metabolism (Vanden Heuvel et al. 1991; Andersen et al. 2008; Clewell 2024). In general, toxicology studies show statistically significant associations for some PFAS (primarily PFOA and PFOS) with specific adverse effects at certain doses in some test species, but extrapolating the results to humans is challenging as human exposures to PFAS typically occur at concentrations several orders of magnitude lower than those used in animal studies.

Epidemiology studies on PFOA/PFOS frequently showed inconsistent, statistically insignificant results and lacked consistent dose-response relationships within or between studies. (U.S. Environmental Protection Agency (EPA) 2024j, 2024k). Some scientists have questioned methodological limitations in key studies, including … (1) the inability to match individual human exposures or circulating blood concentrations with specific effects seen in animal studies, (2) exposure to more than one PFAS does not adhere to a single mechanism, (3) the potential for reverse causality by physiological or disease processes that are occasionally associated with the corresponding PFAS concentration, and (4) the reported effects from these observational studies rely upon clinical biomarker levels that may be considered uninformative for determining a safe dose (Verner et al. 2015; Burgoon et al. 2023; Clewell 2024).

This paper discusses 15 misconceptions concerning the promulgation of the MCL for PFAS and other regulatory actions by global agencies. Different governmental and scientific bodies around the globe have disagreed on what should be considered as a safe dose for PFAS (Burgoon et al. 2023).

Not surprisingly, the definition of what is "safe" varies among agencies and countries:

> "In parallel, another important concern is the costliness of the MCLs. Economic analyses project staggering costs tied to the MCL's implementation. Some estimates suggest compliance and litigation expenses – including upcoming personal injury claims – could approach or exceed $1 trillion over the next decade" and follow with Wolf and Magill references already in text. Some sources believe that the cost of this MCL, including the upcoming personal injury litigation, could well approach or exceed $1 trillion over the next decade (Wolf 2023; Magill 2024). Therefore, it is useful to reflect on the process used by EPA to promulgate these MCLs and any "lessons learned" to ensure similar future regulatory actions that have this level of impact on society are well justified.

While the projected litigation costs will be large, the hidden cost lies in the fact that public support is waning for the ability of governmental agencies to make decisions that are protective of public health using unbiased scientific assessments.

Background on the regulatory approach to these PFAS

It is recognized that all federal regulatory agencies are expected by Congress to establish safe chemical concentration thresholds while economically justifying regulations through cost-benefit analysis of health impacts (Office of Management and Budget (OMB) 2003, 2023). This is a delicate balancing act and it is not our intention to question whether EPA had the congressionally mandated responsibility to set PFAS MCLs by applying some aspects of the precautionary principle (EPA 1974). We support some of the actions and outcomes resulting from the EPA's novel drinking water regulation in the discussion. However, because the cost of this environmental regulation will have a dramatic effect on so many aspects of the economy, including national security, it seems scientifically appropriate to reflect on whether the information that EPA assembled was adequate for rulemaking, whether they used a weight of evidence approach to assess the quality of the studies that they relied upon, whether the data utilized to identify each MCL were adequate, and whether they should have used a more thorough peer review process by one or more capable panels of experts who could have assisted the EPA to assess these matters. This would have ensured that the Agency had met the rigorous scientific requirements for setting an MCL.

The EPA expedited a rulemaking process that normally involves 3-7 years of multi-agency peer review (including EPA/SAB, NAS, and OECD evaluation). George and Birnbaum (2024) analyzed this shift from evidence-based protocols to precautionary policymaking in their recent commentary. They elegantly described why they believed society should be concerned about the presence of these chemicals and why, in their opinion, they deserved aggressive regulatory action. However, their analysis, like most in the field, predominantly examined methodologically projected future dose-dependent hazards of individual PFAS compounds, rather than

addressing empirically established adverse effects at current U.S. population blood concentrations (including high-exposure subgroups).

We believe that an argument could be made that simply initiating a gradual phase-out of the most relevant 50 PFAS would have been a more effective regulatory strategy than setting a four ppt water regulation for PFOA and PFOS. It is important to note that the strategy chosen by the EPA will have a minimal effect on blood concentrations in the U.S., as only 10-20% of the daily intake is from drinking water for those not living near contaminated sites (Sunderland et al. 2019, EFSA 2018).

Given the rapidly evolving body of literature on PFAS, combined with the significant political and stakeholder interest, consumer concerns, and articles in the popular or lay press that take existing science out of context and the numerous uncertainties regarding the adverse health effects of PFAS exposure in humans, it seemed imprudent for the EPA to promulgate this MCL in a hurried manner. This article discusses what have now become fifteen misconceptions about the known hazards of these six chemicals. The rationale for the alleged benefits of this aggressive regulatory decision is also addressed.

Methods: Literature search

We identified 15 major misconceptions through an analysis of stakeholder comments submitted to the EPA's PFAS NPDWR Docket, and conclusions drawn from scientific studies reporting on the adverse effects of PFAS in humans and animals. To thoroughly understand the scientific foundation of these fallacies, we conducted a comprehensive literature search to locate pivotal reviews, studies, and technical documents that delve into the underlying principles. For each misconception, we performed searches using Google, Google Scholar, and PubMed for pertinent studies across various topics related to PFAS, including immunotoxicity, developmental effects, cardiovascular effects, liver effects, carcinogenicity, toxicology and epidemiology studies, and mode of action. We presented evidence that critically examined and elucidated flaws in the purported arguments. In our narrative review, we synthesized our findings to present the misconceptions related to the PFAS MCLs.

Misconception #1: Current epidemiological studies provide adequate evidence that PFOA and PFOS levels found in typical drinking water may pose an immunotoxicity hazard at current or anticipated blood concentrations

In the EPA's final drinking water regulation that was issued in April 2024, the Agency stated that:

> "The available evidence indicates a relationship between PFOA exposure and immunosuppression; epidemiology studies showed suppression of at least one measure of the antibody response for tetanus and diphtheria among people with higher prenatal and childhood serum concentrations of PFOA" (EPA 2024i, pg. 32698).

The EPA identified three papers in their human health toxicity assessments (HHTA) for PFOA and PFOS and the final PFAS NPDWR, that in their view, provided sufficient data to derive draft RfD values for these two chemicals based on their alleged adverse effects on the immune system (EPA 2024j, 2024k, 2024i). Specifically, the EPA stated:

> "… two *medium* confidence epidemiologic studies that reported decreased antibody responses in children exposed to PFOA (Timmermann et al. 2022; Budtz-Jørgensen and Grandjean (2018) were considered for POD derivation …" (EPA 2024k, pg. 4–8).

Similarly, the 2024 Final Toxicity Assessment for PFOS embraced these same studies, with the addition of a medium-confidence study by Zhang et al. (2023). These studies evaluated diminished antibody production in children and adolescents in different countries. They each deserve careful review.

The study by Budtz-Jørgensen and Grandjean (2018) was a benchmark dose (BMD) analysis using two cohorts of Faroese children ($n = 1{,}146$) recruited between 1997 and 2000 and 2007 and 2009. The authors calculated BMDs for five PFAS (e.g., PFOS, PFOA, PFHxS, PFNA, and PFDA) in regards to specific IgG antibodies against tetanus and diphtheria at ages 5 and 7. The study by Timmermann et al. (2022) was a cross-sectional study that recruited a cohort of Greenlandic children ($n = 367$) and collected data between 2012 and 2015. This study examined the effects of seven PFAS (e.g., PFHxS, PFHpS, PFOS, PFOA, PFNA, PFDA, and PFUnDA), PCBs, and total mercury on the concentrations of diphtheria and tetanus antibodies post-vaccination in children aged 7 to 12 years old. Zhang et al. (2023) examined 819 adolescents (aged 12 to 19) who had detectable rubella and measles antibody levels from the 2003-2004 and 2009-2010 National Health and Nutrition Examination Survey (NHANES) cycles and had serum measurements for four PFAS (e.g., PFOA, PFOS, PFHxS, and PFNA).

Budtz-Jørgensen and Grandjean (2018) reported that average U.S. blood PFAS levels exceeded their model estimated BMDL doses for diminished diphtheria/tetanus antibody responses. It should be noted that the authors did not mention how much a reduction in these antibody responses correlated to higher susceptibility to infection or if the observed serum titer values would not be protective of children. For example, a 20% decrease in antibody titer levels does not correlate to a 20% increase in infection incidence. The authors suggested that all five PFAS in their study should be of regulatory interest until additional evidence proves otherwise.

Timmermann et al. (2022) reported that for each 1 ng/mL increase in serum concentrations of PFHxS, PFOS, PFNA, or PFDA, the odds of having unprotective levels of diphtheria antibodies of $\geq$0.1 IU/mL were increased and statistically significant. The authors reported that only a small proportion of children (12%) had tetanus antibody levels below the protective threshold, whereas a substantially higher percentage (52%) had sub-protective levels of diphtheria antibodies. They also noted that a limitation of their results was that for the age group examined, the last tetanus booster that the oldest study participants received was approximately seven years prior (Timmermann et al. 2022, p. 5). This would have allowed for a substantial decrease in antibody concentrations

over time. They also stated that they had the date of the most recent vaccine booster for approximately half of the study participants and that using this restricted dataset yielded different results than applying the estimated date of vaccine booster for the missing dates (Timmermann et al. 2022). However, the authors did not provide these results for the restricted data in the paper or in the supplemental materials. They also did not report if their findings of lower tetanus antibody titers had actually led to an increase in tetanus in the community.

Zhang et al. (2023) reported inverse associations between serum levels of PFOS and PFHxS and rubella antibody levels, between serum levels of PFOA and mumps antibodies, and PFAS mixtures and rubella and mumps antibodies. Critically, these inverse associations were only reported in adolescents with red blood cell folate concentrations $< 66^{th}$ percentile (lower folate group), while no association was reported among adolescents with higher RBC folate levels (upper folate group). In addition, for each quartile increase in serum concentrations of the total PFAS mixture, it was reportedly associated with an 9.84% (95% CI: −15.57%, −3.74%) decrease in rubella antibody levels, and an 8.79% (95% CI: −14.39%, −2.82%) decrease in mumps antibody levels, but only for the lower folate group. No associations were observed in the upper folate group. The authors noted that caution is needed when interpreting their results due to "… the small sample size and the wide confidence intervals of the estimates" (Zhang et al. 2023, p. 2451).

The Timmermann et al. (2022) and Budtz-Jørgensen and Grandjean (2018) studies were thought-provoking and have attracted the attention of many regulatory agencies around the globe. However, many scientists and physicians have presented concerns with the shortcomings of these papers (Food Standards Australia New Zealand (FSANZ) 2021b; Burgoon 2022; Burgoon et al. 2023; Cotruvo et al. 2023; Crawford et al. 2023; Garvey et al. 2023). They believed that the conclusions of the authors should be considered preliminary and far from adequate to be the basis for regulatory action. For example, Burgoon (2022) noted:

> "The EPA based its health advisories for PFOA and PFOS on a 2018 study by Budtz-Jørgensen and Grandjean that looked at the association between PFOS concentrations in blood and diphtheria antibodies in children. This study calculated a PFOS concentration that the authors state is not likely to have an adverse effect on diphtheria antibody production. And that's true – it appears that Budtz-Jorgensen and Grandjean did not see diphtheria antibody levels that were in the adverse health range for any subjects (… none of the children in the study were experiencing diphtheria antibody levels that were too low to yield basic immunity …)" (Burgoon 2022)

As noted by Burgoon (2022), when evaluating the Budtz-Jørgensen and Grandjean's analysis, one can see that their model only goes down to a diphtheria antibody concentration of 0.444 IU/mL. This value is 44x larger than the basic immunity threshold of 0.01 IU/mL that has been identified by the World Health Organization (WHO) and other world authorities on the matter. It must be noted that WHO considers this threshold as a "… minimum putatively protective level …" and should not be considered "… a guarantee of immunity under all circumstances" for tetanus (World Health Organization (WHO) 2018, pg. 13,14). In addition, assessing fluctuation in vaccine antibody titers is only informative when the appropriate covariates are considered (e.g., site of administration, age, gender, concurrent medications, co-morbidities, etc.). In short, based on this analysis, the antibody levels far exceed the minimum punitive protection level.

Regarding the Grandjean et al. (2012) study, which was relied upon by the analysis conducted in Budtz-Jørgensen and Grandjean (2018), Perez et al. (2023) noted the following regarding the use of antibody concentrations:

> "… the mean diphtheria antibody concentration reported by Grandjean et al. (2012) for all participants, pre-booster, was above a clinically protective level of 0.1 IU/mL (mean: 0.12 IU/mL, interquartile range: 0.05–0.40 IU/mL) and the mean tetanus antibody concentration for all participants, pre-booster, was also above the clinically protective level (mean: 0.22 IU/mL, interquartile range: 0.10–0.51 IU/mL). Post-booster antibody titers were well above the clinically protective levels for both vaccines, regardless of PFOS or PFOA serum concentration. More importantly, all median antibody titers reported by Grandjean et al. (2012) appear to be within a normal range of age-specific antibody response following immunization for both tetanus and diphtheria when compared to available literature (Gowin et al. 2016; Schauer et al. 2003; World Health Organization (WHO) 1993). For example, a study by Schauer et al. (2003) reported mean tetanus antibody concentration of 1.65 IU/mL in children at 8 years of age, which is roughly comparable to the mean concentration of 1.59 IU/mL (interquartile range: 0.65–4.60) reported by Grandjean et al. (2012) for children at 7 years of age" (Perez et al. 2023, p. 2).

Additionally, Grandjean et al. (2012) did not address potential covariate exposures to PCBs and mercury in this population, both of which are known to suppress antibody responses and have been identified as immunotoxicants in prior studies of the Faroe Islands Cohort (Heilmann et al. 2006, 2010; Maqbool et al. 2017; Timmermann et al. 2019; Perez et al. 2023).

Another significant observation regarding the Budtz-Jørgensen and Grandjean (2018) study was that there was no evidence of an adverse effect on the ability of the vaccines to protect the persons. In Budtz-Jørgensen and Grandjean (2023), the authors noted several challenges associated with applying the benchmark dose (BMD) approach to observational data: exposures are not assigned by study design and unexposed controls are typically absent; censored data may limit data availability; and model selection can significantly influence the results. This difference is seen when comparing the BMD results of Budtz-Jørgensen and Grandjean (2023) to those of Abraham et al. (2020). Two additional issues that were raised by Perez et al. (2023) regarding the BMD analysis conducted by Budtz-Jørgensen and Grandjean (2018) are (1) the study population did not exhibit a clinically significant reduction in serum antibody levels and (2) by not having a non-exposed control and selecting a benchmark response level (BMR) of 5%, the implicit assumption is that the "… modeled antibody level extrapolated from zero serum PFOA or PFOS is the 'healthy' level from which the BMR departs. Thus, a 5% reduction of antibody levels from the extrapolated zero exposure level results in BMD and BMDL estimates that do not correspond with an actual observed or adverse reduction of antibody levels" (Perez et al. 2023, p. 2).

For decades it has been well documented that a variety of factors including the vaccine type, vaccination procedure used, the recipients' sex, genetics, age, nutritional status, smoking status (of the parents when studying children), psychological stress, and disease state, all play a role in an individual's vaccine titer levels post-vaccination (Weisglas-Kuperus et al. 1995; Van Loveren et al. 2001). An example of the importance of accounting for these confounders was shown by Weisglas-Kuperus et al. (2000). This paper found that there was a decrease in lower antibody responses. This led to a statistically significant negative correlation of antibody titers to measle vaccines with the exposure to PCBs and dioxins as determined from cord blood and a statistically significant negative correlation of antibody titers to rubella with maternal exposure to these compounds (Weisglas-Kuperus et al. 2000; Van Loveren et al. 2001). However, when these results were corrected for sex, early feeding type (formula fed or breast-fed), duration of breast-feeding during infancy, tobacco smoking by one or both of the parents, family history of atopy, and day care or nursery attendance, definitive conclusions could not be drawn (Van Loveren et al. 2001).

For Budtz-Jørgensen and Grandjean (2018) and Timmermann et al. (2022), it was not clear from their papers if all of the relevant confounders were considered in their analysis. If these were adequately considered, it is possible that their conclusions would be different. Thus, these studies are unable to shed light on the blood concentration of selected PFAS chemicals that might have an adverse impact on the effectiveness of these vaccines. This shortcoming was also acknowledged by the WHO, who stated:

> "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 health based guidance value (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" (WHO 2022, p. 79).

Experts have also noted that there is a fundamental shortcoming in the way the authors applied the BMD approach. According to commenters (3M Company 2023; Raptor Pharm Tox Ltd 2023) on the EPA's draft PFAS NPDWR, the EPA did not follow its own guidance document on how to use the BMD approach; this included failing to recognize that the data in Budtz-Jørgensen and Grandjean (2018) was continuous rather than quantal. They noted that the BMD approach used was not appropriate for continuous data like those in the Budtz-Jorgensen and Grandjean (2018) study (3M Company 2023; Raptor Pharm Tox Ltd 2023; Hua et al. 2025) (in press).

It appears that the EPA, as well as Budtz-Jørgensen and Grandjean (2018), misinterpreted the Benchmark Dose Technical Guidance document when they performed their analysis. Specifically, they both referenced the (dichotomous) data section. EPA and others have noted that:

> "… most reproductive and developmental studies with nested study designs easily support at BMR of 5%. Similarly, a BMR of 1% has typically been used for quantal human data from epidemiology studies" (EPA 2017a).

The EPA agreed with using a 5% BMR, justifying it by stating that the health issues being considered, which include serious outcomes like fatalities and impacts on children, warranted this level. However, some commenters (3M Company 2023; Raptor Pharm Tox Ltd 2023) believed this reasoning was flawed. They argued that the level of protection in the blood measurements was already sufficient to safeguard children, so using a 5% BMR might be too cautious or unnecessary.

The EPA's choice to derive a BMD for Budtz-Jørgensen and Grandjean (2018) conflicted with their Technical Guidance, which stated:

> "The ideal is to have a biological basis for the BMR for continuous data, e.g., a consensus scientific definition of what minimal level of change in a continuous endpoint is biologically significant" (EPA 2012, p. 22).

It would appear that the authors of the Budtz-Jørgensen and Grandjean (2018) and Timmermann et al. (2022) articles were not aware that the generally accepted concentration of vaccine antibody titers for diphtheria that is considered to be minimally protective from the disease is 0.01 IU/mL (Liang et al. 2018). Both EPA and the study authors appear to have incorrectly interpreted antibody titers that were five-fold higher than the protective threshold as indicative of an adverse effect. Their argument would have been strengthened if there was an outbreak of disease in this population, which was not reported in the article. In short, both articles claimed that persons were at risk, when, in fact, they were not. As discussed in Perez et al. (2023), the analysis in Budtz-Jørgensen and Grandjean (2018) should have included data from a similarly aged cohort, which would show if the dose-response is actually linear between the serum PFOA and PFOS levels that were reported and the normal variation in antibody levels in individuals (Perez et al. 2023).

This shortcoming in interpreting the vaccine titer blood concentrations commonly occurs in studies lacking physician peer review during publication. Researchers often report statistically significant differences between treatment and control groups without acknowledging that both groups fall within the established physiological range for the parameter being assessed.

The EPA and the authors failed to recognize that there were also serious shortcomings in the design of the Budtz-Jørgensen and Grandjean (2018) and Timmermann et al. (2022) articles. First, both groups failed to compare the vaccine titer data to the range of national background data. Second, they seemed unaware that the blood concentrations that concerned them were still amply protective for diphtheria. Third, Budtz-Jørgensen and Grandjean (2018) did not provide numerical values for the PFAS serum concentration in the population they studied; however, these authors stated the blood levels were comparable to levels reported in the U.S. (ng/mL). Timmerman et al. (2022) noted that PFOS serum concentrations in the Greenlandic cohort were double the

levels measured in American and Faroese children. Lastly, the original papers did not have repeated measurements of blood for each person which can introduce significant uncertainties; even to the point of the study not being reliable.

Interestingly, the alleged immunosuppressive effects observed in the Budtz-Jørgensen and Grandjean (2018) study have also been observed in animal studies that used doses (30 mg PFOA/kg/day) where serum PFOA levels were 150-fold greater than those in individuals living near a PFOA production site (Dewitt et al. 2008). The doses used in this animal study are far greater than what are seen from environmental and occupational exposures, resulting in blood concentrations significantly greater than levels observed in humans (Lucas et al. 2023). In addition, how the endpoints examined translate to adverse health effects in humans are not clearly defined. Statistically different alterations should be secondary when evaluating a health effect. The response (e.g., decreased vaccine titer) should be evaluated in the context of clinical significance. Even with PFAS, there is little evidence to suggest that the effects seen in animal studies at the doses administered translate to clinical relevance in humans.

Several scientific bodies have questioned the findings of the three studies that the EPA relied upon. For example, at least four expert committees, discussed below, have written reports critical of these papers (Food Standards Australia New Zealand (FSANZ) 2021a; Antoniou et al. 2022; WHO 2022; Burgoon et al. 2023; Garvey et al. 2023).

Garvey et al. (2023) and Food Standards Australia New Zealand (FSANZ) (2021a) concluded that vaccine antibody titers were not sufficient to determine immune suppression as a prioritized endpoint, due to the difficulty in controlling for potential confounders. Garvey et al. (2023) reported that a vaccine threshold of 0.01 IU/mL was a surrogate of protection rather than a concentration below which humans were found to be more susceptible to the disease: in the case of diphtheria, given the complexity of immune response, it was recommended that disease incidence be used instead to assess immunotoxicity resulting from PFAS exposure. This was a valuable suggestion that is relevant to future studies. Currently, the animal and human evidence of a relationship between PFAS exposure and risk for infectious disease are mixed and inconclusive (Antoniou et al. 2022; Burgoon et al. 2023).

In immunosuppressed populations (e.g., organ transplant recipients or individuals with HIV/AIDS), certain tumors, such as squamous cell carcinoma of the cervix, EBV-related lymphomas, HHV8-related Kaposi sarcoma, and melanoma occur in excess compared to the non-immunocompromised population (Frisch et al. 2001; Grulich et al. 2007; Engels et al. 2011). However, the epidemiology studies on PFAS exposure have not suggested a significant increase in these types of cancers (Agency for Toxic Substances and Disease Registry (ATSDR) 2021). In addition, translating immunotoxicity findings from rodents to human immune function is inherently complex, and the EPA did not adequately address these interspecies differences. (Mestas and Hughes 2004; Steenland and Winquist 2021).

While the alleged effects of PFAS exposure on the effectiveness of vaccines has captured the attention of the public and regulatory agencies globally, the data are far from being adequate to drive regulations or concern the public. The current body of evidence does not fully support the characterization of PFOS and PFOA as immunotoxic hazards to humans for the following reasons: (1) The methodological shortcomings in the data render virtually all the studies too weak for drawing a solid conclusion; (2) The changes identified, if correct, may be different between controls and PFAS populations, but there were no increases in disease incidence identified in the study populations; (3) The calculation of the safe dose was flawed due to an improper use of BMD methodology; and (4) Peer review panels have generally reached the view that all of these studies are too unreliable, yet EPA and other regulatory agencies still mention them as a cause for concern.

Misconception #2: Current animal and epidemiology studies indicate that exposure to PFOA and PFOS in drinking water is a developmental hazard to humans at current or anticipated levels

In the EPA's Human Health Toxicity Assessment, provided in support of the PFAS NPDWR, the Agency, as of April 2024, stated that the developmental effects observed from exposure to PFOA were evaluated based on:

> "… primary evidence related to decreased birth weight in human infants and decreased offspring survival, decreased fetal and pup weight, delayed time to eye-opening, and related pre-and postnatal effects in animal studies" (EPA 2024k, p. xxi)

Similarly, for PFOS, the EPA stated that:

> "… the available human and animal evidence indicates that PFOS exposure is likely to cause developmental toxicity in humans under relevant exposure circumstances … The conclusion is supported by coherent epidemiological evidence for measures of decreased gestational duration and other biologically related effects (e.g., decreased postnatal growth and birth length) and consistent findings of dose-dependent decreases in fetal and maternal weight …" (EPA 2024j, p. 3–253).

The EPA relied on two human studies for PFOA (Sagiv et al. 2018; Wikström et al. 2020), and three studies for PFOS (Darrow et al. 2013; Sagiv et al. 2018; Wikström et al. 2020) to derive a health endpoint-specific reference dose based on their concerns regarding decreased birth weights.

Before discussing the merits of these studies, it is worthwhile to recognize that decreased birth weight has been rarely defined as a toxic endpoint for regulatory action for an industrial chemical in air, water, or food. There are several reasons for this. First, usually, when one considers historical information from animal testing, it is unusual that decreased birth weight is the only adverse effect observed (i.e., it is usually a secondary effect of direct organ toxicity). Second, unless the lessened birth weight is clinically significant, it is not considered a developmental effect. In other words, in most cases, if birth weight is statistically lower than expected, but not outside the range of normal, the child will more than likely catch up to the expected weight soon after birth (Jain and Singhal 2012; Liu et al. 2019). Thus, this would not be considered an adverse effect. To conduct a proper analysis of

whether birth weight is an adverse effect, the data must be analyzed by quartiles to determine if there are clinically significant differences between weights in an exposed versus unexposed population. In short, birth weight is a challenging parameter to use as an adverse effect, which is why it is rarely used as a justification for regulation.

As has been reported in most other studies of the effects of chemicals on developmental toxicity, if one is relying on epidemiology studies (human data), the following confounding factors need to be characterized and accounted for if the evaluation is to be considered credible:

a. Socioeconomic factors
b. Race and gender
c. Maternal education
d. Home environment
e. Fundamental nutrition of the mother
f. The degree of smoking before and during pregnancy
g. The amount of alcohol intake during the pregnancy
h. The degree of pharmaceutical and non-pharmaceutical drug use
i. The type of pre-natal care and use of vitamins known to affect pregnancy outcomes
j. Any confounding illnesses in the medical history of the mother
k. The family history of pregnancies
l. Maternal exposures to chemicals, including mixtures
m. Statistical methods used to analyze the data

In each of the studies upon which the EPA relied, few, if any, of these factors were addressed by the authors or the EPA in their analysis. Without that information, it is extremely difficult to assign cause to a particular serum concentration of any chemical with respect to developmental effects. Equally important, one needs to measure and consider other so-called developmental toxicants in the blood that may have had an impact on outcome. For example, at certain doses, PCBs, dioxins/furans, organochlorine pesticides, various metals, pharmaceuticals, and some other persistent chemicals have been known to impact birth weight (among other endpoints) but they have not been considered or corrected for in the epidemiological assessments of development effects of PFAS.

The EPA focused on a handful of studies to conclude that developmental toxicity from exposure to PFAS was worthy of regulatory attention. Each study deserves careful examination. Many comments were submitted by stakeholders during the comment period which seem not to have been given serious consideration (Hua et al. 2025) (in press).

The three studies that the EPA relied upon for deciding that PFOA and PFOS are developmental toxicants are Darrow et al. (2013), Sagiv et al. (2018) and Wikstr€om et al. (2020). Darrow et al. (2013) conducted a study involving 1,630 births among women who delivered on or after January 1, 2005 and were enrolled in the C8 Health Project in the Mid-Ohio Valley. The geometric mean maternal serum concentration for PFOA was 16.2 ng/mL (range: 0.6 – 459.5 ng/mL) and 13.2 ng/mL (range: LOD-92.9 ng/mL) for PFOS. The authors stated that "… we observed little or no evidence of an association between maternal serum PFOA or PFOS and preterm birth or low birth weight, positive associations with [pregnancy-induced hypertension] … [and a] negative association between PFOS and birth weight among full-term infants" (Darrow et al. 2013, p. 1211).

Sagiv et al. (2018) measured four PFAS in plasma from 1,645 pregnant women (<22 weeks gestation) in eastern Massachusetts that were pregnant between 1999 and 2002. Plasma concentrations were highest for PFOS (median: 25.7 ng/mL; IQR: 16.0) and lowest for PFNA (median: 0.7 ng/mL; IQR: 0.4). It is important to note that this time frame was during 3M's phase out of PFOS from 2000 to 2002 and before the voluntarily phase-out of other long chain PFAS in the United States that began in 2006 (EPA 2024e). The results showed that for these four PFAS, there was a non-significant change in fetal growth for both male and female births, based upon the 95% confidence intervals crossing zero, and there was a statistically significant decrease in gestational length for PFOS and PFNA in males (Sagiv et al. 2018, p. 800). This decrease in gestational time equates to approximately one to three days, which would not be clinically relevant for a normal pregnancy.

Wikström et al. (2020) measured serum concentrations of eight PFAS in 2,355 pregnant women (3-27 weeks pregnant) between 2007 and 2010 in Sweden, as well as, body weight outcomes in 1,533 infants. Serum concentration results showed that PFOS had the highest measured concentration (geometric mean: 5.3 ng/mL; 95% CI: 5.21, 5.50) and the PFHpA had the lowest (geometric mean: 0.018 ng/mL; 95% CI: 0.017, 0.019). The authors identified a statistically significant negative association between prenatal PFAS exposure and body weight in girls in all cases, but not for boys, and when analyzed by quartile, the largest gender difference was in the upper exposure range. Between the lowest and highest quartiles, there was a 136 g lower body weight observed in girls, but no statistically significant decrease in body weight was seen in boys. At the highest quartile, there was a borderline statistically significant finding for girls being born small for gestational age. Regarding the public health relevance of their findings, the authors reported that while this reduction might have a minor impact at an individual level, it may have consequences at a population level (Wikström et al. 2020, p. 1098). It should be noted that while this decrease was detected in the study population, none of the measured quantiles actually had a birthweight that would be classified as being low body weight (<2500 grams).

The weight of evidence shows that these three studies did not find the adverse effects that were clearly of concern to the EPA. As with the studies relied upon to characterize an immunotoxicity reference dose, the EPA's selected studies for characterizing developmental effects have significant flaws that were not adequately acknowledged. As stated by commenters to the EPA, Wikström et al. (2020) did not identify consistent exposure-response relationships or trends when exposure was categorized into quartiles (American Chemistry Council (ACC) 2023). The study showed an increased risk of low birth weight in girls when the highest exposure was compared to the lowest exposure, while other studies that

sampled early in pregnancy reported decreased risks of low birth weight, including Manzano-Salgado et al. (2017) and Hjermitslev et al. (2020) (AWWA 2023). Collectively, there was little evidence that PFOA and PFOS at typical serum concentrations have any effect on developmental outcomes. Even when there were statistically significant differences, they were minor and almost certainly of no clinical significance as nearly all data were within the normal range for the measured endpoints (Hua et al. 2025) (in press).

The exposure-response coefficients used in the EPA analyses were based on decreases in birth weight reported in Darrow et al. (2013), Sagiv et al. (2018) and Wikström et al. (2020). In particular, the studies used in establishing their views evaluated differences in average birth weight but not risk of low birth weight, as odds ratios were calculated. Odds ratios are a measure of association and are not a direct measure of risk. The EPA's conflation of low birth weight with decreased birth weight is prevalent in the economic analysis as well. While these endpoints are correlated, they are not equivalent, and they should not be evaluated as if they are they are the same (AWWA 2023). Dourson et al. (2019) noted that the Texas Commission on Environmental Quality (2016), the EPA (2016b), and ATSDR (2018) all identified developmental toxicity as a critical effect of PFOA; however, each organization relied upon different endpoints. Overall, it is almost certain that what was observed was not a clinically adverse effect.

As noted by AWWA (2023) regarding the EPA's proposed MCL, bias associated with birthweight and trimester of sampling was investigated in two meta-analyses conducted by Steenland et al. (2018a) and Dzierlenga et al. (2020). Both of these studies conducted specific sensitivity analyses to evaluate bias associated with maternal sampling during late pregnancy compared to maternal sampling during early pregnancy. One commenter reported that:

> "Both meta-analyses reported that essentially no effect on birth weight was seen when maternal blood was sampled early in pregnancy, while a relatively larger effect on birth weight was seen when maternal blood was sampled late in pregnancy. In general, this suggests that any effect of PFOA or PFOS on birth weight is confounded by the time of sampling (Steenland et al. 2018a; Dzierlenga et al. 2020; Steenland et al. 2020)" (AWWA 2023, pg. 11).

All of the studies relied upon by EPA required a good understanding of the various factors that are part of the stages of pregnancy and the outcome at birth. Perhaps most importantly, there was no clear dose-response relationship observed except in the highest quartile of girls in Wikström et al. (2020). In addition, the effect estimates are imprecise with wide confidence intervals among the three studies that the EPA relied upon for adverse developmental effects.

If one does not consider all of the biologically important "moving parts," it is easy to conclude that there are possible associations between blood concentrations and clinically relevant changes that occur during a pregnancy. For example, an increased glomerular filtration rate and maternal plasma volume expansion during pregnancy leads to increased elimination of PFOA and PFOS (which needs to be accounted for). Both of these factors also influence fetal weight (AWWA 2023). When PFAS in the serum is sampled late in pregnancy, the magnitude of the glomerular filtration rate and the plasma volume expansion can distort the association between PFAS in blood and birth weight. This has been observed in several studies (Steenland et al. 2018b; Dzierlenga et al. 2020; Steenland et al. 2020).

In terms of other confounding factors, a meta-analysis of epidemiological studies has suggested that decreased renal clearance during pregnancy is associated with lower birth weight and higher PFAS concentrations (Verner et al. 2015). Women who experienced decreased renal filtration during pregnancy are more likely to deliver infants with lower birth weights and are also more likely to exhibit higher concentrations of PFOA during the third trimester of pregnancy due to altered filtration rates (Verner et al. 2015). However, this was debated because a recent systematic analysis of the PFAS literature by the National Academy of Sciences and Medicine and a cohort study by Sagiv et al. (2018) found that evidence surrounding PFAS and glomerular filtration was insufficient to determine a relationship.

It is likely that EPA should have placed more weight on the paper published by Steenland et al. (2018a) who found that there was no effect on birth weight after including the C8 Science study in their meta-analysis and stated:

> "Our meta-analysis including nine new studies, with an almost equal number of births as prior studies, shows a modest inverse association between maternal or cord PFOA and birthweight, with large heterogeneity across studies. The two studies with exposure above background levels showed no association, and similarly, restriction to studies with blood sampling conducted early in pregnancy or shortly before conception showed little or no association. These findings are consistent with confounding and/or reverse causality being responsible for the inverse association seen in studies with low background exposure levels and blood sampling conducted later in pregnancy, when confounding and/or reverse causality are likely to be more important" (Steenland et al. 2018a, p. 774).

Interestingly, as highlighted by the AWWA in their comments (2023), just the prior year before issuing the MCL, the Agency (EPA 2023b) concluded that there was generally a lack of evidence for exposure-response associations between PFOA and PFOS and various developmental outcomes:

> "Additionally, the magnitude of birth weight changes may be correlated with other developmental outcomes such as preterm birth, gestational duration, fetal loss, birth defects, and developmental delays. As described in Section 6.2, these developmental outcomes have limited epidemiology and toxicology evidence showing associations with PFOA/PFOS exposure and due to this uncertainty, these outcomes were not further assessed" (EPA 2023b, p. 6–36).

In 2022, NASEM examined the relationship of PFAS exposure and birthweight and identified two studies that were rated as having a low risk of bias (Buck Louis et al. 2018; Chu et al. 2020). Neither of these studies were selected by the EPA in their analysis of developmental effects. The authors noted that "[t]he magnitude and precision of the estimates of the impacts of PFAS exposure on birthweight varied across and within studies, but the direction of the effect was consistent" (National Academies of Sciences Engineering and Medicine (NASEM) 2022, p. 72). It should be noted that the

PFOA results reported in Buck Louis et al. (2018) were not statistically significant, as were the female infants and male infants results in Chu et al. (2020). For PFOS, there was only a statistically significant decrease in birthweights for male infants (Chu et al. 2020). Despite these weak findings, NASEM concluded that there was sufficient evidence of an association of PFAS exposure with small reductions in birthweight (National Academies of Sciences Engineering and Medicine (NASEM) 2022, p. 72).

It was important to note that between 2003–2018, the median level of PFOS detected in blood serum decreased substantially by 12 ng/mL, from 14.6 ng/mL in 2003–2004 to 2.6 ng/ml in 2017-2018 (EPA 2024c) in women aged 16–49 years of age (i.e., women of childbearing age). During the same time, the median level of PFOA in blood serum decreased by 2.1 ng/mL, from 3 ng/mL in 2003–2004 to 0.9 ng/mL in 2017-2018 (EPA 2024c). These changes are important because most of the studies the EPA believed were reflective of negative effects on birth weights are no longer applicable to blood concentrations that exist in 2024 (when the rule was promulgated) (AWWA 2023).

While the average birth weight in 2003 was about 30 grams greater than that in 2018, 7.9% of births in 2003 were characterized as low birth weight compared to 8.3% in 2018 (AWWA 2023; CDC 2024). Though PFOA and PFOS concentrations have decreased over time, birth weights have not increased, suggesting that it is unlikely that a change in birth weights was the result of PFAS reduction. (AWWA 2023). Indeed, increased birth weights are not always considered positive outcomes.

Evaluating the data from an entirely different viewpoint

Tilstra and Masters (2020) conducted a study using restricted National Vital Statistics System data that investigated the substantial decline in U.S. birth weights during the 1990s and 2000s. The researchers ultimately attributed it to shifts in gestational age driven by changes in obstetric practices, such as increased rates of induced labor and cesarean deliveries. Using simulation techniques and life table analyses, the findings revealed that earlier deliveries at 37–39 weeks, replacing later-term births, likely suppressed what would have been an increase in average birth weight had obstetric practices remained consistent (AWWA 2023). This shift has affected the average birth weight for the nation, which could erroneously be attributed to PFAS chemicals, or any other xenobiotic, for that matter, had careful research not been conducted to understand this change.

One needs to consider what has been learned from various animal studies. Several developmental toxicity studies in animals reported significantly increased maternal, fetal, and/or pup liver weights associated with gestational PFOA/PFOS exposures; rather than lesser weights. For example, Lau et al. (2006) observed notable increases in the rates of stillbirths and neonatal mortality in CD-1 mice at dosages of 5, 10, and 20 mg/kg/day of PFOA when administered from gestation day (GD) 1 to GD 18, followed by natural deliveries. The highest dosage of 40 mg/kg/day induced total loss of pregnancies. Altered lipid metabolism in dams will have an adverse effect on the fetuses (Nyitray et al. 1980). However, the high exposures in the *in vivo* study were not realistic for the general human population, as these dosages were hundreds to thousands of times greater than the average concentration reported in drinking water in at least one survey in the last two years (Ao et al. 2019). Thus, one has to question the relevance of animal studies to understand the risk of PFAS exposure on reducing birthweights in humans.

Based on the review of the epidemiology data from human studies chosen by the EPA, they do not appear sufficient to accurately quantify the risks associated with PFAS and reduced birth weight, nor do they confirm the existence of a clear dose-response relationship in humans between blood concentration and an adverse effect on development. As mentioned, the epidemiology studies the Agency relied upon to derive the reference doses also showed inconsistent results and they did not adequately consider several other important studies in their evaluation (e.g., Steenland et al. 2018a).

It is acknowledged that, much like the alleged effects on the immune system described previously, effects were identified in animal studies at ~500 to 2,000-fold above the typical range of blood concentrations in humans (EPA 2024k, Tables 4–11, p. 4–59 & 4–60). It should be remembered that all chemicals, at some dose, can impact reproduction and development (Barrow 2016). In addition, scientists need to recognize that the dose at which humans will be exposed needs to be reasonably close to the doses observed in the epidemiology and animal studies for the data to be informative. Extrapolation of adverse effects far below those to which persons or animals are exposed with respect to developmental effects is a far different exercise than for carcinogens or many other adverse effects.

Overall, there is little evidence that PFOA or PFOS at serum concentrations reported in the general population, even for highly exposed populations such as occupational workers and the C8 Health populations, adversely affect developmental outcomes.

Misconception #3: Epidemiology or animal studies show that exposure to PFOA and PFOS in drinking water is a cardiovascular hazard to humans

The EPA identified cardiovascular disease (CVD) risk as a driving factor in supporting the MCL that was promulgated in April 2024. The EPA stated:

> "There is *moderate* evidence for an association between PFOA exposure and cardiovascular effects in humans based on consistent positive associations with serum lipids, particularly [low-density lipoprotein] LDL cholesterol and [total cholesterol] TC" (EPA 2024k, p. 3–193).

The Agency stated that there was "[a]dditional evidence of positive associations with blood pressure and hypertension in adult populations" to support their view about cardiovascular risk as an important hazard (EPA 2024k, p. 3–193). However, in the PFOA Final Toxicity Assessment, the EPA acknowledged that "[t]he evidence for an

association between PFOA and increased risk of hypertension overall and in gender-stratified analysis was inconsistent" (EPA 2024k, pg. 3–194). Similarly, in the PFOS Final Toxicity Assessment, the EPA stated that "[t]he limited evidence for an association between PFOS and an increased risk of hypertension was inconsistent" (EPA 2024j, pg. 3–194). In addition, the studies that investigated hypertensive endpoints were not relied upon for the RfD and the EPA instead focused on what they determined to be the most sensitive cardiovascular endpoint (increased total cholesterol).

The EPA identified two studies used to derive a cardiovascular specific RfD that reported associations between increased PFAS serum levels and increased total cholesterol (Steenland et al. 2009; Dong et al. 2019). The EPA acknowledged the limitations of using this endpoint to support their MCL and stated that the available evidence supports a positive association between PFOS and TC in the general population. "… [a]though PFOS appeared not to be associated with elevated TC and LDL in workers, this conclusion is uncertain …" (EPA 2024j, p. 3–174). Regarding PFOA and TC, the EPA reported that considering medium and low confidence studies together resulted in an observation of increased TC with increased PFOA in adults, although "[s]ome inconsistencies in the direction of association across studies were found" (EPA 2024k, p. 3–179).

The EPA also stated that:

> "Although evidence of associations between [high-density lipoprotein cholesterol] and PFOA and PFOS was mixed, certain individual studies reported robust associations in general adult populations" (EPA 2023c, p. 74–75).

As with claims about effects on the immune system and reproductive outcomes, the EPA did not evaluate or consider all the relevant medical aspects or pertinent studies before reaching their conclusions. Similarly, we saw no evidence that one or more practicing cardiologists were involved in the peer review process. Further, we found no comments submitted by professors of cardiology or by epidemiologists who specialized in this endpoint. There seemed to be an inadequate recognition of importance of whether the identified blood lipid and CVD effects from these studies were clinically relevant.

The EPA cited two studies (Steenland et al. 2009; Dong et al. 2019) for its derivation of the draft health endpoint-specific RfDs for cardiovascular effects that were used in the MCL (U.S. Environmental Protection Agency (EPA) 2023e, 2024j, 2024k).

Steenland et al. (2009) performed a cross-sectional study of lipids and serum PFOA and PFOS concentrations for 46,294 residents (> 18 years of age) who drank water contaminated from a chemical plant in West Virginia. Mean serum PFOA concentrations were 80.1 ng/mL (range: 0.25–17,556 ng/mL), while the mean serum PFOS concentration was 22.4 ng/mL (range: 0.25–759.2 ng/mL). Regarding lipid outcomes, the authors reported a statistically significant increasing trend in cholesterol levels across exposure deciles for both compounds, with predicted increases between 11 and 12 mg/dL from the lowest to the highest decile (Steenland et al., 2009). They reported that a similar trend was not apparent for HDL cholesterol. For both compounds, the odds ratios for high cholesterol (≥240 mg/dL) by increasing quartile was similar and ranged between 1.00, 1.21 (95% CI: 1.12, 1.31), 1.33 (95% CI: 1.23, 1.43) and 1.38 (95% CI: 1.28, 1.50). The authors noted for the total cholesterol, the most important predictors were age, gender and body mass index, not PFOA or PFOS serum levels (Steenland et al. 2009).

Dong et al. (2019) utilized NHANES data collected between 2003 and 2014 to evaluate the associations between exposure to five PFAS (e.g., PFOA, PFOS, PFDE, PFHxS, and PFNA) and cholesterol levels ($N =$ 11,895; 2,987 adolescents and 8,948 adults). In the adolescent population (12-19 years), the mean serum levels of PFOA and PFOS were 3.3 and 12.2 ng/mL, respectively, while in the adults, serum levels of PFOA and PFOS were 3.7 and 15.6 ng/mL. The authors reported that most of the associations for adolescents between PFAS and cholesterol levels were insignificant except for PFOS. Meanwhile in adults, a positive trend was observed between PFOA and total cholesterol and indicated that each 1 ng/mL increase in serum PFOA would result in a 1.5 mg/dL (95% CI: 0.2, 2.8) elevation in total cholesterol. In addition, significant associations were identified between PFOS and total cholesterol, which indicated that each 1 ng/mL increase in PFOS serum levels, would result in a 0.4 mg/dL (95% CI: 0.06, 0.6) elevation in total cholesterol levels. The authors noted that age and gender contributed into differences in PFAS concentrations in the NHANES populations observed.

Both Steenland et al. (2009) and Dong et al. (2019) excluded participants prescribed cholesterol medication to minimize confounding variables that may alter serum total cholesterol levels due to medical intervention. Steenland et al. (2009) used linear regression modeling to evaluate associations between perfluorinated compounds (PFOA and PFOS) and serum lipid outcomes, while Dong et al. (2019) measured serum total cholesterol.

Although the Steenland et al. (2009) and the Dong et al. (2019) studies were cross-sectional and reported positive associations between PFOA and PFOS with increased trends in cholesterol levels, the analyses did not include a measure for cumulative exposure to PFOA and PFOS. Importantly, Steenland et al. (2009) modeled predicted total cholesterol, HDL and LDL cholesterol as a function of median PFOA and PFOS serum levels. As summarized by the Cleveland Clinic, dangerous levels of cholesterol are: Total > 240 mg/dL, LDL > 160 mg/dL; HDL < 40 mg/dL (2022). Steenland et al. (2009)'s model identified largely consistent positive trends of total cholesterol, LDL, HDL, and other non-HDL lipids that they considered troublesome, stating that:

> "… the odds of high cholesterol (>240 mg/dL) increased 40%-50% from the lowest to the highest quartile of PFOA and PFOS serum levels" (Steenland et al. 2009, p. 1276).

When considering the weight of scientific evidence and the data from this study, it is unclear whether the distribution of various lipid markers across the C8 population were outside the range in the U.S. population (background).

As some commenters argued, one can have statistically different concentrations from background, but they may not be clinically or scientifically important (Hua et al. 2025) (in press). For example, the results in Steenland et al. (2009) showed that the predicted LDL cholesterol compared to median PFOA and PFOS serum concentrations had a LDL range between approximately 110 – 124 mg/dL (Steenland et al. 2009, p. 1274). According to Johns Hopkins Medicine, regarding the general guideline for LDL for adults in the U.S., the range of 100 to 129 mg/dL is considered to be "near or above optimal" (Johns Hopkins Medicine 2024). Similarly, Harvard Health reported that individuals should aim for having an LDL below 130 mg/dl if they are not considered high risk for heart attack or stroke, unless otherwise recommended by their physician (Harvard Health 2011). This might explain why the researchers did not find an increase in the risk of cardiovascular disease in this population (Winquist and Steenland 2014; Steenland et al. 2020). Some comments from 3M on the draft PFAS NPDWR indicated that repeated measurements are needed to understand whether the concentrations reported are reliable (3M Company 2023, Hua et al. 2025) (in press).

As mentioned, Steenland et al. (2009) also reported that the predicted increase in cholesterol from lowest to highest decile for either PFOA or PFOS was 11–12 mg/dL. However, it is noteworthy that the concentrations of these blood parameters tended to plateau at serum concentrations of approximately 50 ng/mL for PFOA and PFOS. Considering that the PFOA and PFOS levels in drinking water in the United States are significantly lower than the levels in drinking water detected near a chemical plant in West Virginia, this would suggest that lipid levels will not increase outside of normal ranges.

Steenland et al. (2020) described the results of several cross-sectional studies that had been conducted up to that time, with all of them showing a positive association between PFOA and increasing serum lipid levels of LDL and/or total cholesterol (Sakr et al. 2007; Steenland et al. 2009; Frisbee et al. 2010; Nelson et al. 2010; Steenland et al. 2010), with cohort and/or longitudinal studies also supporting this association (Fitz-Simon et al. 2013; Winquist and Steenland 2014; Lin et al. 2019; Liu et al. 2020). Steenland et al. (2020), on the topic of the reported associations in cross-sectional studies, noted that:

> "Inter-individual variation in enterohepatic cycling of both PFAS and bile acids, the latter affecting serum cholesterol levels, has been postulated as a mechanism for such a correlation between PFAS and cholesterol (EFSA 2018)" (Steenland et al. 2020, p. 3).

Dong et al. (2019) reached similar conclusions regarding the difficulties with relying upon cross-sectional studies to establish causation; however, this is not as large of a concern for the longitudinal studies.

Fitz-Simon et al. (2013) conducted a longitudinal study of 560 adults who lived in Ohio and West Virginia in areas that had been contaminated with PFOA and examined within-individual changes in serum PFOA and PFOS and serum lipid levels between 2005/2006 and 2010. The authors reported that despite the geometric mean serum PFAS concentrations decreasing by approximately half during this time, there was a limited corresponding change (a mean increase of 1.8% (SD: 26.6%) in LDL cholesterol. For a person whose serum PFOA decreased by half, there was only a 3.6% (95%CI: 1.5–5.7%) decrease in serum LDL cholesterol and a 5% (95% CI: 2.5–7.4%) decrease in serum LDL cholesterol for the same reduction in serum PFOS (Fitz-Simon et al. 2013). Unfortunately, the EPA did not rely upon the results of Fitz-Simon et al. (2013) for the derivation of PFOA and PFOS MCLs as the study shows that despite significant decreases in serum PFOA and PFOS levels, it does not translate to any clinical significance as both sets of LDL cholesterol data are within the normal ranges. For comparison, clinical trials of statin medication generally show a 28-35% reduction in LDL cholesterol (Ross et al. 1999).

As stated previously, despite these positive associations, there was no evidence of a concurrent increase of cardiovascular disease (Winquist and Steenland 2014; Steenland et al. 2020). Similarly, in a Swedish nested case-control study by Schillemans et al. (2022), it was found that although exposure to five PFAS, including PFOA and PFOS, was associated with increased cholesterol levels, no associations between higher risk of myocardial infarction, stroke, or cardiovascular disease were observed. This study is not cited or discussed in either the EPA evaluation or the review by the SAB (Science Advisory Board (SAB) 2022).

Several epidemiological studies have reported associations between exposure to PFAS and cardiovascular outcomes, including atherosclerosis, and broader cardiovascular disease endpoints. Notably, these findings were not incorporated into the EPA's risk assessment framework as part of its recent regulatory determinations for PFAS. Specifically, Yang et al. (2024) and Osoiro-Yanez et al. (2021) identified statistical associations between serum concentrations of PFHxS, PFDeA, and PFOS with markers of vascular calcification, including abdominal aortic calcification and coronary artery calcification. It should be noted, however, that Yang et al. (2024) explicitly acknowledged that their analysis did not yield conclusive evidence of a causal relationship. In a separate investigation, Biggeri et al. (2024) reported an increased risk of cardiovascular mortality–including ischemic heart disease–among residents of a PFAS contaminated region in Italy. While these findings contribute to the growing body of literature examining potential cardiovascular effects of PFAS exposure, further research is warranted to assess consistency across populations, clarify dose-reponse relationships and address confounding factors.

As expressed in the comments of the various reviewers of the PFAS MCLs, it is unclear whether the EPA relied upon experienced cardiologists and internists to conclude that these changes in lipids were clinically significant (SAB 2022, Hua et al. 2025) (in press). No mention was made of such in the EPA's Final PFAS NPDWR. The most important shortcoming is that the Steenland et al. (2009) and Dong et al. (2019) studies relied upon only a single blood sample for their analysis. Nonetheless, EPA identified a point of departure (POD) based on these studies when, in fact, no POD existed and the study design was incorrect to establish causation. The myriad of issues surrounding assigning a causal relationship to PFOS and PFOA exposure

and changes in serum cholesterol in humans was discussed in a workshop report by Andersen et al. (2021a). While a mechanism for this response has still not been identified, the authors listed several recommendations for further research that would shed light on this topic.

Failing to be able to quantitatively address confounding variables was another shortcoming in these studies. As Dong et al. (2019) noted:

> "The NHANES data are capable of examining the association but cannot address the issue of causality. Similar to other cross-sectional studies, this study cannot answer whether: 1) exposure to PFASs elevates the cholesterol level; 2) high cholesterol levels allow the storage of PFASs easier; or 3) joint factors simultaneously affect both PFASs and cholesterol … " (Dong et al. 2019, p. 466).

Regarding hypertension, although there is some indication of a relationship between PFOA and PFOS exposure and at least one continuous blood pressure metric, the findings across different studies are not consistent. Perhaps most importantly, as acknowledged by the EPA, an increase in cardiovascular disease incidence or risk has yet to be identified when studying even the most highly exposed PFAS populations (Winquist and Steenland 2014; Steenland et al. 2020; EPA 2024i). As stated in Steenland et al. (2020), "… it is plausible that there is a positive association of PFOA with raised cholesterol, yet no impact on the risk of cardiovascular disease" (Steenland et al. 2020, p. 3). This should have given the EPA pause before considering this endpoint as a driving endpoint in this regulation. Indeed, it was identified by EPA as one of the primary reasons why the MCL would improve public health when, in fact, there was no robust evidence that that was accurate (U.S. Environmental Protection Agency (EPA) 2023f).

There are several shortcomings with relying upon total cholesterol as an endpoint intended to suggest an increased incidence of the actual adverse effect (e.g., heart disease). As some commentators wrote to EPA during the comment period (Hua et al. 2025) (in press):

> "The associations with total cholesterol also are not biologically significant. For example, EPA derives an exposure-response slope factor for PFOA equal to 0.08 mg/dL per ng/mL, which means that for every 1 ng/mL increase in serum PFOA, total cholesterol increases by 0.08 mg/dL. Based on the [the general population blood serum concentrations] as estimated by the EPA, the difference in serum concentrations between 4.0 ppt and 10 ppt was less than 1 ng/mL. A change of 0.08 mg/dL of total cholesterol is not biologically meaningful, since total cholesterol is typically reported in mg/dL as whole integers (e.g., 175 mg/dL or 200 mg/dL); cholesterol is not measured or reported to the hundredths mg/dL. Thus, a potential change in total cholesterol going from a drinking water exposure at 4.00 ppt to 10.0 ppt, would not likely be measurable. For PFOS, the exposure-response slope factor is 1.57 ng/mL per ng/mL, which also does not represent a biologically significant change in cholesterol, especially over small changes in serum concentrations" (3M Company 2023, p. 68–69).

The association between serum lipids and PFOA does not adequately account for recent findings regarding reverse causality. The AWWA noted in their comments (Hua et al. 2025) (in press):

> "An international scientific panel (Andersen et al., 2021b) concluded that correlated net absorption or excretion of bile salts and PFAS in the gut enterocytes could give rise to the apparent associations of cholesterol and PFAS in blood observed in epidemiological studies. It has been demonstrated that several bile acid transporters expressed in enterocytes and hepatocytes can also transport PFAS, suggesting that PFAS could be entrained within the enterohepatic recirculation of bile acids. Co-modulation of the kinetics of bile acids and PFAS at these specific transporters by cholesterol has been shown in the rat. Correlated uptake/biliary excretion of PFAS and bile salts could serve as a confounding link between cholesterol homeostasis and PFAS kinetics, leading to an apparent association between Total Cholesterol (TC) and PFAS concentrations in serum" (AWWA 2023, p. 22).

When characterizing the toxicity of PFOA and PFOS, scientists need to consider reverse causality and uncontrolled confounders, which are particularly important at low exposure levels and when the results are more strongly influenced by complex behaviors (i.e., diet) (Steenland et al. 2020). Andersen et al. (2021a) provides a robust discussion on the state of the science regarding reverse causality and confounding by disease to the interested reader.

In the EPA's analysis, while there was a large quantity of published information available, not all of the available information was considered by the Agency; thus, the weight of evidence assessment used in their evaluation may be biased and potentially flawed. For example, in their analyses, it appears that the EPA did not adequately consider other cross-sectional studies that observed negative or inverse associations between PFAS exposure and effects on blood lipids (Olsen and Zobel 2007; Château-Degat et al. 2010; Donat-Vargas et al. 2019). In one study by Convertino et al. (2018), GEE analysis revealed the average rate of change in total cholesterol with increased PFOA concentration was -1.2×10^{-3} millimol/liter/millimolar, and this decline in total cholesterol levels occurred when serum PFOA levels ranged between 420 and 565 micromolar [175,000 to 230,000 ng/mL]. In addition to these mixed, clinically insignificant, and/or conflicting findings, it is important to note that many other factors affect the concentration of the various types of cholesterol in blood (e.g., genetics, diet, and exercise) (Kanter et al. 2012). While marginal changes in blood lipid levels have been associated with PFAS (Steenland et al. 2009; U.S. Environmental Protection Agency (EPA) 2016a, 2016b; Liu et al. 2018) it should be reemphasized that increased risk of cardiovascular disease has not been associated with increasing PFAS concentrations in the blood (Steenland et al. 2020; Girardi and Merler 2019; Raleigh et al. 2014).

It is well known that for studies using measured cholesterol as an endpoint, if researchers are to identify whether there are any effects due to treatment, a complete medical history of participants and repeated serum measurements are required. Perhaps most importantly, the collection of the blood must occur at regular intervals, and the volunteer needs to either fast or not-fast consistently; otherwise, the data are not informative. A review of the various methods used in the studies indicated that neither a series of measurements nor standardized conditions were in place for the Steenland et al. (2009) and Dong et al. (2019) studies that

the EPA relied upon. The Fitz-Simon et al. (2013) study that did perform repeated measurements and used similar methodologies as the Steenland et al. (2009) article, did not find a significant reduction in lipid levels, despite an approximately 50% reduction in serum PFOA and PFOS concentrations (EPA 2024j, 2024k). As stated previously, no increased incidence of CVD was found in this population, despite their high exposure to PFAS in drinking water (Winquist and Steenland 2014; Steenland et al. 2020).

As noted by the EPA, occupational studies typically represent highly exposed populations. Thus, if these populations do not have increased risk of developing CVD, then one would not expect a response in the general population who are exposed to much lower PFAS concentrations in drinking water or who have lower blood levels of PFAS. As stated in the PFOA Toxicity Assessment (EPA 2024k, p. 3–187), the occupational studies "suggest no association between PFOA and TC in workers", in part due to a lack of statistically significant associations for the observed increases in serum TC and the reported inverse association between changes in PFOA and serum TC (Olsen et al. 2012; 3M Company 2023). EPA stated that:

> "[c]ross-sectional occupational studies … reported positive associations between PFOS and increased serum TC … however, the association was not observed in longitudinal analyses" (EPA 2024j, p. 3–145).

This weakens the claims of a causal relationship, since the significant associations were only observed in cross-sectional analyses that cannot establish a temporal relationship (3M Company 2023) and studies have not yet been conducted that would assist in identifying a causal mechanism in humans (Andersen et al. 2021a).

Concerning animal studies, the EPA asserted that several studies of PFOA exposure "… in rodents provide evidence of alterations in serum total cholesterol and triglycerides, though the effect direction varied with dose" (EPA 2023e, p. 18658), which is consistent with the workshop report by Andersen et al. (2021a). This puts into question the utility of using animal models for this endpoint and the need for further research to be conducted on this topic.

With respect to reliance on animal studies, with human studies already demonstrating mixed findings, it does not seem logical that information from various animal species would shed additional light on this topic. Although it is easier to control for various factors such as diet and the time of blood sampling, there are other confounders that need be accounted for when conducting studies regarding blood lipids. With respect to blood chemistry and compensatory mechanisms, humans are much more complex and have distinct differences in lipid metabolism than the laboratory rodent (Gordon et al. 2015).

In summary, although some research suggests an association between modest cholesterol increases and exposure to PFOA and PFOS, there is no evidence that this leads to an increased incidence of CVD. The studies that support this conclusion (i.e., Winquist and Steenland 2014; Steenland et al. 2020), did not seem to carry much weight with EPA, although it is arguably the most powerful argument against the Agency's claim that the MCL will be protective of cardiovascular health in the U.S.

Despite the lack of epidemiological evidence or plausible biological pathways connecting PFOA or PFOS with CVD, the EPA derived an RfD for both substances based on their potential to raise LDL and total cholesterol. The scientific foundation for that decision remains unclear and seems to be unsupported by the lack of evidence of increasing incidence of cardiovascular disease in highly exposed PFAS populations.

Misconception #4: Epidemiology or animal studies show that exposure to PFOA and PFOS at concentrations found in drinking water may lead to hepatotoxicity in humans

In their final PFAS NPWDR, the EPA stated that "[e]vidence indicates associations between PFOA and PFOS exposure and hepatic effects, such as increases in ALT" (EPA 2024i, p. 32699). Similar to analyses of the other endpoints that the EPA identified, all the relevant data do not appear to have been considered and the clinical relevance (rather than statistical relevance) did not receive adequate consideration.

Many studies identified by the EPA reporting an association between PFOA or PFOS exposure and hepatic diseases were either of low confidence (Girardi and Merler 2019) or had statistically insignificant associations (Jin et al. 2020).

For hepatic effects, the EPA identified three *medium-confidence* epidemiological studies which they reported:

> "… provided all necessary analytical information (e.g., exposure distribution or variance, dose-response data, etc.) for POD derivation, [and] analyzed the outcome of interest in the general population or susceptible population" (EPA 2024k, p. 4–2).

Thus, the EPA concluded that the Gallo et al. (2012), Darrow et al. (2016), and Nian et al. (2019) for PFOA, and Gallo et al. (2012) and Nian et al. (2019) for PFOS, were adequate for the derivation of RfDs using alanine aminotransferase (ALT) elevation as the endpoint. Using these studies, the EPA determined that human serum levels as low as 1.1 to 5.2 ng/mL and 0.57 to 5.0 ng/mL for PFOA and PFOS, respectively, were sufficient to cause adverse hepatic effects. Some commenters mentioned that in order to truly understand the effects on the liver, many other clinical endpoints from the lipid panel and urine analyses should have been considered (3M Company 2023; AWWA 2023; Hua et al. 2025) (in press). For example, SPGT, SGOT, SDH and other markers of cellular damage of the liver are normally considered at the same time ALT is assessed (Bethea and Pratt 2022).

To assess the effects of PFOA and/or PFOS on liver function biomarkers, Gallo et al. (2012) and Nian et al. (2019) employed cross-sectional studies, while Darrow et al. (2016) conducted a cohort study.

Gallo et al. (2012) conducted a cross-sectional study involving 47,092 adults who participated in the C8 Health Study to examine the association between serum PFOA and PFOS concentrations and liver markers (ALT, aspartate aminotransferase (AST), gamma-glutamyl-transferase (GGT), alkaline phosphatase (ALP) and direct bilirubin). The authors

conducted linear regression modeling of natural log (ln) transformed values of ALT, GGT and direct bilirubin and the model was adjusted for potential confounders, and analyses of PFOA and PFOS deciles in relation to high liver biomarkers were reported. The results showed that PFOA and PFOS serum concentrations were positively associated with ALT concentrations, but there was inconsistent evidence for an associations between PFOA/PFOS and GGT or bilirubin (Gallo et al. 2012).

Darrow et al. (2016) recruited 32,254 participants from the C8 Health Project (2005–2006) who had liver disease. 30,723 subjects had available liver biomarkers. They examined associations between modeled PFOA exposure and biomarkers of liver injury (i.e., ALT, GGT and direct bilirubin). The authors found that there was a positive association between the modeled PFOA serum concentration and ALT levels, with an increase from the first to the fifth quintile of cumulative PFOA exposure associated with a 6% increase in ALT levels (95% CI: 4%, 8%), and 16% increased odds of having above normal ALT (95% CI: 1.02%, 1.33%). There was no association between PFOA and elevated GGT, and PFOA was associated with decreased direct bilirubin. Most importantly, there was no evidence that the observed increases in ALT liver enzymes led to an increased risk of liver disease (Darrow et al. 2016).

Nian et al. (2019) conducted a cross-sectional study on a cohort of 1,605 individuals who were enrolled in the "Isomers of C8 Health Project in China" and lived approximately 100 km from one of China's largest fluoropolymer plants. The researchers measured the concentration of 18 PFAAs in the serum of individuals, as well as nine biomarkers, Albumin (ALB), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Total Protein (TP), in alkaline phosphatase (ALP) between Prealbumin (PA), and Cholinesterase (ChE), Total Bilirubin (TB), and Gamma-Glutamyl Transferase (GGT), indicative of liver health. The study conducted linear and logistic regression analysis to determine if there was an association between PFAS concentrations and adverse liver biomarkers. Regarding ALT levels, they reported that a one ln unit increase in PFOA levels was associated with a 7.4% increase in measured ALT levels (95% CI: 3.9%, 11.0%) and a 4.3% increase per one ln unit increase in PFOS serum levels (95% CI: 1.2%, 7.4%). As discussed previously, cross-sectional studies cannot establish causation and the results from Nian et al. (2019) would likely not be considered clinically relevant.

The three studies relied on for the derivation of the RfD reported associations between PFOA/PFOS exposure and increased ALT. As with other human observational studies, those describing potential hepatotoxicity can identify statistically significant associations but are unable to demonstrate causation (e.g., increased incidences of liver injury or disease). Elevated ALT concentrations alone are not clear predictors of hepatic disease. In fact, the available literature fails to associate any incidence of hepatic disease with PFOA and PFOS exposure (EFSA 2018), including from the C8 Health Project (C8 Science Panel 2012a; Darrow et al. 2016; Steenland et al. 2020).

For the EPA to have had confidence that liver toxicity was occurring, they needed to evaluate the six or seven classic indicators that are presented in the standard blood and urine panels. A major shortcoming observed across the EPA's search for evidence of hepatotoxicity is the assumption that a slight elevation in ALT, measured at one point in time, is indicative of liver injury. In fact, ALT is a nonspecific marker of liver function that may be elevated when there is no substance-induced liver injury (Tiller and Stringer 2023), which the EPA did not mention in their toxicity assessment (EPA 2024k, 2024j). ALT levels higher than 45 IU/L are not necessarily reflective of liver injury (Helsper et al. 2012), For example, a bout of strenuous exercise may increase serum ALT for up to a week afterward (Tiller and Stringer 2023). Fluctuations such as these are not reflected in the singular blood sample obtained per participant, as seen in the studies that the EPA relied upon to derive their hepatic RfD.

Interestingly, Convertino et al. (2018) documented a clinical trial assessing the chemotherapeutic potential of ammonium perfluorooctanoate (APFO) in 49 cancer patients. The dosages assessed in this study ranged between 50 and 1,200 mg (which are sometimes higher than those used in animal studies) and did not induce liver, thyroid, or renal toxicity. When the liver effects were evaluated, ALT and other liver enzyme levels were unaffected, and no hepatic functional changes were observed. Lastly, serum creatinine, urea and uric acid were not associated with PFOA serum levels, suggesting PFOA did not affect renal function. In the thyroid, the increase in serum free thyroxine (fT4) levels was attributed to antagonistic hormone binding by PFOA.

The interpretation of liver injury by measured ALT serum levels (IU/L) alone is in discordance with clinical practices. The use of a battery of tests that includes the aminotransferases, alkaline phosphatase, bilirubin, albumin and prothrombin time is needed to diagnose liver disease. Additionally, no single set of liver tests will necessarily provide a diagnosis and it is often necessary to repeat tests for weeks for a diagnostic pattern to emerge (Bethea and Pratt 2022, p. 2556). This contextual information is highly important for assessing the EPA's utilization of elevated ALT levels as a prioritized endpoint "with the strongest overall weight of evidence based on human and animal evidence for POD derivation" (EPA 2023e, p. 18658). As such, from a clinical perspective, focusing on only one liver enzyme (i.e., ALT) will not necessarily result in a reduction in disease, such as was seen in the C8 Health Project (C8 Science Panel 2012a; Darrow et al. 2016; Steenland et al. 2020). According to an authoritative text on the matter:

> "Because values for enzyme activities in serum/plasma from normal (e.g., healthy) people can vary across clinical chemistry laboratories that perform the measurements, increases in the enzyme activities are typically expressed as a ratio normalized to the "upper limit of normal" (ULN) for a particular laboratory in which the measurements are performed. In humans, the serum ALT activity is 3 to 5 times or more above the ULN, suggesting liver injury. Clinically, drug-induced liver injury is often classified as hepatocellular, cholestatic, or mixed (e.g., both), depending on clinical chemistry results" (Roth et al. 2019, p. 733).

Another clinically relevant interpretation of elevated liver enzymes is summarized as follows in one of the most common textbooks used in medical schools:

> "The aminotransferases are normally present in the serum in low concentrations. These enzymes are released into the blood in greater amounts when there is damage to the liver cell membrane, resulting in increased permeability. Liver cell necrosis is not required for the release of the aminotransferases, and there is a poor correlation between the degree of liver cell damage and the level of aminotransferases. Thus, the absolute elevation of the aminotransferases is of no prognostic significance in acute hepatocellular disorders" (Bethea and Pratt 2022).

It would appear that the association between elevated ALT, PFOA and PFOS serum levels, and liver disease, as an endpoint of biological relevance, was not fully understood by the EPA. The lack of association with any clinically diagnosed liver disease and the nonspecific relationship between mildly elevated ALT and hepatic injury weakens the Agency's ability to identify hepatotoxicity as a health endpoint associated with PFOA and PFOS exposure. These types of endpoint changes that are reversible, and that do not equate to liver disease, are not suitable for influencing drinking water standards set by EPA.

Misconception #5: Animal studies on PFAS regarding immune, developmental, cardiovascular, and hepatic effects are appropriate for conducting health risk assessments on humans exposed to drinking water

The health effects ascribed to PFAS often stem from the health effects observed in animal studies. Unfortunately, the dosages used and the mechanisms of action for rodents are not always considered biologically relevant in humans. For PFAS compounds, it has been reported for nearly 20 years that the effects in rodents are not replicated in humans (if related to PPARα) (Ehrlich et al. 2023).

Comparing PFAS toxicity across species and strains is challenging due to inadequate mechanistic data, differences in animal dosing when compared to the exposure of humans (both occupationally and in the general population), and differences in elimination half-lives between species.

In 2021, the Agency for Toxic Substances and Disease Registry (ATSDR) published a comprehensive review of key literature examining the toxicological properties of PFAS and concluded that there was strong evidence of hepatotoxicity, developmental toxicity, and immunotoxicity in rodent models (ATSDR 2021). PFAS exposure was reported to induce hepatic steatosis in mice [PFOA: 10 mg/kg-day, 7 days, oral gavage] (Das et al. 2017), rats [PFOS: 100 ppm; 100,000,000 ppt, 3 weeks, dietary] (Bagley et al. 2017), zebrafish [PFOS: 0.5 μM; 250,000 ppt, 5 months, static water] (Cheng et al. 2016), chickens [PFOA: 2 mg/kg; 2,000,000 ppt; HFPO-DA: 1–8 mg/kg; 1,000,000–8,000,000 ppt, 21 days, embryo incubation] (Xu et al. 2020), frogs [PFOA: 0.01–1 mg/L; 10,000–1,000,000 ppt, 14 days, static solution] (Zhang et al. 2019) and primates [PFOA: 3-20/30 mg/kg-day; 3,000,000–20,000,000/30,000,000 ppt, 26 weeks, oral (capsules)] (Butenhoff et al. 2002). PFAS [PFOA: 0.1–20 mg/kg-day, 100,000–20,000,000 ppt, GD1-17, drinking water) exposure was reported to induce pregnancy loss, decreased pup survival rates, reduced pup body weights, delayed dam mammary gland differentiation, delayed eye-opening times, and altered sexual maturation (Abbott et al. 2007; Fenton et al. 2021).

Agency for Toxic Substances and Disease Registry (ATSDR) (2021) noted that rodent studies reported PFAS exposure resulted in decreased immune organ weights (e.g., spleen and thymus), decreased immunoglobulin responses, and altered lymphocyte subpopulations (Yang et al. 2000, 2001, 2002; Dewitt et al. 2008; Loveless et al. 2008; DeWitt et al. 2009; Son et al. 2009; Qazi et al. 2012; DeWitt et al. 2016; Kim et al. 2016), suggesting PFAS exposure induced immunomodulatory effects.

The EPA suggested the cardiovascular system may be a sensitive target of PFAS toxicity through the modulation of serum levels of triglycerides and cholesterol in animal models (EPA 2023e). In support, one of the several studies cited by the EPA was Das et al. (2017a) in which mice were administered 10 mg/kg-day PFOA for seven days via oral gavage and were found to have increased hepatic triglyceride levels.

Loveless et al. (2008) found that oral gavage of 0.29-9.6 mg/kg-PFOA for 29 days decreased serum triglyceride levels in rats, while 9.6 and 29 mg/kg-day PFOA decreased serum triglyceride levels in mice. The authors also reported that total cholesterol was significantly decreased in rats dosed with 0.3 and 1 mg/kg PFOA compared to control-treated rats, and no differences in serum cholesterol levels were observed in mice. Butenhoff et al. (2002) reported that 10 mg/kg-day PFOA (oral administration of capsule) significantly increased serum triglyceride levels after 5, 10, and 14 weeks of exposure compared to pretreatment triglyceride levels. In addition, the authors noted that cholesterol levels significantly decreased in the monkeys administered 30/20 mg/kg/day PFOA after 14 weeks of exposure compared to the respective baseline cholesterol levels, while significant increases in serum triglyceride levels were observed after 5 weeks of exposure compared to baseline levels in this treatment group (Butenhoff et al. 2002).

One of the primary mechanisms responsible for the adverse health effects observed in laboratory animal models is the activation of peroxisome proliferator-activated receptor-α (PPARα) (Issemann and Green 1990). Unfortunately, variations among species and strains exist surrounding PPARα sensitivity. Rats and mice are known to be among the most sensitive species to PPARα agonists, while guinea pigs, nonhuman primates, and humans are much less responsive (ATSDR 2021). In recent years, more studies utilizing PPARα-knockout (or null) mice exposed to PFAS have provided a clearer picture of the health effects that may be observed in humans. As discussed in Goodrum et al. (2021), the MOA for adverse effects in humans is complex and likely involves multiple nuclear receptors. Therefore, animal studies that are focused on mechanisms solely dependent upon PPARα are likely uninformative in assessing human health risks.

As identified in Clewell (2024), other nuclear receptors (e.g., PPARα-independent) play a role in the health effects observed in both rodents and humans. In the intestine, absorption and biliary clearance are facilitated by organic

anion transporting polypeptides (OATPs) OATP1B1, OATP1B3, and OATP2B1 (Zhao et al. 2017) and the organic anion transporter 4 (OAT4) (Nakagawa et al. 2009). In the kidneys PFOA clearance is facilitated by OAT1 and OAT3, and is reabsorbed by OAT4 and urate transporter URAT1 (Yang et al. 2010). In the blood, PFOA binds readily to albumin (Maso et al. 2021) and to the plasma thyroid hormone carrier, transthyretin (TTR) (Ren et al. 2016). PFOA binds to fatty acid binding proteins (FABPs) in hepatocytes which facilitates the transport and utilization of fatty acids within cells (Luebker et al. 2002).

Dewitt et al. (2008) conducted a two-part study examining the immunomodulatory effects of PFOA in adult female mice. The authors performed a recovery study in which female C57BL/6J mice (6-7 weeks old) were orally gavaged with 30 mg PFOA/kg/day once daily for 15 days. On days 11-15, half of the mice in the treatment group were switched to water vehicle treatment (recovery group), and the other half continued to receive PFOA treatment. The dose-response portion of the study exposed C57BL/6N female mice (6-7 weeks old) to 0-30 mg/kg/day in drinking water for 15 days. In the recovery study, sheep red blood cell (SRBC)-specific IgM antibody titers were reduced compared to controls in both the recovery and the continuous treatment groups. In the dose-response studies, all doses of PFOA decreased SRBC-specific IgM antibody titers compared to the controls. The authors noted in the dose-response study that 3.75 mg PFOA/kg/day resulted in serum PFOA levels of 7.4 x 10^4 ng/mL after one day of exposure. The serum PFOA levels detected in these animals were 150-fold greater than the levels reported in individuals living near a PFOA production site (Dewitt et al. 2008). According to EPA, these studies have helped to provide evidence that PPARα drives hepatotoxicity, immunotoxicity, and developmental toxicity and influences serum levels of triglycerides and cholesterol in rodent models (EPA 2024k). However, these adverse effects are likely not relevant to humans and, even if animals are found to be relevant for predicting the human response, the doses required are thousands of folds higher than what Americans are currently ingesting each day from drinking water (Smalling et al. 2023).

Dose is the fundamental principle in toxicology and, typically, animal studies often examine the toxicity of compounds at doses hundreds to thousands-fold higher than human-relevant intake or blood concentrations. The general U.S. population had blood concentrations of 1.4, 4.3, and 1.1 μg/L [1,400, 4,300, and 1,100 parts per trillion (ppt)] for PFOA, PFOS, and PFHxS, respectively per the 2017 to 2018 NHANES dataset (ATSDR 2021). For comparison, ATSDR reported PFOA-induced hepatic effects in laboratory animals occurs between 1 and 20 mg/kg-day (1,000,000–20,000,000 ppt) (ATSDR 2021). Meanwhile, PFOS was reported to induce hepatotoxicity in mice and rats at 0.05 to 0.1 mg/kg-day (50,000–100,000 ppt) (ATSDR 2021).

In short, the animal studies used by the EPA involve PFAS doses that cause liver toxicity at levels approximately 700 to 14,000 times higher for PFOA and 11 to 23 times higher for PFOS than the blood levels found in the average American in 2018. Meanwhile, adverse developmental effects were observed at serum concentrations of 2,300 ng/ml [2,300,000 ppt] in mice, which is at levels approximately 1,642-fold higher than serum concentrations observed in the United States general population in 2018 (Post et al. 2012; ATSDR 2021). The levels of PFOA and PFOS in serum samples of U.S. residents have decreased appreciably since the phase out of these substances in the United States. The geometric mean serum levels of PFOS have declined over 84% from NHANES survey years 1999–2000 (30.4 ng/mL) to 2013–2014 (4.72 ng/mL) and the geometric mean serum levels of PFOA have declined 70% over the same temporal period, decreasing from 5.2 ng/mL in years 1999–2000 to 1.56 ng/mL for 2015–2016 (ATSDR 2021).

The higher dosages utilized in animal studies have traditionally been used to identify toxic effects, assess dose-response relationships, understand mechanisms of action, protect sensitive populations, and determine margins of safety for exposed humans. High dosages are sometimes useful for identifying potential health effects that may not be observable at lower dosages (Paustenbach 2024b). For non-carcinogenic effects, high dosages are often not useful for predicting the likelihood of adverse outcomes at doses 100 times lower than used in studies (Paustenbach and Cox Jr. 2024).

Researchers have found that animal testing to evaluate the possible acute and chronic effects in humans from PFAS chemicals is often not useful (Rodriguez 2021). For example, ATSDR acknowledged in their comprehensive review that the hepatic health effects observed were specific to mice and rats and did not apply to human health (ATSDR 2021, p. 182). Regarding the developmental effects of PFAS, it has been said that "[t]he effects occurring at the repeated administered oral doses of PFAS most relevant to drinking water; exposures are those from animal developmental toxicity studies involving maternal oral exposures during pregnancy and [during] lactation and result[ing] in adverse effects in the offspring … at blood levels almost 1,000 times higher [than] levels observed among the general human population" (Rodriguez 2021, p. 4).

Two of the most interesting adverse effects observed in animal studies, hepatotoxicity and developmental toxicity, were reported at exposure levels hundreds to thousands-fold greater than what is observed in the general population or in occupationally exposed individuals. Even the EPA noted in their promulgated MCL rule that "[s]everal studies in rodents provide evidence of alterations in serum total cholesterol and triglycerides, though the effect direction varied with dose" (EPA 2023e, p. 18658). Many commenters on the draft PFAS NPDWR, including 3M, AWWA, and ACC noted that the data from animal studies at these doses, especially for non-carcinogenic effects, were only marginally useful since a cascade of adverse effects can occur in several organs at these high doses that would never occur at much lower doses. Overall, with respect to using laboratory animals to assess PFAS chemicals, we still have much to learn as to whether rodents or other animals are good models for humans, particularly at the low doses that the general U.S. population is exposed to.

Misconception #6: PFOA and PFOS are known human carcinogens

In their final regulation of the PFAS MCLs in April 2024, the EPA stated that they:

> " … assessed the weight of the evidence for the available cancer data and determined that PFOA and PFOS are 'Likely to Be Carcinogenic to Humans consistent with the Guidelines for Carcinogen Risk Assessment'" (EPA 2024d, p. 39125).

The EPA assigns this designation to chemicals where "the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor Carcinogenic to Humans" (EPA 2023e, p. 18659, 18718). This statement is not based upon conducting a complete risk assessment for these compounds; rather, it is a hazard identification. Without considering dose, one cannot characterize the risk of any particular exposure scenario.

For PFOA, the EPA stated that there was "evidence of kidney and testicular cancer in humans and LCTs, PATS [pancreatic acinar cell tumors], and hepatocellular adenomas in rats" (EPA 2023h, p. 3–306). The Agency also stated that there was an association between PFOS exposure and liver cancer (EPA 2024i, p. 32699).

Over the past two decades, various scientists have noted that the classic triad of PPARα activated tumors (i.e., Leydig cell tumors (LCTs), pancreatic acinar tumors, and liver heptatocelllular tumors) observed in rats lack relevance to human health (as reviewed in Klaunig et al. 2003). PPARα tumors, regardless of the agent tested, are irrelevant to humans as the mechanisms responsible for initiating uncontrolled cell division for these malignancies after PPARα activation (i.e., the downstream activation of lipid metabolism, peroxisome proliferation, oxidative stress) are different (Klaunig et al. 2003; Corton et al. 2014; 2018; Felter et al. 2018). For PFOS, EPA stated:

> "This determination is based on the evidence of hepatocellular tumors in male and female rats, pancreatic islet cell carcinomas in male rats, and mixed but plausible evidence of bladder, prostate, kidney, and breast cancers in humans" (EPA 2023e, p. 18663).

Often there is a misunderstanding in epidemiology regarding the difference in testicular Leydig cell tumors and testicular germ cell tumors. There are no known environmental agents that induce germ cell tumors in humans (Mulvihill 2012). In rats, the mechanisms of action for germ cell tumors differ from the MOAs for Leydig cell tumors (Klaunig et al. 2012; Steinbach et al. 2015).

The EPA's designations for PFOA and PFOS followed closely on the heels of the International Agency for Research on Cancer (IARC), who, in November 2023, designated PFOA as carcinogenic to humans" and PFOS as "possibly carcinogenic" (IARC 2023). IARC claimed that there was strong mechanistic evidence for both chemicals, sufficient evidence for cancer in experimental animals for PFOA, and limited evidence for cancer in experimental animals for PFOS. Notably, cancer evidence in humans (specifically that for kidney and testicular cancer) was deemed limited for PFOA and inadequate for PFOS (IARC 2023). The basis for these claims seems questionable.

When IARC lists a chemical as likely to be carcinogenic to humans, they do not consider dose, bur rather categorize the chemical by its carcinogenic risk. For some chemicals, it is entirely possible that some chemicals listed by IARC will never be associated with appreciable human intake or ever pose a cancer risk in the citizenry. For example, toxins such as aflatoxins and sterigmatocystin in certain mushrooms are known to be carcinogenic in the liver, and IARC lists them as such (Ezekiel et al. 2013). However, few, if any, individuals actually ingest enough of these compounds to ever pose a increased cancer risk.

The EPA's systematic review identified 22 epidemiological studies and four animal toxicological studies that investigated the relationship between PFOA and cancer (EPA 2024k). For PFOS, 18 epidemiological and one toxicological study were identified that examined this endpoint (EPA 2024j). The SAB advised that toxicity values should be based on medium-confidence studies or higher and requested clarification on the animal bioassay data used for cancer slope factor (CSF) development (Science Advisory Board (SAB) 2022). The SAB noted the EPA's unclear stance on kidney tumor mechanisms so EPA favored reliance on human data.

Epidemiological studies have generally shown associations, but no significant increased cancer risk associated with PFOS exposure in occupational settings, or the general population. Two highly cited reviews have been conducted that examined the association between PFAS exposure in the general population and cancer (Steenland et al. 2020; Steenland and Winquist 2021). Steenland et al. (2020) examined the PFOA literature regarding thyroid disorders, cancer, immune and auto-immune disorders, liver disease, hypercholesterolemia, reproductive outcomes, neurotoxicity, and kidney disease. For cancer, the authors identified 19 epidemiology studies regarding PFOA exposure, six of which involved occupational cohorts. Since the 2012 C8 Science Panel's determination that there was a probable link between PFOA exposure and testicular and kidney cancers (C8 Science Panel 2012b), the authors stated that "[t]he modest evidence that has accumulated since that time does not generally strengthen the conclusion that PFOA is carcinogenic for any given site, also there is somewhat stronger evidence for kidney cancer" (Steenland et al. 2020, pg. 2).

Steenland and Winquist (2021) examined 16 cohort (or case-cohort studies), 10 case-control studies (4 nested within cohorts and 6 non-nested), one cross-sectional study and one ecological study that related to PFAS exposure and cancer. From their analysis of these studies, the authors reported that "[w]hile there are no associations between PFAS and cancer that have been both marked and consistent across studies, there is some evidence for an association of PFOA with testicular cancer" (Steenland and Winquist 2021, pg. 26). Fortunately, testicular cancer is rare and is not fatal, and only three studies have reported on this association (Barry et al. 2013; Vieira et al. 2013; Mastrantonio et al. 2018). The authors reported that for kidney cancer and PFOA exposure, the evidence was suggestive based upon several studies (Steenland and Woskie 2012; Barry et al. 2013; Vieira et al. 2013; Mastrantonio et al. 2018; Shearer et al. 2021), but there was

no evidence of an association with exposure to other PFAS (Steenland and Winquist 2021).

There are conflicting results in non-occupational contexts, particularly with breast cancer (Cohn et al. 2020; Itoh et al. 2021; Mancini et al. 2020; Omoike et al. 2021), where a recent study has suggested a potential association between PFOS exposure and estrogen receptor-positive tumors (Mancini et al. 2020). However, these studies often had small sample sizes and variable PFOS levels, limiting the strength and relevance of findings. EPA did not consider the data sufficiently reliable to identify breast cancer as a hazard (at any dose).

The EPA did not find sufficient evidence of a relationship between either liver or pancreatic cancer and PFOA exposure, with the exception of excess liver cancer seen in Girardi and Merler (2019). However, this study did not account for alcohol consumption as a confounder (Girardi and Merler 2019), which is a significant risk factor for this disease. In the final NPDWR, the EPA estimated that "… an expected value of $4.79 million in benefits via the reduction in liver cancer cases …" can be realized in the decades following the promulgated MCLs (EPA 2024i, p. 32712). As there is no association between PFAS exposure and this disease, it is unclear how these savings will be realized. Subsequent analyses that have been performed have not added any additional conclusive evidence that PFAS exposure are casually linked with cancer (Law et al. 2023; Rhee et al. 2023; Biggeri et al. 2024; George and Birnbaum 2024; Zahm et al. 2024).

The EPA's benefits estimate used to justify the MCL included projected reductions in human kidney cancers from decreased PFOA and PFOS drinking water concentrations, despite warnings from the Science Advisory Board (SAB) about the reliability of the Shearer et al. (2021) study. Additionally, the estimate anticipated a reduction in bladder cancer cases linked to lower levels of disinfection byproducts (DBPs), specifically trihalomethanes (THM), from new treatment technologies that would be used to remove PFAS in drinking water (EPA 2023e). However, the decision to promulgate the PFAS regulation should not be based upon another unrelated contaminant's adverse health effects.

It is noteworthy that the EPA's promise of a reduced cancer incidence due to removing PFOA and PFOS as they were labeled as carcinogenic and possibly carcinogenic, respectively, from drinking water, even though there was no causal evidence that the chemicals have caused any type of cancer in humans, also encompasses benefits assumed from the removal of other contaminants. While EPA believes PFOA is likely to be carcinogenic, a study by Convertino et al. (2018) conducted a phase 1 trial to determine the safety, dose limiting toxicity, and maximum tolerated dose of ammonium perfluorooctanoate (APFO) as a potential chemotherapeutic agent. Based on what EPA has presented, it is apparent that one reason for aggressively regulating PFAS in drinking water is that the technology for removing these chemicals will also remove other contaminants, (i.e., pesticides, heavy metals, organic contaminants, disinfectants and disinfection byproducts) in drinking water that the EPA is mandated to remove under the SDWA (U.S. Environmental Protection Agency (EPA) 2020a). Based upon the lack of any causal relationships (instead of statistical associations) between PFAS exposures and the endpoints selected in the peer-reviewed literature, it is likely that these ancillary benefits are a primary driver behind these MCLs. This is a policy decision, but it should be made clear by the EPA that it is a policy decision, rather than a decision based upon robust science.

A significant amount of genotoxicity and mechanistic data supports the view that PFOA is not mutagenic and may only cause genotoxic effects at cytotoxic concentrations (Butenhoff et al. 2014). This is generally supported by epidemiological and animal studies (Temkin et al. 2020). For example, it is widely accepted that PFOA induces tumors in laboratory animals through non-genotoxic or epigenetic mechanisms and at doses of approximately 1.1 to 4.6 mg/kg/day in rats (NTP 2023). In addition, 3M noted in its submitted comment to the proposed MCL that EPA concluded "most of the evidence [regarding PFOA] for mutagenicity is consistently negative" (3M Company 2023, p. 30). Studies, including those summarized by Klaunig et al. (2012), have shown that PFOA-activated genes associated with PPARα activation (important for fatty acid balance and cell cycle regulation) induced peroxisome proliferation and liver enlargement primarily through a hypertrophic response. Additionally, PFOA has been shown to increase DNA synthesis and promote the clonal expansion of preneoplastic hepatic lesions, supporting its proposed mode of action in liver tumor development in rats (Klaunig et al. 2012). These substances typically do not lead to tumor formation in humans due to significant differences in biological parameters between species (Klaunig et al. 2012).

Using the evidence mentioned above, the independent scientific committee that advises the United Kingdom's governmental agencies and health departments concluded that there was "no evidence for a link between exposure to PFASs and cancer risk" (Committee on Toxicity (COT) 2022, p. 26). In addition, the European Food Safety Authority's (EFSA) 2018 Panel on Contaminants in the Food Chain (CONTAM Panel) found little evidence linking PFOS or PFOA exposure to increased cancer risk (EFSA 2018). As previously discussed, subsequent studies up to the present have not changed that conclusion, as there has yet to be sufficient evidence to support the human carcinogenicity potential of PFOA and PFOS. As discussed in Perez et al. (2023), for carcinogenicity, it is unlikely that of the nine Hill criteria, that only plausibility would be met. In addition, the SAB noted that in the weight of evidence discussion, the Hill criteria should be referenced in the epidemiological data.

Based on all the data that has been evaluated in the peer-reviewed literature, it is our view that the perception by the American public that PFOA and PFOS pose a considerable carcinogen risk, at the concentrations detected in the vast majority of drinking water, is not supported by the epidemiology studies conducted to date. Based on the weight of scientific evidence, there is minimal support for the agency's decision to set an MCLG of zero for the PFAS identified in the MCL, and setting the MCLs at the practical quantification limits (PQLs) appears unwarranted.

Misconception #7: The four PFAS in the MCL regulation can be considered additive for all adverse effects as a mixture

In the promulgated MCL, the EPA considered the dose of PFHxS, HFPO–DA, PFNA, and PFBS, to be additive and set a drinking water limit for this mixture (EPA 2024i). The Agency reported that, due to dose additive concerns and likely co-occurence in drinking water, the Hazard Index approach was necessary to protect public health.

In December 2023, the EPA published a document titled "*Advances in Dose Addition for Chemical Mixtures: A White Paper*" that was developed to:

> "… advance cumulative risk assessment, specifically chemical mixtures risk assessment within the broad field of cumulative risk assessment, informed by U.S. EPA's experience and scientific progress since 2000" (EPA 2023a, p. xi).

This 2023 white paper discussed the use of MOA, toxicity pathways, and adverse outcome pathways (AOPs) to group chemicals together upon their similar toxic action(s) (EPA 2023a).

The EPA stated that for PFAS, including PFOA and PFOS, while these compounds were not toxicologically identical, they did "… elicit similar toxicological effects across different levels of biological organization, tissues/organs, life stages, and species" (EPA 2024l, p. 33). As such, they concluded that it would be health protective to assume that PFAS would have additive effects in mixtures if they shared one or more molecular/cellular pathway events and/or adverse health outcomes (EPA 2024m). This is the same assumption that the EPA used to set the MCLGs for HFPO-DA, PFNA, PFHxS, and PFBS in the promulgated PFAS NPDWR (EPA 2024i).

In a recent paper by George and Birnbaum (2024), the authors echoed the EPA by referring to the dioxins as a good example of how to use the toxic equivalents approach to regulate mixtures of chemicals which appear to be similar. A key difference in the suggested regulatory approach, however, was that PFAS would be better regulated with relative potency factors, rather than toxic equivalents, given the uncertainty surrounding PFAS MOA. They acknowledged that the diversity of PFAS chemicals was so vast that identifying toxic equivalencies (TEQs) would be a challenge; especially since there is no MOA that appears similar and there are very few known clinically adverse effects even at doses much higher than what most Americans receive (George and Birnbaum 2024; Steenland et al. 2020).

It appears that the decision to use dose additivity for these PFAS in the hazard index was a policy decision, not based upon scientific evidence that there is a shared MOA for the health endpoints selected by the EPA. The approach that the EPA used regarding dose additivity has been used by other regulatory bodies, including EFSA. ATSDR, Health Canada, the National Institute for Public Health and the Environment in the Netherlands (RIVM) and the WHO (Meek et al. 2011, Meet, 2013, WHO 2017, EFSA Panel on Contaminants in the Food Chain, 2020). However, the Australian Environmental Health Standing Committee and the Food Standards Australia and New Zealand (FSANZ) took the position that there was not sufficient evidence to assume concentration additivity between perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) (Food Standards Australia and New Zealand (FSANZ) 2022). At this time, there is no evidence of a single unifying factor for assessing PFAS mixtures.

Teuschler (2007) described several key questions that should be addressed prior to conducting a chemical risk assessment on mixtures, including: (1) When is it appropriate to generalize and assume dose or response additivity?; (2) What information is needed to determine that two or more chemical components of the mixture share a common MOA or have similarly shaped dose-response curves?; (3) What evidence is needed to estimate the toxicity of the mixture if whole mixture toxicity study data are lacking?; and (4) How should the fraction of unidentified chemicals that may be present in a mixture be addressed (Goodrum et al. 2021)?

As discussed in Rosato et al. (2022), there has been increased interest in exposures to mixtures of PFAS and there are a variety of statistical methods that have been utilized to assess their toxicity. While it is beyond the scope of this review to discuss the strengths or weaknesses of the various methods, the appropriate statistical method should be based upon the research question and there should be a robust discussion regarding the strengths and limitations of the selected statistical method (Vuong et al. 2020; Rosato et al. 2022).

With respect to the challenges of regulating PFAS chemicals as a class, there have been several strategies proposed for grouping PFAS chemicals together, rather than treating them all as a single class (Ritscher et al. 2018; Cousins et al. 2020a; Anderson et al. 2022). Cousins et al. (2020a) offered several potential strategies for grouping PFAS, with the selected method depending on whether the intent is to regulate them based upon intrinsic properties or to make informed decisions for risk assessments. As expected, each of the proposed grouping strategies has varying data requirements as well as advantages and disadvantages (Cousins et al. 2020a).

Anderson et al. (2022) discussed the results of an expert panel that was convened to examine how to group PFAS to conduct human health risk assessments. The authors noted that there were key data gaps that needed to be filled to conduct a PFAS mixtures risk assessment, including understanding the relevant critical effects, the mechanisms of PFAS toxicity, and better dose-response information. They additionally stated that studies were needed to define the relevant MOAs for PFAS that were necessary to inform grouping strategies to aid in the assumption of additive risk (Anderson et al. 2022). Ultimately, the authors concluded that "… the lack of knowledge about exposure, dose/body-burden-response relationships, relevant health effects, mode(s) of action, and potential interactions, does not allow for a science-based grouping of PFAS for the purposes of human health risk assessment" (Anderson et al. 2022, pg. 8).

In a study conducted by Barutcu et al. (2024), which re-analyzed published *in vitro* gene expression studies from human primary liver spheroids, it was revealed that with treatment times ranging from 10 to 14 days, shorter-chain PFAS (those with 6 or fewer fluorinated carbon atoms in the

alkyl chain) showed enrichment for pathways of fatty acid metabolism and fatty acid beta-oxidation with upregulated genes. Longer-chain PFAS compounds, specifically PFOS, PFDS (perfluorodecane sulfonate), and higher doses of PFOA, were reported to enrich pathways involved in steroid metabolism, fatty acid metabolism, and biological oxidation for downregulated genes (Barutcu et al. 2024). The transcriptomic analysis indicated that the biological MOAs of PFAS compounds differ according to chain length and dose, showing that risk assessments for PFAS must take these into consideration when evaluating PFAS mixtures. There are significant data gaps on how to handle mixtures (Peters and Gonzalez 2011; Cousins et al. 2020b; Bil et al. 2021; Goodrum et al. 2021). Given the extensive data gaps, it seems premature for the EPA to have promulgated a regulation that is based upon such extensive uncertainties.

There are claims that PPARα might give some insight as to how the PFAS chemicals produce adverse effects on various tissues, despite decades of research showing that these nuclear receptors are not relevant in humans (Klaunig et al. 2003; Corton et al. 2014, 2018; Felter et al., 2018). Further, there does not appear to be a clear target organ for these chemicals or a consistent adverse effect (certainly at the doses to which humans have been exposed). The EPA is aware of the limited relevance of the PPARα pathway to humans (EPA 2020b); even though it certainly is important in understanding some responses in rodents, but it doesn't seem to have altered their thinking in promulgating the MCL. The agency stated that:

> "The extent of PPARα activation is likely to differ by PFAS type, making it harder to apply read-across (specifically, drawing conclusions for one PFAS based on findings for another PFAS) or related approaches" (EPA 2020b, pg. 2-26).

Few scientists would likely disagree with that; nonetheless, it appears that EPA decided to embrace additivity for some of the PFAS that they chose to regulate in April 2024 (EPA 2020b, 2024m).

The EPA has not identified consistent MOAs for the noncancer effects for these compounds nor has there been any identified in the peer-reviewed literature (Corton et al. 2018; Felter et al. 2018; Chappell et al. 2020; Heintz et al. 2023; Clewell 2024; Li et al. 2024), with the Agency noting only some molecular and cellular similarities (EPA 2024m). While the primary molecular initiating event (MIE) identified from both *in vitro* and *in vivo* studies is the activation of PPARα (EPA 2024l), Corton et al. (2014) found significant species differences in response to PPARα activators, with rodents showing high sensitivity and guinea pigs, hamsters, nonhuman primates, and humans showing fewer and lesser biological response.

The key events of PPARα activation in liver tissue include gene regulation and hepatocyte proliferation; the succession of which can potentially lead to tumors (Li et al. 2024), but among these, only PPARα receptor activation is shared between humans and rats (Klaunig et al. 2012; Corton et al. 2018; ATSDR 2021; Li et al. 2024), while the most important event, cell proliferation, is not. Consequently, using PPARα-based adverse outcome pathways (AOPs) for assessing PFAS effects in humans is inappropriate because the key event that leads to any permanent adverse effect is missing. For cell proliferation to be biologically important, especially in the liver, it needs to occur to a degree that is clinically significant and chronic since this effect is reversible (much like phenobarbital). No epidemiology studies have identified an increase in liver disease, even in highly exposed populations (Darrow et al. 2016).

As discussed in Meek (2013), in the context of mixture assessment, it is reasonable to group chemicals together if there is a biologically plausible sequence of key events for both or all chemicals that leads to an observed effect supported by observations and mechanistic data. Pohl et al. (2024) reached the same conclusion in their recent book chapter. Goodrum et al. (2021) argued that in humans, the MOA for PFAS is complex and likely involves over two dozen nuclear receptors, which makes it improbable that focusing solely on PPARα would suffice for human health protection. As stated by the authors, this means that it is unlikely that there is a singular nuclear receptor or molecular initiating event that explains the toxicological responses of PFAS with differing chain lengths (Goodrum et al. 2021). This should have given the EPA pause in assigning dose additivity for these chemicals, as it most directly applies when the individual chemicals act of similar biological systems and elicit a common response. Thus, the relevance of PPARα-independent effects observed in animals to human PFAS exposure remains uncertain, highlighting the limitations in understanding PFAS-induced toxicity for human health assessments. This is an active area of research; however, it must be noted from the epidemiology data that no causal relationships between PFAS exposure and increased incidence of disease have been identified (Steenland et al. 2020; Steenland and Winquist 2021).

Based on the totality of the available mixture toxicity data and the lack of a clearly defined MOA for PFAS effects in humans, especially across various organs, the EPA's assumptions regarding the dose-additive model for PFAS are not well supported.

Misconception #8: PFAS are forever chemicals in the environment, so it is logical that they have extremely long biological half-lives in humans

The common perception of PFAS as being "forever chemicals" stems from their strong carbon-fluorine bonds, which contribute to their persistence in the environment and resistance to degradation (National Institutes of Health (NIH) 2019; Green Science Policy Institute 2024). The label suggests that these chemicals do not break down naturally and can remain in the environment and living organisms indefinitely (EPA 2023d). However, "forever" is an inaccurate term when discussing the biological half-lives of specific PFAS compounds like PFOA and PFOS in the environment and especially in regard to human exposures.

Serum PFAS concentrations can originate from either direct exposure to these compounds or from the metabolism of precursor compounds to PFAS within the body (reviewed in

Kudo 2015). Understanding the toxicokinetics (i.e., absorption, distribution, metabolism and excretion) of PFAS is especially important. Unlike dioxins, which have a high affinity for adipose tissue, PFAS are water soluble and are reported to mainly accumulate in the blood, liver and kidney and preferentially bind to proteins (i.e., serum albumin (HSA) and liver fatty acid-binding proteins (LFABP)) (Bischel et al. 2011; Lau 2012; Kato et al. 2015; Lau 2015; Fan et al. 2020; Lu et al. 2024).

Metabolism of PFAAs, GenX chemicals and 4,8-dioxa-3H-perfluorononanoate does not occur, except for some PFAS that are PFAA precursors that can be metabolized into PFAS (Kato et al. 2015; Interstate Technology and Regulatory Council (ITRC) 2023a). PFAS are eliminated in urine and in feces, with breast milk, transfer to the fetus, bile, and menstrual blood also found to be substantial routes of excretion (De Silva et al. 2021; Dourson et al. 2024). Additionally, individual determinants (e.g., sex, age, genetics, overall health) can also contribute to interindividual variation in PFAS half-lives (Bois et al. 2010; Chiu et al. 2022). It is believed that PFOA can be mistaken as an essential fatty acid in humans and, as a result, this compound is resistant to endogenous fatty acid metabolism which can give it a longer apparent half-life (Clewell 2024).

Urinary excretion is considered to be the predominant elimination route for most PFAS in both animals and humans, with fecal elimination also playing a role (Lu et al. 2024; Rosato et al. 2024). For long chain PFAS, the longer half lives are believed to be due to a saturable transport process in the proximal tubule of the kidney and the presence of active renal reabsorption. However, it should be noted that there is currently a lack of studies in humans, especially of certain subgroups (e.g., children and adolescents) that focus on the toxicokinetics of PFAS (Lu et al. 2024; Rosato et al. 2024). An additional challenge is that there is a data deficiency regarding the half-lives of short-chain PFAS and how exposures to PFAS mixtures could influence their half-lives (Rosato et al. 2024).

The differences in elimination pathways between PFAS types affect their biological half-lives (Li et al. 2018). Half-life estimates reflect the time it takes for the concentration of these substances in the blood to be reduced by half, assuming normal excretory function. They most likely exhibit alpha and beta elimination half-lives but, for sake of simplicity, these are generally blended into a single value.

As reviewed in ATSDR (2021), PFAS half-lives are species, sex, and compound specific. The half-life of PFOA in non-human primates ranged from 20.1 to 32.6 days, whereas in female and male rats it ranged from 1.9 to 322 h (ATSDR, 2021 pg. 5). In addition, for the half-life of PFOS ranged from 110 to 170 days in non-human primates, and between 179 to 1968 h in rats and mice (ATSDR, 2021 pg. 5).

In humans, PFAS half-lives are generally longer for the long-chain PFAS (estimates of several years), compared to shorter chain PFAS (e.g., PFBA, PFHxA, and PFBS), where the half-life is estimated to be between several days to months. It has been shown that the estimated mean half-life for PFOA in humans ranged from 1.5 to 5.0 years (Li et al. 2018; Xu et al. 2020; Dourson and Gadagbui 2021; Rosato et al. 2024). For PFOS, the mean half-life was estimated to range between 3.40 and 5.70 years in humans (Olsen et al. 2007; Li et al. 2018; Nilsson et al. 2022; Rosato et al. 2024). The mean half-life of PFHxS in humans ranged between 2.84 and 6.00 years (Li et al. 2022; Olsen et al. 2007; Xu et al. 2020; Nilsson et al. 2022). Yu et al. (2021) estimated that the human half-life of PFNA was 3.52 years when using a mixed model of 68 high-exposed participants (individuals $>95^{th}$ percentile of the 2015–2016 NHANES cycle) and controlling for physiological covariates. Olsen et al. (2009) reported a geometric mean serum elimination half-life of 25.8 days for PFBS in a study of six individuals. ECHA determined the half-life of Gen-X in humans was 81h when analyzing blood from 25 industrial workers (GenX Exposure Study 2021).

In general, it is believed that the shorter the carbon chain, the shorter the half-life (Han et al. 2012; Nicole 2020). The four other PFAS addressed in the final rule (e.g., PFHxS, PFNA, PFBS, and HFPO-DA) have biologic half-lives that are also far from "forever". For example, "next generation" PFAS, such as Gen-X (HFPO-DA), have faster elimination rates from the human body compared to PFOA and PFOS (Shea 2018).

Examining PFOA and PFOS, two out of the thousands of PFAS to consider, illustrates the complexity of discussing them as a single class. For example, PFOS is primarily excreted through bile, whereas PFOA is more commonly eliminated via urine (Fletcher et al. 2022), which can explain differences in their biological half-lives. If factors such as excretion are not fully accounted for, this can result in overestimating the half-lives of PFOA and PFOS, leading to misconceptions about their persistence and the associated health risks of PFAS exposure (Dourson and Gadagbui 2021).

In summary, the widely held belief that PFAS are "forever chemicals" within humans is not supported by the evidence in the peer-reviewed literature. In humans, they do have partially long biological half-lives, compared to some substances, as they do not permanently reside in human tissues and are gradually eliminated. Indeed, their biological half-lives in humans are shorter than some of the classic long-lived chemicals like the PCBs, the dioxins and furans, DDT, and other highly lipid-soluble chemicals.

Misconception #9: The PFAS MCLs promulgated in April 2024 will significantly reduce blood concentrations in American citizens in the coming years

In the general population, the primary exposure to PFAS and their precursors are likely from diet, particularly seafood, as well as food packaging, consumer products, and household dust (ATSDR 2021; ITRC 2023a; Sunderland et al. 2019). Occupationally, persons have been exposed to aqueous film-forming foam used in fire suppressant systems and while working in production facilities or industries that make PFAS (EPA 2024l). Individuals living near contaminated sites may have higher exposure through drinking water (ITRC 2023a). For these reasons, along with its persistence in the environment, virtually all Americans have measurable concentrations of PFAS in their blood serum.

In the authors' opinion, the most important misconception in the promulgated PFAS MCL is that after it is implemented, blood concentrations of these PFAS in Americans will significantly decrease, thereby having clinically relevant improvements in the health of Americans. Unfortunately, as discussed in this article, when one critically evaluates the peer-reviewed literature on the endpoints that the EPA selected for this regulation, there is no causal evidence that the current blood concentrations of the American population pose a health hazard (Emmett et al. 2006). In addition to no appreciable decrease in blood concentrations at 4 ppt in water, it is not clear what additional health benefits will be realized from this PFAS MCL.

Since 1999-2000, NHANES has been monitoring serum PFAS levels in the U.S. general population in two-year cycles (Agency for Toxic Substances and Disease Registry (ATSDR) 2024a). The most recent data is from 2017–2018 and includes eight perfluoroalkyl acids (PFAAs) (PFOA, PFOS, PFNA, PFHxS, PFHxA, PFDA, PFUnDA, PFHpS) and four other PFAS (GenX, ADONA, 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid, MeFOSAA), while five PFAS (PFBS, PFHpA, PFDoDA, PFOSA, EtFOSAA) were no longer monitored as they were infrequently detected in earlier rounds of NHANES cycles (Interstate Technology and Regulatory Council (ITRC) 2023a).

The available data show that serum PFAS concentrations in the U.S. population have declined over time, with the most significant changes being for PFOS concentrations.

Blood concentrations of PFOA and PFOS in Americans have considerably decreased in the past two decades, which is consistent with their biological half-lives of approximately 1.5 years and 4 to 6 years, respectively (Zhang et al. 2013; Dourson and Gadagbui 2021). To be specific, blood PFOA and PFOS levels have declined by more than 70 and 85 percent, respectively, since 2002 (Agency for Toxic Substances and Disease Registry (ATSDR) 2024a).

There are several reasons why blood concentrations for these six regulated PFAS will not decrease measurably for the U.S. general population, with the exception of persons living in highly contaminated areas, even if drinking water utilities achieve the specified MCLs. Near contaminated sites, drinking water PFAS concentrations have been reported in the 1,000 μg/L (1000 ppt) range (Emmett et al. 2006; Landsteiner et al. 2014; Worley et al. 2017; Hu et al. 2019), which at these sites, the relative source contribution (RSC) could account for upwards of 75% of total PFAS exposure (Emmett et al. 2006; Vestergren and Cousins 2009; Hu et al. 2019). In areas that do not have a point source, the RSC for PFAS in drinking water is significantly lower (Vestergren and Cousins 2009; Hu et al. 2019).

An initial assessment of PFAS in the U.S. was conducted between 2013-2015, as part of the EPA's Unregulated Contaminant Monitoring Program (UCMR3), which found that approximately 4% of water systems had detectable PFAS (Hu et al. 2016). It should be noted that for the UCMR3 dataset, the method reporting limit (MRL) for the six PFAS (i.e., PFBS, PFHxS, PFHpA, PFOA, PFOS, and PFNA) used in the Hu et al. (2016) ranged from 10 ng/L to 90 ng/L, which is a limitation of this analysis. Andrews and Naidenko (2020) analyzed publicly available datasets of PFAS occurrence in drinking water in the U.S. The authors estimated that between 18-80 million people in the U.S. received tap water containing >10 ng/L (combined PFOA and PFOS) and that over 200 million receive tap water with concentrations for these PFAS >1ng/L. Data are lacking for approximately 100 million Americans who obtain water from small public water supplies that serve less than 10,000 individuals and from private wells (Sunderland et al. 2019).

A recent analysis by Smalling et al. (2023) of 716 locations (269 private wells, 447 public water supplies) in the U.S. between 2016-2021, found that median cumulative PFAS concentrations were similar among private wells and tap water. The authors found that the MCL value (4 ng/L) for PFOA and PFOS was exceeded in 6.7% and 4.2% of samples, respectively, of all tap water samples collected, but were exceeded in 48% and 70%, respectively, of tap water samples when there the PFAS were detected (Smalling et al. 2023). In private well tap water, PFOA and PFOS detections exceeded the MCL in 63% and 67% of samples collected. Lastly, the authors reported that for the hazard index for the mixture of PFBS, PFNA, PFHxS and HFPO-DA, there was only a 4.6% exceedance for tap water (Smalling et al. 2023).

An additional challenge is that the human PFAS biomonitoring studies often provide data for a single point in time; for mixtures, there is a simplifying assumption that exposures to the co-occurring chemicals have not changed over time (Goodrum et al. 2021). This is especially relevant to PFAS with the phase out of long-chain compounds and the increasing use of short-chain PFAAs, which can change the kinetic parameters for human data (Goodrum et al. 2021). Based on the trends in PFAS blood serum concentrations over time, it is clear that the PFAS mixtures that the general population is exposed to are changing. Also, analytical limitations for PFAS often lead to a discrepancy between the concentrations of PFAS detected through targeted analytical methods and the total PFAS present in an environmental sample (Baqar et al. 2024). Feasibility of targeted analysis for individual PFAS has been demonstrated for approximately 80 out of the thousands of existing PFAS (Baqar et al. 2024). Other conventional methods such as Extractable Organic Fluorine (EOF) and Total Oxidizable Precursor (TOP) may provide a more comprehensive picture of total PFAS, but do not identify individual PFAS compounds (Baqar et al. 2024). In reviewing mass balance studies of total fluorine content in environmental samples, Yanna et al. (2019) concluded that between 50% to ≥99% of total fluorine content is labeled unidentified organic fluorine in environmental samples.

The intake of these PFAS by Americans via drinking water represents only approximately 10%-20% of the total PFAS that people are exposed to on a daily basis for those not living near PFAS contaminated sites (Haug et al. 2011; Sunderland et al. 2019; Kougias et al. 2024). As previously mentioned, the majority of PFAS exposure in the general population is from ingestion of contaminated food, especially seafood, migration from food packaging, and in certain rare circumstances inhalation of dust from indoor air (ATSDR 2021; DeLuca et al. 2022; ITRC 2023a; Kougias et al. 2024; Sunderland et al. 2019). EPA was aware of this via numerous

comments and their own funded studies (Hua et al. 2025) (in press).

In the final rule, the EPA defaulted to a RSC of 20% for PFAS in drinking water for the general population (EPA 2024i). This means that the EPA assumed that 80% of the daily dose was from sources other than drinking water. The Agency indicated that a default was used because there were insufficient data to estimate exposure attributable to drinking water accurately. Although this default assumption may be justified in circumstances where exposure sources are not well characterized (DeWitt 2015); the RSCs for various populations exposed to PFAS have been well characterized in the peer-reviewed literature. Various studies have estimated that in the general population, the RSC for drinking water is between <1%–22% of total exposure for PFOS (Egeghy and Lorber 2011; Gebbink et al. 2015; Shan et al. 2016) and between approximately 1%–37% for PFOA (Vestergren and Cousins 2009; Tian et al. 2016). Hu et al. (2019) estimated that the median RSC for PFNA and PFHxS was 13% and 34%, respectively. It is important to set a RSC that uses the best available data in order to be protective of human health. For example, if the RSC is overestimated, the total exposures of the general population may exceed the reference dose, even if PFAS concentrations are below the regulatory limit (Hu et al. 2019). Based on the available data, although it is limited, there is evidence that using a default RSC value of 20% will likely greatly underestimate exposure to certain PFAS and overestimate it for others.

Other potential RSCs of PFAS include dietary ingestion and household dust inhalation, meaning that even if the MCLs were set to zero, blood concentrations of PFAS could not reach the desired blood concentrations (Vestergren et al. 2012; Tian et al. 2016; Sunderland et al. 2019). Vestergren et al. (2012) estimated dietary intake of PFAS in the general population of Sweden using archived food market basket samples from 1999, 2005, and 2010. The authors reported that dietary exposure to PFOS (860-1440 pg/kg-day), PFUnDA (90–210 pg/kg-day), PFDA (50–110 pg/kg-day) and PFNA (70–80 pg/kg-day) was dominated by the consumption of fish and meat. In addition, the authors reported that PFOA (350-690 pg/kg-day) dietary exposure to frequently consumed foods, such as cereals, dairy products, vegetables, and fruit. Vestergren et al. (2012) reported dust ingestion accounted for a significant contribution (27–49%) of the total exposures to PFHxA, PFHpA, PFNA, perfluorotridecanoic acid (PFTrDA) and perfluorotetradecanoic acid (PFTeDA). This was a highly unusual scenario.

Overall, the authors reported that dietary intake (dust and food ingestion) of PFOS and PFOA was estimated to comprise 85 and 83% of the total average intake for the average Swedish population, respectively. Seafood has been identified as a "major contributor" of PFAS exposure, as fish and other seafood can contribute up to 86% of chronic PFOS exposure in adults (EFSA, 2018) Further, up to 80% of the seafood consumed in the US is imported from Canada, South America, and Asia (Economic Research Service 2024).

Tian et al. (2016) evaluated the estimated daily intakes of total PFAS via house dust ingestion for toddlers and adults in Korea. In these populations, indirect exposure to PFOA and PFOS via house dust ingestion to precursors accounted for 5 and 12% of exposure, respectively. The authors concluded that hose-dust ingestion was a minor contributor in this population as it accounted for 5% of the estimated daily intake for PFOS in toddlers and less than 1% of the overall estimated daily intake of PFOS in adults, as well as PFOA exposure in toddlers and adults. It should be noted, however, that this study had a small sample size. Only 15 indoor dust samples were collected from homes in South Korea.

As noted, there are other sources of information that could have informed EPA in their exposure assessment. For example, the 2011-2014 NHANES data indicated that approximately 20% of U.S. adults did not drink any plain water (e.g., tap water or bottled water) on a given day (Rosinger et al. 2018). This is further contextualized when considering that it is reasonable to estimate that less than 5% of the hundreds of gallons of tap water that are utilized by the average American household daily is actually ingested (Water Research Foundation 2016). The majority of processed water is used in washing machines, dishwashers, toilets, showers, watering lawns, washing cars, watering gardens, and a significant portion is lost to evaporation and leaking pipes. When one accounts for how little tap water is actually consumed as drinking water, one would not expect that EPA's final rule would significantly lower PFAS blood concentrations in the American population. This could be considered an example of inadequate risk communication by EPA to the press, the public and elected officials.

Not only is lowering the drinking water concentration of PFAS unlikely to have an appreciable impact on the blood concentrations of nearly all Americans, but it is unclear how much lower blood concentrations of these six chemicals can further decline in the coming years. For example, blood concentrations of PFOA, PFOS, and other legacy PFAS (e.g., PFHxS and PFNA) in human blood have been decreasing since 1999-2000 (ATSDR 2024c) due to the voluntary phase out of these compounds from manufacturing and production. Due to the phase out of the legacy compounds, the introduction of newer short-chain alternatives such as PFBS and HFPO-DA in manufacturing and production may increase blood levels of these compounds over time. Therefore, ongoing and future biomonitoring studies are essential to understand and assess the trends in human exposure to emerging PFAS alternatives.

When the new NHANES data for PFAS in blood serum are released, it is expected that for the general population, the concentrations will be much lower than when they were last reported by the CDC in 2018. The blood levels of PFNA and PFHxS in the U.S. population are equal to or less than 1.1 μg/L (ATSDR 2024c) and data from the 2014 NHANES survey showed that the average American's blood serum concentration of PFBS was at or below the limit of detection of 0.1 ng/L (Olsen et al. 2017; EPA 2022a). That was nine years before the MCL was promulgated, and given the short half lives of PFNA and PFBS (Olsen et al. 2009; Zhang et al. 2013), one would appreciably lower serum concentrations today.

HFPO-DA was detected in the serum of a small portion of individuals (1.2–1.5% of subjects in one NHANES study) with a concentration ranging from 0.07 to 0.4 μg/L (Calafat et al. 2019). The already declining (or nondetectable) blood serum concentrations of the six PFAS (PFOA, PFOS, PFNA, PFHxS, PFBS, HFPO-DA) in the absence of MCLs calls into question

the necessity of recent aggressive regulation that may not decrease PFAS blood serum concentrations for the average American. Since the blood serum levels may not decrease further, after the rule has been implemented by the various water providers, it is unclear how the EPA intended to support its claims about the reduction in diseases that they expected to occur following the promulgation of this ruling.

To test the assumption that lowering the previous health advisory from 70 ppt to the promulgated 4 ppt for PFOA and PFOS would cause a meaningful decrease in blood serum concentrations, we used the ATSDR PFAS Blood Level Estimation Tool (ATSDR 2024b). Assuming that a 38-year-old male (median age of men in the U.S.) who weighed 200 pounds (average weight for men) was exposed to a combined 70 ppt (35 ppt PFOA + 35 ppt PFOS) of PFAS and using the NHANES dataset, consumed 56% of their total plain water intake from tap water, their estimated blood serum concentration for PFOA would be 2.77 μg/L and 6.61 μg/L for PFOS.

Using the same assumptions but using a 4 ppt exposure to both PFOA and PFOS, their estimated blood serum concentration would be 1.6 μg/L for PFOA and 5.0 μg/L for PFOS. Assuming the same exposure scenario for a woman who was 40 years old (average age of women in the U.S.) and weighed 170 pounds (average weight of a U.S. woman), who had not breastfed in the past five years, their estimated PFOA blood concentrations would be about 2.6 μg/L at 70 ppt and 1.4 μg/L at 4 ppt. For PFOS, their estimated blood concentrations would be 5.0 μg/L at 70 ppt and 3.4 μg/L at 4 ppt.

While mathematically, there is an approximate 25% reduction for PFOS in men and a 33% reduction in women, and for PFOA, a 42% reduction in men and a 46% reduction for women, the estimated blood concentrations at 4 ppt are almost certainly not going to be statistically different than before the rule except for only highly contaminated water districts. If one uses 1/2 the LOD for censored data, the predicted concentrations will be massively higher than measured. Based upon first principles, reducing drinking water concentrations to 4 ppt is not likely to lead to measurable changes in blood serum concentrations because intake from drinking water is low for the average person, and diet is the predominant source of PFAS exposure, except in highly contaminated areas. In short, an expensive and strict water guideline will not change blood levels in a measurable way for most of America.

Given that this promulgated rule will cost the country hundreds of billions of dollars, thereby removing funds from being invested in other pressing challenges facing the nation, one might question whether such an investment would have been better spent on education, healthcare access, and lifting people out of poverty. The obvious "winners" associated with this rule are consultants, engineering firms, government agencies, and the legal community. This is precisely what happened during the first 20 years following the passage of the Superfund rule where, ultimately, it was found that a significant amount money allocated to remediating Superfund sites was consumed by lawyers rather than going toward improving public health (Hurley 2011).

Misconception #10: The 2024 PFAS drinking water rule "will prevent thousands of deaths and reduce tens of thousands of serious PFAS-attributable illnesses"

The EPA has claimed that:

> "… over many years the final rule will prevent PFAS exposure in drinking water for approximately 100 million people, prevent thousands of deaths, and reduce tens of thousands of serious PFAS-attributable illnesses" (EPA 2024i, p. 32532).

For those who have studied these chemicals and the exposures attributable to drinking water, it is understandable that they would question whether this claim is scientifically accurate. Indeed, the data indicate, as has been shown in the prior misconceptions, that there is minimal scientific or medical bases for making this claim.

In its basis for the MCL final rule for PFOS and PFOA, the EPA asserted that:

> "… the quantifiable annual benefits of the final rule will be $1,549.40 million per year and the quantifiable costs of the rule will be $1,548.64 million per year. The EPA's quantified benefits are based on the agency's estimates that there will be 29,858 fewer illnesses and 9,614 fewer deaths in the communities in the decades [through the year 2105] following actions to reduce PFAS levels in drinking water" (EPA 2024i, p. 32533).

Upon careful review of the basis for these claims, it is challenging to demonstrate that nearly any population in the United States, who is currently exposed to PFAS in drinking water, is at risk of an increased incidence of cancer or at risk of an increase in non-cancer effects.

Currently, the weight of evidence of the epidemiological data does not indicate a clinically relevant increase in cancers or other diseases, even in highly exposed communities, much less the general population. When one evaluates the available animal studies and considers the typical PFAS blood concentrations in Americans, it seems implausible that there should be any changes in health status due to the further lowering of the concentration of PFAS in blood serum, as the doses commonly used in animal studies are orders of magnitude higher than exposures from drinking water in the general population (Agency for Toxic Substances and Disease Registry (ATSDR) 2021). Furthermore, no MOA has been identified that can explain how these chemicals cause either cancer or non-cancer effects in humans (Corton et al. 2018; Felter et al. 2018; Chappell et al. 2020; Heintz et al. 2023; Clewell 2024; Li et al. 2024).

The EPA's quantifiable annual benefits are based on the number of theoretical cases of cancer, illnesses and premature deaths expected to be avoided due to the promulgation of the PFAS MCLs. While the EPA identified several health outcomes as discussed in Misconception 1 through 5, the EPA quantified or monetized only a subset of these potential health effects. The EPA reported that it was only able to provide a quantitative analysis when "… there is evidence of an association between PFAS exposure and health effects, if it is possible to link the outcome to risk of a health effect, and if there is no overlap in effect with another quantified endpoint in the same outcome group" (EPA 2024i, p. 32672). Therefore, any potential health effects, including the immune,

liver, endocrine, metabolic, reproductive, musculoskeletal, or other cancers noted by the EPA were not quantified or monetized in the economic analysis.

Rather, the estimated morbidities and mortalities avoided are from the EPA's expected reductions in incidences of cardiovascular disease, low birth weight, renal cell carcinoma, liver cancer, and bladder cancer (mostly as a result of removal of disinfection by-products through PFAS-related treatment technologies (EPA 2024i)). For example, for renal cell carcinoma (RCC), cancer benefits estimated by the EPA include 2,028 RCC-related deaths avoided, 6,964 RCC non-fatal cases avoided, and over $350 million in annualized benefits accrued as a result of reduced PFOA exposure in drinking water (EPA 2024i, p. 32691). However, these estimates were fundamentally flawed because of inadequate epidemiological data for this disease.

Also, the weight of evidence does not support the idea that RCC is currently caused by PFAS exposure. As this paper and some stakeholders, including 3M, ACC, and AWWA discussed, the EPA failed to consider all datasets relevant to understand the current cancer risk for RCC (Hua et al. 2025) (in press). When evaluating carcinogenicity, the EPA did not consider several occupational exposure studies (Steenland and Woskie 2012; Raleigh et al. 2014) or community exposure studies (Barry et al. 2013; Vieira et al. 2013; Mastrantonio et al. 2018; Rhee et al. 2023) that were relevant to understanding the risk of this disease.

Instead, the EPA relied on Shearer et al. (2021), a nested case-control study on kidney cancer (324 cases; 324 matched controls) from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial to evaluate the risk of RCC in relation to pre-diagnostic serum concentrations of PFOA and seven other PFAS. The authors observed a positive association between RCC risk for PFOA, PFOS and PFHxS, but after adjustment for all three chemicals, only the association with PFOA remained. It should be noted that this study had several limitations, including the potential for reverse causality from pharmacokinetic confounding, where RCC induction results in impaired kidney function (Clewell 2024). While Shearer et al. (2021) adjusted their results for estimated glomerular filtration rate (eGFR), this was based upon one data point per individual, which is inadequate for evaluating glomerular filtration rate (Clewell 2024). Also, this is only one of many renal transporters that are involved with the clearance of PFOA (Burgoon et al. 2023; Clewell 2024). Thus, correcting for eGFR alone would not be sufficient to correct for confounding by impaired kidney function. This study also relied upon measured PFAS exposure that was collected nearly a decade prior to cancer diagnosis and the contrasts between the upper quartile of PFOA (>7.3 μg/L) and the lower quartile (<4.0 μg/L) were modest (3M Company 2023). These exposures are approximately 10-100-fold less than the PFOA exposures in Raleigh et al. (2014), which did not find an increase in kidney cancers in this population.

By using a serum PFOA concentration from a single time point in Shearer et al. (2021), based upon the long half-life of PFOA, to provide an accurate measurement of a person's long-term exposure to PFOA is also not consistent with fundamental considerations of the connection between toxicodynamics, toxicokinetics and time (Rozman et al. 1996; 3M Company 2023). For example, if the average serum PFOA measurement was collected, on average, 8.8 years prior, it would be approximately three half-lives away from the diagnosis of kidney cancer. This limits the reported serum concentrations and validity of the results from Shearer et al. (2021). Furthermore, the nested case-control study design cannot be used to establish causation, only associations between exposures and outcomes.

It is surprising that EPA did not give much weight to the information presented in the studies of occupational cohorts that had significant PFAS exposure in its final ruling (Steenland and Woskie 2012; Barry et al. 2013; Raleigh et al. 2014). Steenland and Woskie (2012) is a cohort mortality study of 5,791 workers at a DuPont chemical plant in West Virginia. The workers had an estimated average annual serum concentration of 350 ng/mL (median 230 ng/mL, with a total of 12 kidney cancer deaths reported. This study observed a significantly elevated risk of kidney cancer death only in the highest exposure quartile, with exposures greater than 2,384 ng/mL-years (Steenland and Woskie 2012). This finding is questionable because kidney cancer death in the highest quartile was likely confounded by occupational exposure to tetrafluoroethylene (TFE), a known rodent renal carcinogen (Steenland and Woskie 2012). The EPA identified this as a medium-confidence study but stated that it did not consider it for dose-response analysis because of the small number of observed cancer cases and because "… the exposure levels reported in the study population (average annual serum concentration of 350 ng/mL) are less comparable to the U.S. general population than the levels reported by Shearer et al. (2021) and Vieira et al. (2013)" (EPA 2024k, p. 44–68). To some this lacks a logical thought process.

However, as pointed out by 3M Company (2023), the Steenland and Woskie (2012) article had known shortcomings, including

> "EPA did not acknowledge that the observed kidney cancer cases could have been confounded by occupational exposure to tetrafluoroethylene (TFE), a known rodent renal carcinogen. EPA also failed to address that Steenland communicated in a recent publication (Bartell and Vieira 2021) that there was a major error in the cumulative ppm-years quartile analyses where the quartile PFOA exposure categories should have been defined as cumulative ng/mL-years (ppb-years) and not ppm-years. Therefore, the exposures in this study were actually lower (i.e., more relevant to the general population) and the reported cancers may have been due to TFE exposures and not PFOA" (3M Company 2023, p. 31)

Barry et al. (2013) is a community/worker cohort study of 32,254 residents (28,541 community members; 3,713 DuPont workers) who either lived in the Mid-Ohio Valley or worked at a local DuPont chemical plant. The authors reported that the median PFOA serum level in 2005-2006 was 24.2 ng/mL (range: 0.25 – 4,752 ng/mL) in the community and was 112.7 ng/mL (range: 0.25-22,412 ng/mL) in the workers. There were a total of 113 validated kidney cancers (community: 93; workers: 19). This study also did not find a significant association of kidney cancer cases among workers who had serum concentrations that were 10-fold greater than the community population in Shearer et al. (2021); although, a general

monotoic increase across quartiles was observed when the population was not stratified by occupational status. For the occupational results, the authors stated that it was due to the low sample size for the cancers of interest (Steenland and Woskie 2012). The EPA excluded this study from their analysis because they said that it was not suitable for dose-response analysis and that it lacked the necessary data to perform a cancer slope factor calculation (3M Company 2023, p. 32); however, Bartell and Vieira (2021) reported the necessary exposure data from the Barry et al. (2013) study, which should have necessitated its inclusion by EPA (3M Company 2023, p. 31–32).

The EPA also excluded Raleigh et al. (2014). EPA was concerned about the exposure assessment methods and study quality, as well as, the small number of kidney cancer cases reported (EPA 2024k). Raleigh et al. (2014) examined the mortality and cancer incidence in a cohort of 4,668 workers at a 3M APFO production facility and 4,359 employees from a 3M non-APFO production facility (as a reference population). Compared to the Minnesota Cancer Surveillance System (MCSS) and the Wisconsin Cancer Reporting System (WCRS), the APFO-exposed cohort were at or below the expected mortality rates for cancer, including kidney cancer (24 cases reported), and non-cancer effects. Unlike the results in Steenland and Woskie (2012), which were possible confounded by TFE exposure, this study cohort had a near absence of this exposure (Raleigh et al. 2014).

The EPA also stated that it excluded this study because it used model estimates of PFOA air concentrations in the workplace rather than biomonitoring measurements (EPA 2024k). In addition, EPA did not appropriately consider the totality of other studies that found that these workers had high PFOA exposures consistent with the higher PFOA serum concentrations (Olsen et al. 2000; 2003). Therefore, the EPA mischaracterized the quality of these data from Raleigh et al. (2014), resulting in an unwarranted exclusion of this study from their analysis.

The EPA's inability to collectively synthesize evidence from the occupational exposure studies resulted in a misinterpretation of the weight of evidence on adverse health effects. Though individually, as noted by 3M Company (2023), the three occupational studies may not have been suitable to calculate a CSF, the EPA failed to consider that, collectively, the PFOA exposures in these three worker studies were one to two orders of magnitude greater than the general population serum PFOA concentrations reported in Shearer et al. (2021), yet showed little to no association with kidney cancer. In Shearer et al. (2021), 324 kidney cancer cases originated from a cohort of 150,000 adults aged 55–74, with kidney cancer cases representing 0.22% of the cohort. In the three occupational cohort studies (Steenland and Woskie 2012; Barry et al. 2013; Raleigh et al. 2014), which had cohorts of 5,791, 3,713, and 4,668 (total = 14,172) workers, respectively; there were a total of 52 kidney cancer deaths and cases, representing 0.37% of the combined three cohorts (3M Company 2023, p. 32). Though the EPA labels each of these as small studies, they are collectively comparable to Shearer et al. (2021) in the percentage of kidney cancer cases.

Among these three occupational analyses, which likely represent the highest exposed individuals based on overall reported biomonitoring data, only one analysis, Steenland and Woskie (2012), showed a statistically significant association with kidney cancer. The EPA did not synthesize the evidence across these studies, as is recommended by the IRIS Handbook and EPA Guidelines for Carcinogenic Risk Assessment, to inform its approach to setting the CSF, and, as a result, did not appropriately assess the overall weight of evidence for RCC.

As discussed previously, given that the evidence for a relationship between PFOA and cancer remains sparse despite several epidemiology studies and reviews, the EPA's decision to quantify the number of RCC cases and deaths avoided (6,964 and 2,028, respectively) with the MCL and MCLG appears to be a clear case of "data overreach" and it yielded misleading results that were a foundation for justifying their PFAS MCL (EPA, 2024i, p. 32691). Renal cell carcinoma is just one health outcome identified by the EPA when it quantified the deaths that they expected to prevent and the magnitude of healthcare costs to be saved. The EPA's quantification of the benefits from preventing developmental effects, liver cancer, and cardiovascular disease goes well beyond what can be addressed in this paper, but the assumptions and the calculations of lives saved were difficult to follow and seemed to suffer from the same shortcomings observed in the kidney cancer "cost-benefit" analyses.

Misconception #11: Water purveyors will have adequate federal grants needed to meet the newly promulgated MCL

In the EPA's final rule on the PFAS MCLs, the Agency stated that it disagrees with commenters on the proposed MCLs regarding that funding will be insufficient to implement the mandated compliance actions. The final PFAS drinking water regulations state that:

> "The EPA estimates that the initial capital costs of the rule in undiscounted dollars is approximately $14.4 billion (see Appendix P of the EA for more information). Given the BIL [Bipartisan Infrastructure Law] appropriations of $11.7 billion in DWSRF [Drinking Water State Revolving Fund] and an additional $5 billion for emerging contaminants, the EPA reasonably anticipates BIL funding is likely to be able support a substantial portion of the initial capital costs of the final rule. BIL funding appropriations began in the Federal Fiscal Year (FFY) 2022 and appropriations are anticipated to continue through FFY 2026." (EPA 2024i, p. 32639)

It is difficult to understand how the EPA could have concluded, in 2022, that future federal funding was going to be adequate to handle the costs of complying with this rule as only about 25% of the data on the national prevalence of PFAS in water systems was known in April 2024 (EPA 2024g). It is unclear how OMB could have fulfilled its duties to conduct a proper cost-benefit or risk-benefit analysis when the majority of the data were not yet available prior to the EPA launching and promulgating the PFAS MCLs.

In brief, the EPA relied upon data from the Unregulated Contaminant Monitoring Rule 3 (UCMR3) which covered the period between 2013 and 2015 and tested five of the

regulated PFAS (i.e., PFOA, PFOS, PFNA, PFHxS, PFBS) (EPA 2017b). So, at the time the rule was evaluated by OMB and EPA in March 2024, the data were already nine years out of date. A major shortcoming of these data was that minimum reporting levels ranged from 20 to 90 ppt. The reporting limits were two- to ten-fold greater than the concentrations that were promulgated in the regulation (EPA 2024h). This range of concentrations, due to its high minimum reporting levels, was uninformative for detecting PFAS, (except for PFBS, which has a hazard quotient of 2000 ppt) at the new regulatory limits of four to 10 ppt (EPA 2024h).

Although the EPA supplemented the UCMR3 data with state-reported data (EPA 2024i, p. 32649), the need for a more representative national dataset was imperative if EPA and OMB were to accurately estimate the number of water systems that would require upgrades to meet this new regulation.

The UCMR5, the latest ongoing iteration of the contaminant monitoring program, is intended to measure the concentration of 29 different PFAS nationally (EPA 2024h). This program is to be completed in late 2026, at which point the EPA should have the necessary occurrence data to make an informed decision regarding PFAS concentrations in drinking water. The EPA decided to regulate at least 18–36 months before they would have had adequate data to understand the economic impact of the rule on America. Knowing whether just one public water system that would report PFAS concentrations less than the MCL could mean the difference of millions of dollars spent on equipment and filtration upgrades that would not be needed.

Many water providers were quite concerned that EPA did not fully understand the true costs of attempting to meet the demands of the proposed PFAS MCLs (American Water Works Association (AWWA) 2023; Birmingham Water Works 2023; Cleveland Water 2023; Plymouth Village Water & Sewer District 2023). For example, some water systems conducted an individualized cost analysis, using their own experience with treatment costs and infrastructure upgrades, to demonstrate the extent by which the EPA had underestimated the economic burden of meeting the PFAS MCLs for just one system (American Water Works Association (AWWA) 2023; American Water Works Company Inc. 2023; Birmingham Water Works 2023; National Rural Water Association 2023; Water & Health Advisory Council 2023). For example, DC Water, a water and sewer service provider for the District of Columbia, noted that the EPA's capital cost estimate for their treatment facilities was likely to be less than one-third of the actual cost of treatment (District of Columbia Water and Sewer Authority 2023). The EPA's capital cost models estimated that it would cost DC Water's water purveyor treatment plants $103.4 million dollars to install GAC; however, the plant's own 2023 estimate of the upgrade cost was $200 million ($316.6 million in today's dollars).

A February 2024 cost analysis conducted by WSSC Water, the country's eighth-largest water and wastewater utility, estimated that the capital costs required for PFAS treatment at the Potomac WFP would fall between $1.4 billion to $2.9 billion depending on the type of treatment, not including annual operating costs (WSSC Water 2024). This estimate is almost twenty-fold higher than the EPA's estimated annual cost of $16 million to $67 million for a water treatment facility of their size (WSSC Water 2024). WSSC Water further estimated that ratepayers would need to bear an additional $108 million in annual operating costs in the absence of external funding (WSSC Water 2024). This is likely an underestimation, as it only considered upgraded treatment costs at one of the facility's two plants. This water provider's historic PFAS testing indicated that drinking water samples had typically fallen beneath the recently finalized PFAS MCLs, but were close enough that natural variation or analytical error could require them to spend billions of dollars in upgrades (WSSC Water 2024). Based on their limited data, the EPA claimed that:

> "the costs for public water systems and primacy agencies to implement this regulation are approximately $1.548 billion per year. EPA believed that this amount would cover the costs if water system monitoring (e.g., sampling and analytical testing), communicating with customers, and if necessary, obtaining new or additional sources of water or installing and maintaining treatment technologies to reduce levels of the six PFAS in drinking water" (EPA 2024f, p. 2).

The AWWA conducted an independent investigation with Black and Veatch to estimate the cost of the regulation to drinking water purveyors and found that public water systems (PWSs) would require $50 billion dollars in upgrades over the next 20 years ($2.5 billion annualized) AWWA (2023). These estimates did not consider that if PFOA and PFOS are designated as CERCLA Hazard substances, additional disposal costs could add up to $3.5 billion dollars a year (Association of Metropolitan Water Agencies (AMWA) et al. 2023). As of May 8, 2024, PFOA and PFOS were designated as CERCLA Hazardous substances (U.S. Environmental Protection Agency (EPA) 2024c, p. 39124).

The EPA estimated that 6%–10% of regulated water systems (4,100 to 6,700) would have to take action at a 4 ppt MCL (EPA 2024o) and 66,000 regulated water systems would have to complete monitoring and notification requirements (EPA 2024f). However, according to the estimate by Seidel et al. (2023), a 4 ppt MCL could impact 15%-20% of water systems (using EPA Method 533). Similarly, an estimate by the AWWA predicted 16% of regulated groundwater PWSs would be affected – (based on the information in the EPA's March 2023 proposed regulation package) (AWWA 2023). Given this information, the recent debates about the true economic impact of the promulgated PFAS MCLs are understandable.

In a recent press release, the EPA discussed the Biden Infrastructure Law (BIL), which will allocate $9 billion dollars to communities for investment in the infrastructure necessary to meet the finalized MCL (U.S. Environmental Protection Agency (EPA) 2024b). Based on our analyses of the alleged health benefits of these PFAS MCLs, this seems to be a large sum of money to invest in a rule that has yet to demonstrate any significant benefits to Americans' health. Perhaps even more importantly, this amount of money is not likely to cover even a small fraction of the costs of compliance for all

impacted water systems. This view is based on the data collected thus far in the UCMR5 initiative.

While additional funds are available through programs such as The Drinking Water State Revolving Fund and The Emerging Contaminants in Small or Disadvantaged Communities Grant Program, they are diluted by infrastructure and other contaminant treatment needs (EPA 2024f). Some have reported that the totality of the EPA's cited funding sources hardly approaches 60% of the 20-year cost of the regulation (AWWA 2023).

A former EPA assistant administrator overseeing the Office of Water, Radhika Fox, stated that the current funding is merely a "down payment" and, ultimately, states and federal government are going to have to "continue to identify additional resources" to meet the MCLs (Magill 2024). Assuming that no other monies are available, the result will be ratepayers paying the difference to PWSs in the form of water bills that could amount to over $3,570 annually per household, in addition to their current water bill, in smaller communities (e.g., 25 to 100 individuals), whereas in larger communities (e.g., 3,301 to 10,000 individuals) the cost was estimated to range between $305 and $327 annually (AWWA 2023).

Misconception #12: The economic impacts of the promulgated MCLs will be modest even after these chemicals are labeled as hazardous under CERCLA. Furthermore, this rule will not adversely impact America's economic competitiveness

In their final NPDWR, the EPA described that in the PFAS Strategic Roadmap (EPA 2021) the agency was proposing to designate certain PFAS as Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) hazardous substances and provide updated guidance on destroying and disposing of certain PFAS and PFAS-containing materials (EPA 2024d, p. 32538). Less than two weeks later, PFOA and PFOS were both designated as hazardous substances under CERCLA on May 8, 2024 (EPA 2024d).

From reviewing the final NPDWR, the main economic issues that were stated regarding CERCLA were that there could be non-quantified costs from their designation stemming from limited disposal options for drinking water contaminated residuals (e.g., spent media and/or concentrated waste streams) and/or other potentially increased costs (EPA 2024i, p. 32713) and that several commenters mentioned that the EPA "… failed to consider the costs and impacts of the proposed MCLs in non-drinking water contexts, such as its potential uses as CERCLA clean-up standards" (EPA 2024d, p. 32577).

Regarding disposal of contaminated residuals, the Agency stated that their economic analysis showed that the disposal costs would only increase public water systems treatment costs marginally (EPA 2024i). They also stated that:

> "A CERCLA designation as a hazardous substance does not restrict, change, or recommend any specific activity or type of waste (EPA 2022) … The EPA does not expect spent drinking water treatment residuals containing PFAS to be released into the environment at or above the reportable quantity [defined as one pound or more within a 24-hour period] as a part of standard residuals management practices used by water systems. This is because the PFAS loading onto sorptive media is very small" (EPA 2024i, p. 32625; p. 32627).

As described in the NPDWR, this is likely to be an accurate assessment of the costs of disposal of contaminated residuals.

Regarding CERCLA, the EPA responded that, as required by the SDWA, they only included costs that were likely to occur as the results of compliance with the MCL and that they could not consider costs that resulted from compliance with other regulations that were either proposed or promulgated (EPA 2024i). In the EPA's final rule for designating PFOA and PFOS as CERCLA hazardous substances, the Agency stated that they considered the direct costs of complying with the release notification requirements, indirect costs (e.g., potential costs for existing and future National Priorities List (NPL) sites and potential costs that may arise from enforcement actions taken at non-NPL sites) and qualitative costs arising from potential litigation and liability (EPA 2024d). Similar to the NPDWR, the EPA stated that the benefits of the CERCLA regulation would be from the quantified and unquantified health benefits, as well as from transferring response costs from the EPA to potentially responsible parties (PRPs) (EPA 2024d). However, as discussed previously in this article, the health benefits that the EPA has identified from these regulations was based on flawed interpretations of the underlying science, and as such, the expected benefits will likely not materialize – despite the billions of dollars spent on this regulation.

The U.S. Chamber engaged third-party experts who utilized economic modeling to derive a reasonable estimate of potential private cleanup costs (using the EPA's data) resulting from a CERCLA designation for PFOS and PFOA. This analysis revealed that private sector annualized cleanup costs at Superfund sites, following the hazardous substance designation of PFOA and PFOS, could range between $700 million and $800 million, translating into present value costs of $11.1 billion to $22 billion—well above the $100 million threshold that necessitates a regulatory impact assessment (RIA) (U.S. Chamber of Commerce 2022). A Minnesota Pollution Control study from 2023 estimated that the total cost to remove PFAS from waste streams could amount to over $14 billion over 20 years (at least $700 million annualized) in that state alone (Barr Engineering Co. and Hazen and Sawyer 2023), We have not identified any other state-specific estimates regarding groundwater remediation, but this will likely be a massive burden, and it is unclear who will bear these costs at this time.

In addition to groundwater and Superfund cleanup costs, Bloomberg recently reported that the 4 ppt MCL is expected to lead to tens of billions of dollars in alleged liabilities (Wolf 2023). In the realm of toxic torts, any EPA-set MCL, whether seen by the scientific community as a safe limit or not, could be perceived in court as a threshold for adverse health effects, potentially leading to personal injury litigation costs that will almost certainly exceed those associated with

asbestos or talc. Legal matters are projected to surpass one hundred billion dollars (Francis 2004).

The financial impact of the MCL on U.S. manufacturing competitiveness has not been determined. The U.S. Chamber of Commerce has noted that many essential products (e.g., aircrafts, automobiles, semiconductors, and medical equipment) rely on PFAS. They are assuming, as EPA has stated, that many more PFAS will be regulated in the not so distant future. For many of these industries, there are no suitable alternatives to PFAS compounds (DOD 2023; U.S. Chamber of Commerce 2023). This could significantly impact these industries' operations, business models, and supply chain dynamics. As such, it is reasonable to expect that the production of these needed chemicals will effectively move overseas.

Overall, while the EPA estimated superficial ratepayer and regulated community costs and quantified benefits-specific costs, the full economic effects of the MCL are vast and are likely to become one of the most financially significant regulations in the history of the environmental movement, which began in 1962. Individual analyses indicate that the total expenses over a couple of decades could quickly amount to hundreds of billions of dollars when one considers the costs at public water systems (estimated by AWWA to be $50+ billion over the next 20 years) and cleanup at other sites (with superfund costs predicted to be ~$70 billion over the next 20 years). Not to mention the broader economic implications of litigation costs and reduced manufacturing competitiveness in the U. S. compared to foreign markets. Ultimately, these costs will be inevitably borne by taxpayers.

Misconception #13: Current technologies can efficiently and cost-effectively remove PFOA and PFOS from drinking water

The water treatment technologies endorsed by the EPA, such as granular activated carbon (GAC), anion exchange, reverse osmosis, and nanofiltration, have been shown to reduce concentrations of PFAS in water systems (EPA 2024g). However, these methods encounter significant limitations when evaluated against the stringent MCLs for PFAS. One of the main challenges is variable effectiveness across the diverse family of PFAS compounds. Techniques like GAC and anion exchange may effectively remove certain PFAS compounds, such as PFOA and PFOS, but their efficiency diminishes against others, particularly shorter-chain PFAS (McCleaf et al. 2017). For example, a study by Sun et al. (2016) found increased removal of PFAS with increasing chain lengths, with removal values <40% for the majority of short-chain PFAS while long-chain PFAS removal was >80%. This discrepancy in treatment capability could result in noncompliance with MCLs, especially as regulations begin to encompass a broader range of PFAS types.

A variable which has yet to be understood is that competitive adsorption with other water constituents might significantly reduce the efficiency of these treatment technologies (AWWA 2020). For example, if there are higher than average concentrations of solvents, motor oil, and other organics (often due to rainfall, runoff, and spills), these chemicals will prevent the adsorption of the PFAS chemicals onto charcoal because the active sites (pores) will be occupied. This phenomenon is one reason that, in some water treatment facilities, they have been unable to use activated carbon to remove other organics found in groundwater or surface water following spill incidents or heavy rainfalls (which remove oils embedded in roads). Should GAC be used in major metropolitan cities to capture PFAS, the economic burden due to storm water runoff could be astronomical.

Financial and operational considerations also present significant barriers to a cost-effective treatment system. Implementing and maintaining technologies like reverse osmosis and anion exchange require substantial capital investment and incur higher operational costs than traditional treatment technologies due to energy demands and frequent replacement of filtering media (AWWA 2023). These economic constraints can hinder adoption, particularly in smaller or under-resourced communities, where funding for necessary upgrades to water treatment facilities may be limited. This financial burden can result in inconsistent application of these technologies, leading to disparities in water quality and difficulties in achieving MCL compliance (AMWA 2023).

Interestingly, the environmental impact of waste generated by these treatment methods has yet to be quantified in a thorough manner. For example, the EPA has designated PFAS as "hazardous substances" under CERCLA (EPA 2024d). This now requires that activated carbon associated with the removal of PFAS have special disposal or different regeneration technologies (because any emissions are now regulated differently than in the past). Water systems are already experiencing one year or greater lead times for GAC equipment. When commenting on the EPA's proposed PFAS NPDWR, Cleveland Water (a water utility) noted that "[w]ith only a select few GAC reactivation facilities in the country, significant transportation costs, often time across state lines, will be required" to meet the increased operation and maintenance demands for GAC treatment technology (Cleveland Water, 2023. p. 14–15).

Treatment systems such as reverse osmosis and nanofiltration produce a concentrated waste stream containing high levels of PFAS and other contaminants (AWWA 2023). Safe disposal of this waste to prevent further environmental contamination presents a significant challenge if the CERCLA requirements are to be met. Current disposal methods, including incineration and landfill storage, may not effectively contain or neutralize PFAS, potentially creating secondary contamination sources (Stoiber et al. 2020). This ongoing cycle of contamination could undermine the overall effectiveness of EPA-endorsed technologies in reducing PFAS concentrations in drinking water to within the established MCLs.

On a more positive side, challenging regulations do drive science to innovate. A newly developed technology may offer a cost-effective alternative to the clean-up of contaminated groundwater. For example, the ART-PFAS technology, which was developed between 2019 and 2023, uses in-situ groundwater circulation and foam fractionation/stripping to remove PFAS, diverging from conventional pump-and-treat methods (Rabah 2024). The ART-PFAS system has shown significant

reductions in PFAS concentrations, with PFOS and PFOA levels dropping below regulatory limits within a few months of operation. Other novel approaches will surely be released now that the MCL has been promulgated.

In summary, while the EPA-endorsed water treatment technologies should successfully reduce PFAS to concentrations approaching the MCLs, they are questionable with respect to ensuring compliance without considerable complications. The variable effectiveness, financial and operational burdens, and environmental impacts of waste disposal necessitate a more comprehensive approach to managing PFAS contamination.

Misconception #14: The EPA followed best practices for developing methodologies to derive the 2024 PFAS MCLs

The Safe Drinking Water Act (SDWA) was first passed by Congress in 1974, with various amendments being passed in the ensuring decades, to protect public health by regulating the nation's drinking water supply (EPA 1974; EPA 2024a). The SDWA empowers the EPA to establish national health-based standards, with the exception of private wells that serve fewer than 25 individuals, in order to "… protect against both naturally-occurring and man-made contaminants that may be found in drinking water" (EPA 2004, p. 1).

The EPA is required to use the best available, peer-reviewed science to inform its decisions on setting standards (EPA 2024i). This ensures that the regulatory actions are grounded in the latest and most reliable scientific research. In the author's opinions, the EPA did not rely on best practices for determining the appropriate PFAS MCLs for the six PFAS regulated in the 2024 PFAS NPDWR. Perhaps most importantly, the EPA fell short of its responsibility to conduct sound scientific processes that were both transparent and reproducible. This shortcoming was evident in the EPA's literature review protocol and approach to assessing the overall weight of evidence for health effects attributable to PFOA and PFOS. More than 1,600 comments were submitted to EPA during the allocated window for stakeholder comment and at least 150 of them addressed the shortcomings in their evaluation of the scientific data (Hua et al. 2025) (in press). An analysis shows the agency only seriously considered about 10% of public feedback. Even well-supported suggestions weren't formally addressed in the final policy.

A proper systematic review of the relevant scientific literature was necessary because the MCL values for the six PFAS were supposed to be "health-based" rather than arbitrary or based on the best available treatment technologies. As described in the EPA's IRIS Handbook, the EPA must review the full body of available scientific information, identify the subset of that information that is the most appropriate, explain the basis for that decision, and then analyze the remaining literature to draw a reasonably sound conclusion (EPA 2022b).

The EPA's Scientific Advisory Board (SAB) noted that the EPA failed to publish a pre-defined PFAS review protocol during their 2022 review of the draft PFAS NPDWR. The panel noted that they had:

> "… significant concerns that the reviews for PFOA and PFOS do not appear to have established a predefined protocol. The lack of a protocol led to a lack of clarity across each of the major systematic review steps for both chemicals and was seen as a major deficiency of the reviews" (SAB 2022, p. 3).

Further, the SAB "… found that the inclusion and exclusion of epidemiologic and animal studies was inconsistent across endpoints, leading to confusion about the criteria being used" (SAB 2022, p. 2). Similarly, the SAB concluded that the EPA's literature review ignored studies that should have been considered, including some of those the EPA relied on for its 2016 health effects support documents (HESD) for PFOA and PFOS, and some of which may have changed the Agency's conclusions regarding the potential hazard of exposure to PFOA and PFOS at low levels.

Heeding the SAB's warning, the EPA took steps to address this issue by expanding its assessment to include epidemiological and animal studies used in its 2016 Heath Effects Support documents. However, even though the EPA claimed to have considered all the relevant epidemiology data, it appears that they were not successful, resulting in having not considered many important and relevant papers (Hua et al. 2025) (in press). Had the EPA taken the time to evaluate the wealth of information contained in more than 1,600 submitted comments sent to them by stakeholders, they would have seen that many of the papers that they relied upon were fatally flawed and that they ignored studies with better data (Hua et al. 2025) (in press).

Another area where the EPA could have been better aligned with its regulatory guidance practices was in its determination that PFAS exposures were associated with numerous non-cancer health effects including, but not limited to:

> "… developmental effects, cardiovascular effects, hepatic effects, immune effects, endocrine effects, metabolic effects, renal effects, reproductive effects, musculoskeletal effects, hematological effects, other non-cancer effects, and COVID-19 (EPA 2024i, p. 32634–32635)."

For each type of health effect listed, the EPA did not follow its own guidance (i.e., the ORD Staff Handbook for Developing IRIS assessments) in evaluating the weight of evidence addressing each endpoint, which showed, at best, inconsistent associations of adverse health effects with these exposures (EPA 2022b). At worst, the Agency would have found that the various studies point to different critical effects and virtually none of the studies consistently identify plausible dose(s) that cause a particular effect in the general population. When there is such diversity of results among studies, the history of toxicology has shown that more research is needed using study designs and methodologies that can answer the question(s) of interest. For example, this contrasts with continuing to fund studies on PPARα induction and PFAS exposures in rodents, when this research is not relevant to humans.

There is no better example than the EPA's experience with dioxins, where over the course of many decades, the Agency identified no less than seven different "critical endpoints" for the adverse effects of 2378-TCDD (NRC 2006). Ultimately, none of them were found to be accurate

(Gough 1986; Crummett 2002). In a 2006 public report, NRC noted:

> "Fortunately, background exposures for most people are typically much lower than those seen in either Vietnam veterans or occupationally exposed workers. The potential adverse effects of TCDD, other dioxins, and DLCs from long-term, low-level exposures to the general public are not directly observable and remain controversial. One major controversy is the issue of estimating risks at doses below the range of existing reliable data. Another controversy is the issue of appropriately assessing the toxicity of various mixtures of these compounds in the environment." (NRC 2006, p. 1)

In 2009, the EPA introduced a plan with milestones to address two dioxin-related priorities: assessing human health risks and exposure to dioxin ("dioxin reassessment") and reviewing national dioxin soil cleanup standards (EPA 2009).

By February 2012, the EPA finalized the *Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments, Volume 1*, which was added to the IRIS database detailing hazard identification and dose-response data for 2,3,7,8-TCDD, establishing an oral reference dose (RfD) of 0.7 pg/kg b.w. per day for TCDD (EPA 2010). This was more than 30 years after the concerns about the dioxins surfaced.

No adverse effect was ever clearly identified or verified for those exposed to typical environmental concentrations or concentrations 10–100 fold higher. The U.S. EPA and other agencies, over the years, found that, aside from chloracne, elevated GGT, and altered testosterone levels, most health effects linked to TCDD exposure remained inconclusive or required further study, with no significant associations identified for adverse pregnancy outcomes, diabetes, or other endpoints, despite extremely high exposure levels in incidents like Yusho and Yu-cheng (Greene et al. 2003; Wesselink et al. 2014; Hsu et al. 2005; Kerger et al. 2012; Tuomisto et al. 2016; Tuomisto and Tuomisto 2012; Cole et al. 2003; Bofetta et al. 2011). Nonetheless, because draconian regulatory action was threatened over the course of 30 years, all the stakeholders worked aggressively, spending billions of dollars, to prevent the release of these chemicals to the environment.

To many, this is considered a victory for the environmental community and the EPA. To others, they are not convinced that spending those significant sums of money yielded an outcome worth the cost, especially as no adverse health effects were identified at the concentrations to which the general population had been exposed.

Similar to the dioxins, the EPA believes that they have identified several different critical adverse effects for PFAS (EPA 2024i). As described in this article, the evidence is that the Agency improperly associated modest changes in certain biomarkers (e.g., antibody responses, cholesterol (i.e., total cholesterol and LDLC), blood lipids and liver enzymes) with clinical effects – even though none of the endpoints described by the Agency were shown to be clinically relevant in the studied populations. As discussed above, in many cases, a study identified changes in a biomarker that they associated with PFAS exposure(s), but they failed to note that their results were still within the range of normal results.

As courts have ruled over the years, agencies cannot disregard available scientific evidence that is better than the evidence on which it relies (Kern County Farm Bureau v. Allen 2006). However, this seems to be what the EPA did in promulgating this rule. The EPA disregarded legitimate studies for unclear and unjustified reasons and did not adequately address the shortcomings of the studies that they did rely upon. Key scientific evidence and uncertainties for each health endpoint, as well as the EPA's failure to properly review and evaluate the evidence, have been addressed throughout this article.

It is clear from reading the press releases during 2022 and 2023 that the EPA was under pressure from Congress, the White House, environmental groups, and international agencies to quickly promulgate the MCL rules, as well as CERCLA action before end of May 2024 before the next presidential election (Elliott 2024). The pressures resulted in a hurried SAB review (where the committee never met face-to-face), a failure to consider all the critical concerns of the EPA SAB panel (SAB 2022), a lack of a thorough appreciation for the work of standards for drinking water set by many other countries (e.g., Germany, EU, Australia, NZ, WHO, etc.) and a failure to address, individually, all of the comments that were submitted (as they are expected to do to satisfy EPA policy).

Misconception #15: The EPA's MCLs for PFOA and PFOS are similar to those adopted by other countries

As noted in Misconception #14, the EPA seemed to be under considerable pressure to promulgate the PFAS MCL rule and the CERCLA action before the end of May 2024. The reasons for this have been discussed in an op-ed piece written by the former general counsel to the EPA (Elliott 2024). Apparently, due to the desire to rush the process, the EPA decided not to carefully analyze all the scientific data and studies relied upon by the five to seven international organizations that have recommended drinking water standards over the last three to five years (Elliott 2024). In the author's opinion, this resulted in the EPA promulgating an MCL with many scientific shortcomings.

The promulgated PFAS MCL identified a limit of 4.0 ng/L (4.0 ppt) for PFOA and PFOS each, 10 ng/L (10 ppt) for PFHxS, PFNA and HPFO-DA independently, and a Hazard Index (HI) value of 1.0 (unitless) for any mixture that contains two or more of either PFHxS, PFNA, HFPO-DA and PFBS (EPA 2024i). In August 2018, the Food Standards Australia New Zealand (FSANZ) organization identified the Australian tolerable daily intake for PFAS in drinking water. This group established a limit of 0.7 μg/L (700 ppt) for the sum of PFOS and PFHxS levels and 0.56 μg/L (560 ppt) for PFOA (Australian Government 2018). On January 12, 2021, the European Union's Recast of the Drinking Water Directive included a recommended level of 0.5 μg/L (500 ppt) for total PFAS in drinking water (European Chemicals Agency (ECHA) 2024). Shortly after, in June 2021, the Danish Environmental Protection Agency established that drinking water could not contain more than 2 ng/L (2 ppt) for the sum of PFOA, PFOS, PFNA, and PFHxS, making it one of the strictest regulatory decisions worldwide (DHI Group 2021).

More recently, in January 2023, Sweden introduced a new regulatory limit value of 4 ng/L (4 ppt) for PFOA, PFNA, PFOS, and PFHxS and 100 ng/L (100 ppt) for the 20 other PFAS detected in contaminant monitoring of drinking water (Life Source 2023). Germany is in the middle of a two-phase process of introducing and establishing new PFAS regulatory limits (Umwelt Bundesamt 2023). On January 12, 2026, a 0.1 μg/L (100 ppt) limit will be enforceable for 20 different PFAS detected in drinking water and in 2028, a limit of 0.02 μg/L (20 ppt) will be enforceable for PFHxS, PFOS, and PFOA.

The WHO has recommended provisional guideline values of 0.1 μg/L (100 ppt) for PFOA and PFOS individually and a provisional guideline value of 0.5 μg/L (500 ppt) for the 30 PFAS detected using available technologies (Schlea 2022). These provisional guidelines are significantly higher than the EPA's promulgated PFAS MCLs of 4 ppt for PFOA and PFOS and 10 ppt for PFHxS, PFNA, and GenX (EPA 2024i). These limits in other countries are being revisited due to the promulgation of the MCL by the U.S. EPA.

The Burgoon et al. (2023) paper contains one of the most compelling discussions of how differently the EPA, compared with other nations, has chosen to regulate PFAS in drinking water. They formed three international teams of eight scientists each to systematically evaluate mechanistic plausibility, study consistency, animal-human epidemiological data coherence, and dose-response robustness, establishing evidence-based safe exposure thresholds for PFOA. The teams found it challenging to identify MOAs for PFOA, with several potential MOAs being identified, but none of them had enough supporting evidence to establish one with any certainty. After evaluating the data on critical effects, a consensus statement was "[e]xisting human observational studies cannot be used reliably for developing the critical effect in the absence of mechanistic data relevant to humans at serum concentrations seen in the general public" (Burgoon et al. 2023, p. 5).

After evaluating the available evidence, the authors suggested that a provisional safe dose for PFOA should be 0.01–0.07 μg/kg-day (10–70 ppt) (Burgoon et al. 2023). Knowing what we know today, if one assumes that only 20% of the daily dose comes from drinking water, then an acceptable MCL would be about 40–300 ppt for PFOA and PFOS combined. Due to EPA's decision to regulate these PFAS at such low concentrations, many nations are wondering whether they should lower their drinking water targets.

Positive Impacts of EPA's Pursuit of PFAS MCL

Although the authors of this paper have taken issue with much of the science supporting the EPA's choices for the MCLs for these six PFAS, we want to acknowledge the intended or unintended consequences of this landmark regulation. Not only may it be the most expensive of all of EPA's regulations with respect to compliance and toxic tort litigation, but it has and will continue to stimulate hundreds of millions of dollars in health related research. This will involve toxicology research, in-vitro work on the mechanisms of action, epidemiology studies and additional clinical studies of workers.

In this paper, we identified what we perceived to be misconceptions related to the MCLs for these PFAS, focusing on the rather weak scientific basis for the Agency's views about the threat of adverse health effects at low concentrations in drinking water to the general population. While we presented numerous criticisms, we recognize that the EPA's role is to protect public health and, at the same time, be sure that their actions are economically reasonable from the standpoint of cost-benefit. The following are some examples of where the EPA's actions will have intentionally or unintentionally "advanced the ball" in allowing citizens, here and abroad, to better understand whether the PFAS, at concentrations normally encountered in the environment, are likely to produce adverse health effects.

1) The EPA Acknowledged that PFAS are a Persistent but Manageable Challenge

The authors recognize that the EPA attempted to turn what is widely viewed as a fairly significant global environmental challenge into a problem which can probably be mitigated through regulatory action. In their final rule, the EPA stated that:

> "PFAS tend to break down slowly and persist in the environment, and consequently, they can accumulate in the environment and the human body over time" (EPA 2024i, p. 32532).

The EPA's final rule regarding PFAS underscores an important truth: these compounds are resistant to degradation and widespread in both environmental and human systems. Their persistence in the environment and detectability in the human population calls for continued attention, informed risk management, and innovative solutions.

As the Agency noted, PFAS are remarkably stable, resisting breakdown through conventional environmental processes such as hydrolysis, photolysis and biodegradation. This stability has led to long environmental half-lives, which can range from years to a century, depending on environmental conditions, the specific PFAS compounds, and the matrices such as soil, water, and sediment (ITRC 2023b; Brunn et al. 2023; Washington et al. 2019).

The Agency, in its various announcements, reminds us that human exposures to PFAS are well-documented, with their presence detectable in the blood of virtually all Americans. The persistence of some PFAS in the human body, such as PFOA and PFOS, reflects their longer biological half-lives (1.5–5 years), but this characteristic varies widely across the diverse PFAS family. Emerging PFAS compounds, such as HFPO-DA, exhibit much shorter biological half-lives (on the order of hours or days), indicating that the body eliminates them relatively quickly (Shea 2018). In addition, many PFAS, especially fluoropolymers, exhibit limited bioavailability, further reducing exposure (Ankley et al. 2021).

Environmental contamination by PFAS from sources such as industrial facilities and airports have contributed to their ubiquity. The Agency noted that these point sources also represented an opportunity for intervention in its PFAS Strategic Roadmap (EPA 2024). With the development of advanced remediation technologies, including adsorptive

filtration, incineration, and chemical destruction, as well as ongoing improvements to wastewater treatment technologies, there are plenty of opportunities to prevent PFAS from entering the environment (Arvaniti and Stasinakis 2015).

2) The EPA Advanced PFAS Exposure Characterization and Mitigation Efforts

The EPA's recent decision to establish drinking water standards for six PFAS chemicals under the NPDWR was a significant step in reducing exposures to persistent environmental contaminants such as PFAS and their mixtures (EPA 2024i). While there are varying opinions regarding the specific concentrations chosen for the MCLs, this regulatory action highlights the Agency's commitment to addressing PFAS contamination and promoting scientific understanding through enhanced exposure characterization. As discussed in this paper, the Agency should have selected MCLs that were at least 5–40-fold greater than those promulgated and would likely have still achieved safe concentrations in humans blood concentrations.

The detection of PFAS at varying concentrations in public water systems, as revealed by the UCMR3 and UCMR5 datasets, furthers the scientific community's understanding of these contaminants' prevalence. The UCMR5 program required sampling for 30 chemical contaminants, including 29 PFAS compounds and lithium, in public water systems across the nation (EPA 2024h). Although the UCMR5 survey was incomplete when the Office of Management and Budget (OMB) conducted its economic impact analysis, the data collected had already elevated awareness of potential PFAS contamination in water supplies. While it is yet unclear what the data will indicate, as the dataset grows, it will serve as a vital resource for future cost-benefit assessments and regulatory refinements. If Congress were to re-evaluate the PFAS MCLs, the UCMR5 dataset would offer a solid foundation for informed regulatory decision-making.

The EPA has appropriately emphasized the co-occurrence of PFAS in drinking water, recognizing the unique challenges presented by mixtures of these chemicals. Many PFAS compounds are found together, persisting in the environment and raising concerns about potential additive health effects. While the current state of science fails to support that these chemicals all share a common MOA, by raising this issue, the EPA has drawn attention to the disproportionate exposure of some communities to mixtures of PFAS, and the need for additional research to characterize these exposures (and their human health risk, if any).

3) The EPA seemed to recognize that PFAS regulation was going to be costly; although it is unclear if they understood the magnitude of the final price tag

The EPA was correct to acknowledge the significant financial challenges associated with complying with the finalized PFAS MCLs for drinking water. In its final ruling, the Agency recognized that substantial investments in infrastructure and technology will be required over the next decade to meet these stringent standards. They recognized that small and disadvantaged communities are expected to face a disproportionate burden, as they often lack the economies of scale that larger systems benefit from and must allocate a larger percentage of their budgets to water treatment.

To help mitigate these financial pressures, the Infrastructure Investment and Jobs Act (IIJA), also known as the Bipartisan Infrastructure Law (BIL), represents a commendable effort to support communities on the frontlines of PFAS contamination. As noted by the EPA, this legislation allocates billions of dollars over a five-year period, including $11.7 billion for the Drinking Water State Revolving Fund (DWSRF) General Supplemental, $4 billion to DWSRF specifically for emerging contaminants, and $5 billion in grants targeting small or disadvantaged communities. These funds are critical for assisting communities with the cost of installing advanced treatment technologies that might otherwise be financially prohibitive.

While these appropriations represent a significant step forward, drinking water agencies have highlighted that truly significant additional funding will be required on a much larger scale to support all the necessary infrastructure and personnel which will be necessary to comply with the PFAS MCLs (Water Coalition 2023). This underscores the importance of continued advocacy and resource allocation to address remaining funding gaps. It also highlights the need for innovative partnerships between federal, state, and local governments, along with private sector stakeholders to ensure that funding is distributed equitably. Perhaps, if Congress chooses to reevaluate the MCLs, the goals will be more reasonable and the costs will decrease appreciably; yet, still protect the public health.

4) The EPA Recognized and Highlighted Several Knowledge Gaps in PFAS Toxicity to Humans

The EPA has played a valuable role in identifying significant knowledge gaps regarding the toxicity of PFAS to humans. While regulatory efforts have largely focused on well-characterized compounds like PFOA, PFOS, PFHxS, PFNA, HFPO-DA, and PFBS, the Agency has acknowledged that thousands of other PFAS remain insufficiently studied. This limited understanding has led to calls for broader research to explore the environmental and human health impacts of lesser-studied groups and subgroups of PFAS, including sulfonamides, ether acids, phosphate esters, and emerging perfluoroether classes such as perfluoroalkyl ether carboxylic acids (PFECAs) and perfluoroalkyl ether sulfonic acids (PFESAs) (Strynar et al. 2015; ITRC 2023c). These compounds present unknown environmental persistence and potential toxicity challenges that merit further investigation.

A particularly pressing issue raised by the EPA is the absence of a unifying mechanism of action for PFAS, which complicates efforts to evaluate their health impacts comprehensively. It is alleged that there are as many as 3,000 PFAS in our environment and there will be many voices who will want some type of regulation for each of them.

While only a small fraction of PFAS are present in drinking water or the diet at concentrations high enough to pose a human health risk, simultaneous exposure to dozens of uncharacterized PFAS compounds, which are detected in water or human blood, may have additive effects. The Agency's decision to establish MCLs for six PFAS compounds has placed additional pressure on the scientific community to develop tools for assessing the biological activity of PFAS mixtures. Grouping PFAS through shared characteristics, such

as certain biological endpoints, chemical properties, or mechanisms of action (if elucidated) to assess potential health hazards may be a promising means of regulation in the future (George and Birnbaum 2024); though achieving this will be a substantial scientific challenge.

One can imagine that, at some time over the next 10 years, that all 3,000 chemicals will be placed in ten or more categories based on various properties, toxicities or mechanisms of action. At that point, some type of TEQ system will hopefully be developed that would allow for regulatory guidance for all of them.

Nonetheless, despite decades of occupational studies on PFAS, a unifying mechanism of toxicity or family of genuine adverse health effects across highly exposed populations has not been identified. Workers in industries with significant PFAS exposure, such as manufacturing or firefighting, have failed to identify evidence of adverse effects associated with certain PFAS exposures; certainly not in a uniform, dose-dependent, or consistent basis. These inconsistencies in occupational epidemiological studies underscore the complexity of PFAS toxicity or it indicates that we are "chasing our tails" in search of an adverse effect that is simply not present; only time will tell. Most scientists will advocate for continued research to unravel how these compounds interact with biological systems individually and, in mixtures, and this will almost surely occur.

Discussion

The EPA's promulgation of the final PFAS NPDWR on April 26, 2024 has been met with significant scrutiny due to its methodological shortcomings and an overall lack of transparency in the decision-making process. As discussed in this article, the EPA's intent to regulate these chemicals was based on the application of the precautionary principle rather than a robust understanding of the entirety of the scientific evidence regarding the toxicity of these chemicals.

The economic implications of the EPA's MCL and CERLCA regulations have been reported by many entities to be profound, with potential costs rising toward tens of billions of dollars over the next several decades (AMWA et al. 2023; AWWA 2023; U.S. Chamber of Commerce 2022). This includes costs for upgrading public water systems, personal injury litigation, and the broader effects on U.S. manufacturing competitiveness. The EPA also acknowledged that there are nonquantifiable costs that will follow the promulgation of the PFAS MCLs that will increase the economic burden of the final rule (EPA 2024i).

It is entirely possible, when one considers the avalanche of personal injury claims that the MCL and the coverage in the press have stimulated, that the cost to the nation will be well over one trillion dollars (Wolf 2023). Not only may this rule impact America's competitiveness internationally, but it will surely stifle innovations that require the use of PFAS. The Department of Defense (DoD) issued a report on the subject, where it was concluded that "PFAS are critical to DoD mission success and readiness and to many national sectors of critical infrastructure, including information technology, critical manufacturing, health care, renewable energy, and transportation" (Department of Defense 2023). The promulgated MCLs may also drive PFAS-related production overseas, affecting industries that currently have no viable alternatives to these chemicals and requiring international dependence for these critical chemistries.

In light of their physical and chemical properties, and because PFAS can be found in nearly every living creature on the planet, thoughtful actions needed to be taken by the EPA. The question raised in our analysis is whether EPA promulgated too stringent of a regulation too quicky, which resulted in the movement from the EPA's Lifetime Health Advisory (LHA) of 70 ppt in 2016, although this level was non-enforceable, to one that is approximately 94% lower to 4 ppt. In our view, as discussed in a recent lecture before the Toxicology Forum in Washington, DC, it would have been much wiser for EPA to have implemented a tiered and phased regulatory scheme (Paustenbach 2024a). That would have faced little resistance from any stakeholder.

A phased approach, where a less stringent MCL could be set and periodically revisited to assess any reduction in human exposure, is a prudent alternative regulation the EPA should have considered. Over time, the EPA could have continued to lower exposure limits until they were satisfied that the general population's exposures to drinking water were posing no future health concerns. In the United States, we rarely adopt such an approach, but in many ways, this would be highly effective and would largely eliminate the huge sums of money spent on litigation (Paustenbach 2024b).

As discussed in the Interstate Technology and Regulatory Council (ITRC) (2023c), although there have been decades of research into the health effects of PFAS exposure in humans, there are still important data gaps and research needs that remain. One could argue that several of these data gaps needed to have been addressed prior to the promulgation of these MCLs. Several of these important data gaps and research needs include:

- Human half-lives and other toxicokinetic data are not available for many PFAS found in drinking water and other environmental media.
- Strategies for grouping PFAS for use in risk assessments need to be developed and validated
- The majority of the many thousands of PFAS, including those in commercial use, have very limited or no toxicity data. This is a critical data gap in health effects information for PFAS.
- There are relatively few epidemiological studies of communities exposed to AFFF, PFOS, and/or other PFAS in drinking water. Epidemiology studies involving multiple study designs that are focused on clinically relevant endpoints need to be conducted.
- Additional toxicology data are needed for some PFAS found in environmental media, including drinking water, especially at the concentrations that humans could be exposed to via this route.
- There is a need for additional toxicological studies on the effects of PFAS mixtures, although humans are exposed

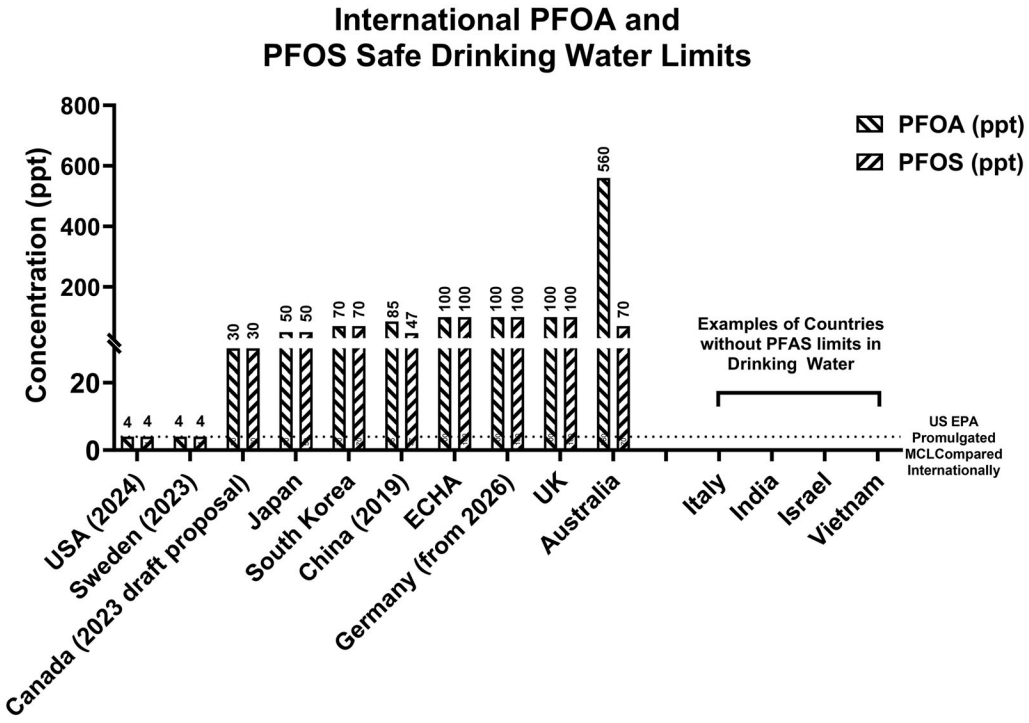

Figure 1. PFOA and PFOS safe drinking water limits across countries and international regulatory agencies.

to multiple PFAS, information on toxicological interactions of PFAS is limited.

- Similarly, current NHANES biomonitoring in blood serum includes only 11 PFAS, primarily PFAAs, and breast milk biomonitoring data for these PFAS are limited. There are limited or no biomonitoring data in blood serum or breast milk for many other PFAS produced or used in the United States, some of which are known to be bioaccumulative in humans.
- Long-term epidemiology studies are needed to determine if the PFAS MCL actually results in lowered incidence of disease in the general U.S. population

In conclusion, while the EPA's efforts to regulate PFAS in drinking water were allegedly driven by public health concerns, the scientific basis for the recently promulgated MCLs is debatable and, almost certainly, on a shaky scientific foundation. The economic fallout from these regulations could be one of the most significant in the history of the environmental movement. Perhaps EPA and other agencies can learn from this journey and improve on it as all regulatory bodies across the globe wrestle with how to properly regulate the thousands of other PFAS. This will require discipline and a balanced approach to analyzing scientific data.

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Declaration of interest

The authors report no conflict of interest. However, Paustenbach and Associates has provided consulting services to at least one firm who has manufactured PFAS. Paustenbach and Associates received partial funding for the Advancing Community Values organization, a nonprofit group that sought to have safe drinking water at affordable prices. We are not aware of the persons or firms who supported Advancing Community Values, but when the MCL was promulgated by EPA, it appears that that organization was dissolved.

All authors are employed by Paustenbach and Associates, a consulting firm that provides scientific advice to the government, corporations, law firms, and various scientific/professional organizations. The firm has been engaged by manufacturers and distributors of PFAS and PFAS-containing compounds in various litigation matters and advisory roles. Dr. Dennis Paustenbach, who has been involved as an expert in many personal injury cases as an expert witness may, along with others, be called upon in the future to serve as an expert in PFAS litigation. The study design, execution, results, and interpretation of the current work are the sole responsibility of the authors. This manuscript was prepared and written exclusively by the authors.

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