Response for public review - submitted November 11, 2022

Document title: PFOS and PFOA in Drinking Water, ***Draft background document for development of WHO Guidelines for Drinking Water Quality***

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Please choose one of the alternatives below: My name can be disclosed to the authors and included in the background document to the GDWQ.

Responses to the questions on the comment form are below. Please note the following:

- A citation or link for references not mentioned in the draft WHO document is provided the first time the reference is mentioned in the comments.
- The major points in the comments are summarized in the **Additional Comments** section beginning on p. 47.

1. **Does this text respond to an issue of concern?**

PFAS in drinking water is currently an issue of high interest and concern, and the New Jersey Department of Environmental Protection (NJDEP) supports WHO's efforts to develop drinking water guidelines for PFOA and PFOS. Because WHO's recommendations will likely receive extensive attention from regulatory agencies, the public and others, it is particularly important that all information in this document be accurately and clearly presented and that the document's conclusions be scientifically/technically sound and public health protective.

The draft WHO document was reviewed by NJDEP scientists and technical staff with extensive expertise and experience in development of drinking water standards for PFAS, including health effects, analytical limitations, and treatment removal capabilities. NJDEP began evaluating PFAS in drinking water in 2006, conducted the first statewide study of the occurrence of PFAS in drinking water in the U.S. in 2006, and established the first drinking water standard (Maximum Contaminant Level) for a PFAS in the U.S. in 2015. As shown at https://www.nj.gov/dep/dsr/pfas.htm, NJDEP scientists and technical staff are the authors and co-authors of numerous peer-reviewed publications on PFAS in drinking water including several highly cited reviews.

In its review of the draft document, NJDEP identified the need for many revisions so that the information presented is factually accurate and the conclusions are scientifically/technically supportable and public health protective. NJDEP also noted additional areas where clarification

is needed. For these reasons, NJDEP concludes that extensive revisions are needed for the document to appropriately respond to the issue that it is intended to address.

Please be aware that NJDEP's detailed comments are submitted with a constructive intent, and we hope that these comments are helpful to WHO as it revises the draft document. NJDEP notes that the WHO website where the draft document is posted for comment states that PFAS, particularly PFOS and PFOA, are being "considered" for the next update of the WHO drinking water quality guidelines. Unless the draft document is extensively and appropriately revised, NJDEP recommends that WHO remain silent on this topic and not proceed to establish drinking water quality guidelines for PFOA, PFOS, or PFAS.

2. Does this text compete or complement other publications in the area – if so, which?

In addition to the authoritative reviews by ATSDR, EFSA, Health Canada, and USEPA mentioned in the box on p. 1, lines 1-8, several other authoritative reviews provide thorough and well-organized information relevant to the topics presented in the draft document. It is recommended that WHO review and cite as appropriate the following additional authoritative reviews (listed in alphabetical order), most of which are cited in our comments below:

- CalEPA. 2021. California Environmental Protection Agency. Public Health Goals - Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water. First Public Review Draft. July 2021. https://oehha.ca.gov/sites/default/files/media/downloads/crnr/pfoapfosphgdraft061021.pdf. The information in this document is relevant to Sections 3, 4, 5, 6, 7, and 9 of the WHO draft.
- ITRC. 2022. Interstate Technology & Regulatory Council. PFAS Technical and Regulatory Guidance Document and Fact Sheets PFAS-1. Washington, D.C.: Interstate Technology & Regulatory Council, PFAS Team. https://pfas-1.itrcweb.org/ This document information in this document is relevant to all sections of the WHO draft, particularly Sections 1 and 2.
- NJ DWQI. 2015. New Jersey Drinking Water Quality Institute. Recommendation on Perfluorinated Compound Treatment Options for Drinking Water. June 2015. https://www.state.nj.us/dep/watersupply/pdf/pfna-pfc-treatment.pdf. The information in this document is relevant to Sections 8 and 9 of the WHO draft.
- NJDWQI. 2016. New Jersey Drinking Water Quality Institute. Addendum to Appendix C: Recommendation on Perfluorinated Compound Treatment Options for Drinking Water. June 2016. https://www.nj.gov/dep/watersupply/pdf/pfoa-appendixc.pdf
- NJ DWQI. 2017a. New Jersey Drinking Water Quality Institute. Health-Based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA). February 15, 2017. https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf. The information in this document is relevant to Sections 3, 4, 5, 6, 7, and 9 of the WHO draft.
- NJ DWQI. 2017b. New Jersey Drinking Water Quality Institute. Second Addendum to Appendix C: Recommendation on Perfluorinated Compound Treatment Options for Drinking Water. November 2017b. https://www.state.nj.us/dep/watersupply/pdf/pfos-

recommendation-appendix-c.pdf. The information in this document is relevant to Sections 8 and 9 of the WHO draft.

- NJ DWQI. 2018. New Jersey Drinking Water Quality Institute. Health-Based Maximum Contaminant Level Support Document: Perfluorooctane Sulfonate (PFOS). June 5, 2018. https://www.state.nj.us/dep/watersupply/pdf/pfos-recommendation-appendix-a.pdf. The information in this document is relevant to Sections 3, 4, 5, 6, 7, and 9 of the WHO draft.

3. Is the level of guidance and information provided appropriate? Please consider the practical aspects and conclusion sections in particular.

As discussed in detailed comments below, insufficient justification is provided for selection of the treatment-based pGVs of 0.1 ug/L (100 ng/l) for PFOA and PFOS. The choice of these values appears to be arbitrary.

Also, the draft document often emphasizes relatively unimportant details from one or a few studies (which may not be the most important ones) without overall synthesis of the available information on the topic.

4. Are there major omissions that should be corrected?

In its review of the draft document, NJDEP noted several important omissions. These are also noted in the line-by-line comments on "errors of fact or interpretation" below, and are summarized here:

- As a general comment, the draft document lacks an overview of the multiple reasons why PFOA, PFOS, and other PFAS differ from other drinking water contaminants and why they are of high concern when present in drinking water. This information is provided in the **Additional comments** section below as "Additional key points not included in the draft document that should be emphasized by WHO."

- p. 1, line 21. Bioaccumulation and health effects, along with persistence, are major reasons for concern about PFAS and should be added to the sentence, as shown in bold here: "… their persistence, **bioaccumulation in humans and other species, and numerous health effects are** ~~can be~~ of concern regarding environmental and human health."

- p. 14, line 16. Section 2.2 on PFAS in food. Recent data from the US Food and Drug Administration should be included. See https://www.fda.gov/food/chemical-contaminants-food/analytical-results-testing-food-pfas-environmental-contamination

- p. 18, line 46, and elsewhere where PFAS in breast milk is discussed. The discussions of PFOA and PFOS in breast milk throughout the document omit the key point that infants receive higher exposures than older individuals from contaminated drinking water. These higher exposure and serum levels are of particular concern because infants are a sensitive subpopulation for developmental effects of PFOA and PFOS.

- p. 23, lines 21-24, and throughout. The important concepts that the difference in male versus female half-lives in rats is much greater than for other species and that this difference is important in interpreting toxicology studies of PFOA conducted in rats, particularly studies of developmental effects, are omitted from the draft document.

- p. 26, lines 33-34, regarding the study of advanced cancer patients given high doses of PFOA. The draft document states: "The test article [PFOA] was described as non-toxic at all dose levels tested" and omits stating that toxicity attributed to the test article was reported in this study.

- p. 32, lines 20-21. The draft document does not include information on the numerous epidemiology studies reporting associations of small for gestational age (or related intrauterine growth retardation endpoints) and/or low birth weight with PFOA and PFOS, and it cites a source that stated that no associations of PFOA or PFOS with these effects have been reported.

- p. 37, line 40 – p. 38, line 7. The draft document does not include information that supports the use of increased serum levels of the liver enzyme ALT in humans as an endpoint for health-based guideline development. In summary, the increased ALT associated with PFOA and PFOS is indicative of liver damage. PFOA is associated with increased incidence of clinically-defined elevated ALT (not just a numerical increase in ALT), and it is well established that relatively small (<2-fold) increases in ALT are associated with pathology-confirmed liver disease such as non-alcoholic fatty liver disease.

- p. 38, Section 4.2.8.1, Carcinogenicity of PFOS. Two additional recent human studies that are relevant to this topic that should be cited are Cao et al. (2022, https://doi.org/10.1016/j.chemosphere.2022.134083) and Goodrich et al. (2022, https://doi.org/10.1016/j.jhepr.2022.100550).

- p. 67, line 29, and/or elsewhere. The document does not mention that there is concordance between laboratory animals and humans for most of the major toxicological effects of PFOA and PFOS, including developmental, immunological, hepatic, and carcinogenic effects.

- The document does not mention that PFOA and/or PFOS were associated with increased risk of clinically defined abnormal values for each of the four most well-established human non-cancer health effects (increased cholesterol, increased ALT, decreased birth weight, decreased response to vaccination) in one or more epidemiology studies.

- The document omits at least two much lower values from the ranges of "health-based drinking water values derived by authoritative agencies" on p. 79, lines 27-32. These include the drinking water guideline of 2 ng/L for the total of PFOA, PFOS, PFNA, and PFHxS

established by Denmark in 2021 and the interim Health Advisories of 0.004 ng/L for PFOA and 0.02 ng/L for PFOS established by USEPA in 2022.

5. Is there superfluous information that could be omitted?

Some of the toxicokinetics sections (e.g., 3.1, 3.2) and some of the sections on health effects in humans and animals (e.g., section 4.2.1.3 on epidemiology studies of preterm birth and pregnancy loss, and others) include more details on design and results of various studies than may be necessary. A synthesis and overall conclusions of the information on these and other topics would be more useful.

Additionally, the detailed discussions in several sections, and the number of citations, of publications by Dourson and co-authors (Dourson et al., 2019, Dourson and Gadabudi, 2021, Campbell et al., 2022) regarding the half-life of PFOA and other topics, and the Elcombe et al. (2013) study of cancer patients administered very high doses of PFOA, is excessive and inappropriate especially since it is stated (p. 1, lines 1-8) that the information in the draft document is "…. primarily drawn from existing authoritative reviews…Examples of robust studies from the primary literature are also described to provide an overview of health effects in humans and animals. However, this document is not intended as a comprehensive summary of the primary literature and not all studies are cited."

As discussed in the line-by-comments below, the cancer patient study is not appropriate for consideration in development of a drinking water guideline for PFOA. Additionally, as WHO notes, the Campbell et al. (2022) conclusion that the half-life of PFOA is shorter than two years is subject to debate, and it is likely more accurate that this conclusion is not accepted by the general scientific community. Importantly, the variability within the range of potential human half-lives for PFOA, including the values suggested Campbell et al. (2022), is not large, and this relatively small range does not warrant so much emphasis. The potential variability of the half-life is similar or less than the variability of many other factors and assumptions routinely used in development of drinking water guideline development including body weight, drinking water ingestion rate, and others.

6. Are there errors of fact or interpretation that should be corrected – if so, what?

This section includes specific (line-by-line) comments related errors of fact or interpretation that need to be corrected or clarified. General comments are provided in **Additional comments** starting on p. 46 below.

Section 1. General Description

p. 1, line 21. Bioaccumulation and health effects, along with persistence, are major reasons for concern about PFAS and should be added to the sentence, as shown in bold here: "… their persistence, **bioaccumulation in humans and other species, and numerous health effects are** ~~can be~~ of concern regarding environmental and human health."

p. 1, line 22, states: "PFAS compounds are distributed across the globe and their degradation products occur in biota and environmental media, often at great distances from their original source."

This sentence is unclear, and it is not completely accurate. "PFAS" include both terminal PFAS (e.g., perfluoroalkyl acids such as PFOA and PFOS) and precursor PFAS (e.g., fluorotelomer alcohols) that can degrade to terminal PFAS such as perfluorocarboxylic acids (PFOA and others). The sentence should be reworded to convey that terminal PFAS in biota and environmental media can result from degradation of precursor PFAS that have traveled great distances from their original source. Also, "PFAS compounds" is redundant ("compounds" should not be included) since PFAS stands for per- and polyfluoroalkyl substances.

p. 1, lines 32-34. Regarding the definition of PFAS, the sentence beginning with "In these cases, …" is confusing and does not appear necessary. Suggest deleting it.

p. 2, lines 1-2, states: "Complex PFAS that can yield these highly persistent PFAS are referred to as precursor substances."

This sentence is not completely accurate and needs to be revised to indicate that the precursors are PFAS, and that precursor PFAS also include smaller molecules such as fluorotelomer alcohols and others that degrade to terminal perfluoroalkyl acids.

p. 2, line 28, Table 1.1. Physical state of PFOA should say "ammonium salt" not "ammonia salt".

p. 2, line 28. In Table 1.1, the "Half-life in Water" information (PFOS – 41 years; PFOS – 92 years) does not accurately reflect the information from the primary source cited by ATSDR (2021) and incorrectly indicates that PFOA and PFOS are known to break down in water over time.

ATSDR (2021), which is cited in the draft document, attributes the PFOS half-life information to OECD (2002) which states (bold added): "The analytical results indicate no degradation of PFOS or dependence on pH. The study indicates that the hydrolytic half-life of PFOS in water is **greater than** 41 years."

Similarly, the PFOA information is attributed by ATSDR (2021) to OECD (2006b) which states (bold added): "From the data pooled over the three pH levels, it was estimated that the hydrolytic half-life of PFOA at 25°C is greater than 92 years, with the most likely value of 235 years. From the mean value and precision of PFOA concentrations, it was estimated the hydrolytic half-life of PFOA to be **greater than 97 years**."

It is suggested that WHO also check the primary source of the air half-life values.

p. 3, lines 1-13. Suggest combining these two paragraphs since they repeat the same information for PFOA and for PFOS.

Section 2. Environmental Levels and Human Exposure

p. 8, line 26. Throughout Section 2.1.2 on occurrence in drinking water, frequency of detection data are not meaningful unless accompanied by the Reporting Level (or other term used for the concentration below which detections were not reported). This comment also applies to all other sections where occurrence data are presented.

p. 9, lines 34-38. When discussing USEPA Unregulated Contaminant Monitoring Rule 3 (UCMR3) data here and in Section 2.1.2.1, it is important to mention that the Reporting Levels in UCMR3 were high (PFOA – 20 ng/L; PFOS – 40 ng/L) and therefore did not report many occurrences of PFOA and PFOS at lower levels. It should be noted that UCMR5, to be conducted in 2023-2025, will monitor for 29 PFAS at much lower Reporting Levels, with a Reporting Level of 4 ng/L for both PFOA and PFOS. See Table 2 at https://www.epa.gov/dwucmr/fifth-unregulated-contaminant-monitoring-rule

p. 9, line 48. The current USEPA Health Advisories for PFOA and PFOS are no longer 70 ng/L. USEPA has issued newer interim Health Advisories for PFOA and PFOS and has stated that they supersede the 2016 Health Advisories of 70 ng/L. See: United States Environmental Protection Agency. Lifetime Drinking Water Health Advisories for Four Perfluoroalkyl Substances. Fed. Reg. 87(18): 36848-36849. June 21, 2022. https://www.govinfo.gov/content/pkg/FR-2022-06-21/pdf/2022-13158.pdf

p. 10, line 31. Section 2.1.2.1 on UCMR3 monitoring study. The comment on p. 9, lines 34-38 above applies here as well.

p. 11, Table 2.1. The arithmetic means for UCMR3 data are shown here. It might be more informative to show the medians or modes of the data set because the averages shown here may be highly impacted by a few extreme values (e.g., average PFOS in GW of 0.199 ug/L is very high; what is the median and mode?)

p. 14, lines 4-11. It should be mentioned that the data and estimated intakes for PFAS in food in this paragraph come from older studies. If newer data are available, they should be discussed. Also, the LOQs should be mentioned as in the discussion of the EFSA study above.

p. 14, line 16. Section 2.2 on PFAS in food. Suggest including recent data from US Food and Drug Administration. See https://www.fda.gov/food/chemical-contaminants-food/analytical-results-testing-food-pfas-environmental-contamination

p. 15, line 20. "DuPont 3M works, West Virginia" is incorrect and should be revised to "DuPont Washington Works, West Virginia."

p. 15, lines 26-28. While the information on atmospheric formation of PFCAs from precursors in this section is accurate, this concept should also be included in the earlier discussion of precursor compounds in air in Section 1.5. Also, the same PFAS are referred to with different nomenclature here than in Section 1.5, and the nomenclature should be made consistent.

p. 16, line 1. Although it is stated on lines 13-15 that >75% of PFOA and PFOS exposure can come from drinking water when it is contaminated, a more general statement in (Section 2.5.

Relative contribution of drinking water to total exposure levels) that the percent contribution from drinking water is dependent on the concentration of PFOA or PFOS in the drinking water should be added.

It is also important to mention that exposure to PFOA and PFOS from even relatively low concentrations in drinking water is greater than exposures from sources prevalent in the general population (e.g., food, consumer products) (DWQI, 2017a, 2018; Post et al. 2012, https://doi.org/10.1016/j.envres.2012.03.007; Post et al., 2017, https://doi.org/10.1371/journal.pbio.2002855), and that the relative contribution from drinking water to total exposure can be predicted with a Clearance Factor, which relates intake to serum levels, and the average daily water intake rate (DWQI, 2017a, 2018; Post, 2021, https://doi.org/10.1002/etc.4863).

p. 16, lines 27-30. If Relative Source Contribution (RSC) is to be mentioned here, several points need to be added and/or clarified. First, 20% is the "floor" value for RSC recommended by USEPA guidance (USEPA, 2000); a lower RSC value cannot be used even if supported by data. Second, and more importantly, the RSC is not the percent of exposure that actually comes from drinking water, which obviously varies in different locations with different drinking water concentrations. Rather, the RSC is the percent of exposure that can come from drinking water so that total exposure from drinking water and non-drinking water sources does not exceed the Reference Dose (USEPA, 2000). Relevant to this point, the USEPA Science Advisory Board (SAB, 2022) reviewed the USEPA (2021b,c)[1] PFOA and PFOS assessments cited here and recommended that USEPA clarify that the percent of the Reference Dose from non-drinking water sources, not the actual exposure from drinking water, is relevant to the determination of the RSC. Additionally, the USEPA SAB (2022) recommended that USEPA clarify that general population exposure from non-drinking water sources far exceeds the draft PFOA and PFOS Reference Doses from USEPA (2021b,c), supporting the use of the "floor" RSC of 20%.

p. 16, line 36-37. "is possible" should be changed to "occurs" since there is no doubt that bioaccumulation takes place in the species mentioned. Also, birds should be added to the list of species mentioned.

p. 16, lines 41-44. It is not clear if the increase in BAF with increasing chain length mentioned here is for perfluoroalkyl carboxylates or perfluoroalkyl sulfonates. Also, the values cited appear to be log BAFs, not BAFs.

p. 17, lines 2-4. Sentence beginning with "Ongoing…" The intended meaning of this sentence is not clear as written. This is the relevant quote from ATSDR (2021): "Ongoing monitoring of perfluoroalkyl levels in animals may help to determine whether efforts to phase out these substances will have had an effect on their biomagnification. A data need exists to determine the bioaccumulation potential of the new replacement substances used in place of PFOA and PFOS."

[1] Note: These are listed as USEPA, 2021a,b in the citation list of the draft WHO document.

Section 3. Toxicokinetics and Metabolism in Humans and Animals

p. 17, line 11, and throughout Section 3. A high-level synthesis of information on each of the topics included in this section would be helpful to the reader and should be added. Throughout Section 3, many details that may not be necessary or meaningful to the reader are included, while the general conclusions that would be most useful are often not provided. The following is an example of synthesis of information on PFOA absorption from DWQI (2017a) that is provided as an illustration:

> "PFOA is well absorbed by the oral route (Lau et al., 2007). More than 95% of a single dose of 0.1 to 25 mg/kg APFO (the ammonium salt of PFOA) was absorbed in male and female rats (Kemper, 2003). It was also well absorbed in mice, rats, hamsters, and rabbits in studies by Hundley et al. (2006). About 98.7% of an oral dose given to pregnant rats on gestation day (GD) 8 or 9 was excreted in the urine within 24 hours (Gibson and Johnson, 1983). Additionally, a recent study in mice (Fujii et al., 2015) estimated the oral absorption of PFOA as 98.7% in males and 99.8% in females. The extent of oral absorption was determined by comparing fecal excretion after intravenous dosing (representing biliary excretion of PFOA into the gastrointestinal tract) and oral dosing (representing both unabsorbed PFOA and biliary excretion). PFOA is well absorbed by humans exposed orally, as demonstrated by elevated serum concentrations in residents of communities with contaminated drinking water (discussed above)."

p. 17, line 42 – p. 18, line 6. The level of detail presented for the Elcombe et al. (2013) study described here and much of the information presented (e.g., time to reach steady-state) are not relevant to absorption, the topic of this section. In addition to the issues that are noted about cancer patients as the study group, the very high dose levels used are not relevant to the lower exposures from drinking water, the topic of this draft document. If Elcombe et al. (2013) is mentioned here, it should simply be stated that the data indicate oral absorption of PFOA in humans.

p. 18, lines 6-8. Serum levels are elevated in studies of many communities with drinking water contaminated with PFOA and/or PFOS. A more general reference that synthesizes this information should be cited, or, at a minimum, the sentence should be revised to state that Emmett et al. (2006) is one of many studies showing elevated serum levels from drinking water exposure.

p. 18, lines 9-11. The following is an example of the need for improved synthesis of the information in the Toxicokinetics section: The information from Cui et al. (2010) about oral absorption in rats (which were males, although this is not stated in the draft document) in the sentence beginning with: "A carbon tracer…," should be synthesized with the information on absorption in male rats from Dzierlenga et al. (2019) on lines 20-21. Also, Cui et al. (2010, https://doi.org/10.1007/s00244-009-9336-5) is not listed in the Reference list.

p. 18, line 21. The relevant statement from Dzierlenga et al. (2019) refers to both sexes of rats, not just males, as follows: "Oral bioavailability for all three chemicals [including PFOA and two

others] was calculated to be >100%, which may be due to increased reabsorption by intestinal transporters." Also, this paper is not listed in the citation list.

p. 18, line 27, Section 3.2 on Distribution. A general statement that the distribution of PFOA, PFOS, and other PFAS is different than other persistent organic contaminants because PFAS do not preferentially accumulate in fat but rather bind to proteins in blood serum and other tissues is needed. For example, see summary in Post et al. (2012).

p. 18, lines 35-36. Sentence starting with: "Other tissues…" After thorough searching, this information on cerebrospinal fluid and thyroid was not found in ATSDR (2021). Was this statement intended to say that ATSDR (2021) did not mention these as partitioning sites, rather than that ATSDR stated that these are not partitioning sites?

p. 18, lines 42-26. Suggest citing a review of placental transfer in humans, such as Appel et al. (2021, https://pubmed.ncbi.nlm.nih.gov/34930098/) instead of a few individual studies.

p. 18, line 46. The discussion of PFOS in human breast milk that begins here should emphasize the important fact that serum PFOS levels in breastfed infants increase several-fold during the first 6 months after birth, as reported by Fromme et al. (2010, https://doi.org/10.1021/es101184f) and others. Also, since it is well established that PFOS is ubiquitously present in human breast milk, the sentence should be revised to say that postnatal exposure via breast milk "occurs," rather than that it "is possible". It is recommended that the recent review of data on PFAS in breast milk from around the world by Fromme et al. (2022, https://doi.org/10.1016/j.scitotenv.2022.154066) be cited here.

p. 19, line 31. It is important to mention that there is also exposure to PFOS via lactation in rats. As stated by DWQI (2018): "PFOS is also transferred to offspring through breast milk in rodents, as shown in a cross-fostering study (Luebker et al., 2005a) in which PFOS was detected in pups born to unexposed dams who were nursed by dams that had been dosed during gestation, with a pup:maternal PFOS serum ratio of 0.27."

p. 20, line 8-10. The fact that toxicokinetics at the very high doses used in this study (Elcombe et al., 2013, as cited in and evaluated by Dourson et al., 2019) are likely different than at the lower exposure levels from contaminated drinking water should be added to the sentence about the "relevance of the data to drinking-water exposure in the general population."

p. 20, paragraph starting on line 12. The reviews of placental transfer of PFAS cited in the section and comments (e.g., Appel et al., 2021) on PFOS above should also be cited here for PFOA, and the range of reported maternal:cord blood ratios for PFOA should be mentioned.

p. 20, line 20. As for PFOS above, it should be stated that postnatal transfer of PFOA through breast milk "occurs" rather than that it "is possible." Additionally, the important facts that serum PFOA levels in breastfed infants increase several-fold between birth and 6 months, and that 6 month old breastfed infants have much higher serum PFOA levels than their mothers (Fromme et al., 2010; Goeden et al., 2019) should be emphasized.

p. 20, lines 40-45, starting with: "Gender difference in…" and ending with "…for 7 or 17 days." This information on sex differences in PFOA distribution in rats versus mice provided here is not informative to the reader because it provides details without explanation or synthesis. It should be explained that the sex differences observed in rats, but not in mice, that are mentioned here are due to the fact that PFOA is very rapidly excreted in female rats (half-life of a few hours) while it has a long half-life in male rats, and male and female mice. This information is highly important in the interpretation of toxicology studies of PFOA in rodents.

p. 21, lines 7-9, beginning with "In neonatal pups..." and ending with "…USEPA, 2021b." More comprehensive information on lactational exposures to PFOA in rodents should be added. See DWQI (2017a, p. 50-51); Fenton et al. 2009. Reprod. Toxicol .27, 365–732; White et al. 2009. Reprod. Toxicol. 27, 289–298.

p. 21, lines 27-30. The information on the study group in Olsen et al. (2007) is not accurate. This study included 23 workers retired from the 3M facility in Decatur, AL, and 3 workers retired from the 3M facility in Cottage Grove, MN.

p. 21, lines 40-42. The sentence on the "contribution of urinary excretion of PFOS to half-life in humans" is unclear. The basis for the statement that renal clearance is impacted by isomeric composition and gender/age is not provided. Also, does "contribution of urinary excretion to half-life" mean relative extent of urinary versus fecal excretion?

p. 21, footnote 5. This information is confusing as written and needs clarification. Also, citation(s) are needed. Suggest revising to say that precursor PFAS that are not fully fluorinated can be metabolized to terminal perfluoroalkyl acids such as PFOA and PFOS and that the metabolites of these precursor PFAS may also include reactive intermediates.

p. 22, line 14-15. Sentence on enterohepatic circulation beginning with "It has been suggested that…" This paragraph should be reorganized so that the information in this sentence is combined with the sentence on enterohepatic circulation starting with "Biliary and fecal…" at the end of line 3.

p. 22, lines 15-17. This sentence about the contribution of blood loss to the difference in half-lives in males and females is not meaningful because the human half-lives in males versus females has not been discussed. Also, "females" should be changed to "females of childbearing age."

p. 22, line 31. Olsen et al. (2007) is the same study mentioned on p. 21, lines 27-30. As stated above, the study group was retired workers from industrial facilities in Decatur, AL and Cottage Grove, MN, not "Ohio, USA" as stated here.

p. 22, lines 37-40. As written, these two sentences about human half-life incorrectly imply that half-life data with background adjustment are not available for the Li et al. study group in which a longer half-life was reported than in the Xu et al. study group. The information for Li et al. (2018) should be replaced with information from Li et al. (2022, https://doi.org/10.1016/j.envint.2022.107198) which provides refined and updated half-live data for the same study group as Li et al. (2018). It is important to mention that Li et al. (2018, 2022)

and Xu et al. (2020) are studies of two different populations that were performed by the same research group, and that Li et al. (2022) adjusted for background PFOA exposure in the same manner as Xu et al. (2020), a much smaller and shorter study than Li et al. (2022). Specifically, Li et al. (2022) studied the decline in serum PFOA in 114 subjects for 4 years, while Xu et al. (2020) followed serum PFOA in 26 subjects for 5 months. The mean and median PFOA half-lives and confidence intervals reported by Li et al. (2022) were 2.47 years (2.27, 2.7) and 2.69 years (1.37, 5.4) respectively.

p. 22, line 41. Lin et al. (2016) is not listed in the Reference list, and it was not located with online searching.

p. 22, lines 42-46. This sentence states that it has been proposed that some half-life estimates reported in the literature are overestimates due to unmeasured PFOA exposures from environmental media, but that this issue is the subject of further debate. If this is to be mentioned at all, it must be emphasized that the potential effect of such "unmeasured PFOA exposures" on their half-life estimates was addressed by the authors of three of the studies cited above (Olsen et al., 2007; Li et al., 2022; Xu et al., 2020). Furthermore, Bartell et al. (2012, https://www.nature.com/articles/jes20122) evaluated the potential effects of background exposure on the half-life estimates for three of the studies cited above (Olsen et al., 2007; Brede et al., 2010; Bartell et al., 2010). All of these evaluations support a human half-life for PFOA of greater than two years.

p. 22, lines 46-48. Suggest removing this sentence. This hypothesis does not appear to be plausible because PFOA is found in blood serum and plasma, which do not contain blood cells including plasma membranes. Little if any PFOA is found in the blood cell fraction of blood.

p. 23, lines 10-11. The part of the sentence beginning with "…it was shown that major branched isomers…" is copied verbatim from the abstract of Zhang et al. (2013). However, in the abstract, this statement applies to both PFOA and PFOS. The fact that phrase this is taken directly from the abstract explains why it says "linear isomers" instead of "linear isomer" here, since there is only one linear isomer of PFOA while PFOS has multiple isomers. If this is included, it should be mentioned that the data from Zhang et al. (2013) indicate that the half-life of the branched isomer of PFOA is only slightly shorter than for linear PFOA and that the branched isomer was only 3% of total PFOA.

p. 23, lines 21-24. Again, it needs to be clearly emphasized that the difference in male versus female half-lives in rats is much greater than for other species. This difference is important in interpreting toxicology studies of PFOA conducted in rats, particularly studies of developmental effects. As stated in this sentence, the half-life in female rats is 0.12 days (3 hours), and it should be added that this very short half-life means that PFOA does not bioaccumulate in female rats as it does in male rats and in both sexes of mice, monkeys, and humans. Because the half-life of PFOA in female rats is so short, an administered dose results in a much lower internal dose in female rats than in male rats, or in males and females of other species, and there are large fluctuations in internal dose (serum levels) in female rats with once per day dosing. For this reason, the female rat is not an ideal model for developmental effects of PFOA in humans.

Because PFOA bioaccumulates in both female mice and humans, mice are a more suitable model.

p. 23, line 35. Section on PBPK modeling. Additional information needs to be added to this section to indicate that the models discussed, including models that account for gestational and lactational exposure, are used to relate the external doses (ng/kg/day) to the internal doses (e.g., serum/plasma concentration, ng/ml) from human epidemiology studies as well as for interspecies extrapolation from laboratory animals. This approach was used to develop the toxicity factors for PFOA and PFOS from human data by USEPA (2021a,b), EFSA (2020), and California EPA (2021).

p. 24, lines 9-12. It should be mentioned that the serum concentrations are converted to a human equivalent dose with a clearance factor, as follows: Serum Concentration (mg/L) x Clearance Factor (L/kg/day) = Human Equivalent Dose (mg/kg/day).

Importantly, here or elsewhere, it should be mentioned that another approach for interspecies extrapolation is to apply a clearance factor to relate measured (rather than modeled) PFOA or PFOS serum levels from animal studies to the administered doses that would result in the same serum level in humans (i.e., the Human Equivalent Dose). This approach has been used by several authoritative agencies in development of toxicity factors for PFOA and PFOS from animal toxicology data, as discussed in Post (2021).

p. 24, line 13. If EFSA (2020) is discussed here, it should be mentioned that the EFSA (2020) Tolerable Daily Intake is based on the maternal PFAS dose resulting in a serum PFAS level associated with decreased antibody response to vaccination in one year old offspring who have been breastfed. It should also be mentioned that a model that considers the impact of maternal exposure on prenatal (transplacental) and postnatal (breast milk) exposure to the infants on serum PFAS levels at age one year was used. Simply citing Loccisano et al. (2011) without mentioning what their model encompasses is not informative to the reader.

p. 24, lines 20-23. Most of the more recent publications cited here are not about PBPK modeling, and it is unclear why they are mentioned here. If recent half-life studies are to be mentioned, Li et al. (2022) (discussed in comments above on p. 22, lines 37-40), must be included. In contrast to what is stated here, Elcombe et al. (2013), Dourson et al. (2019), and Dourson and Gadagbui (2021) are not PBPK modeling studies, and it is suggested that these citations be removed.

p. 24, lines 38-40. The data from Elcombe et al. (2013) are not relevant to environmental (e.g., drinking water) exposure levels and therefore are not relevant here. Aside from issues related to the very poor health of the subjects (advanced cancer patients), renal reabsorption was saturated at the very high doses used such that the Cmax:administered dose ratio would be much lower than at environmentally relevant doses. Although these issues are alluded to in the sentence starting with “However, ….” on line 45, it is strongly recommended that this discussion of Elcombe et al. (2013) be removed as it is not relevant, especially since the document is not intended to be a comprehensive review and is stated to cite only a limited number of the most important studies.

p. 25, two paragraphs on lines 4-26. This discussion does not include the most important general points about the prenatal and lactational exposures that were modeled by Verner et al. (2016) and Goeden et al. (2019). It is critical that the following information be added: Exposures to PFOA and PFOS (ng/kg/day) and serum PFOA/PFOS levels (ng/ml) are much higher in the breastfed infant than in their mother. For example, Goeden et al. (2019) predicts peak serum PFOA levels 6 times higher in the breastfed infant than in their mother. These higher exposure and internal doses are of particular concern because infants are a sensitive subpopulation for developmental effects of PFOA and PFOS.

p. 25, lines 22-26. Starting with sentence beginning with: "The study authors…" As above, this is not the most relevant information. The fact that peak serum PFOA levels are 6-times higher in the infant than the mother is what is most relevant. If this information is to be included, it should added that this model was similarly used by the Minnesota Department of Health to develop a drinking water guideline for PFOS and by several other U.S. states' agencies to develop drinking water guidelines for PFOA and PFOS. See Post (2021).

p. 25, line 30. Suggest explaining what ToxCast is since this sentence will not be meaningful to an uninformed reader without an explanation.

p. 25, line 42-44. Sentence about human biomonitoring of PFAS other than PFOA and PFOS starting with: "Although…" This sentence implies that human biomonitoring of PFAS other than PFOA and PFOS is a recent development. However, other PFAS have been routinely included in human biomonitoring studies for many years. For example, NHANES has included PFNA, PFHxS, and other PFAS since it began biomonitoring for PFAS in 1999.

p. 25, line 47-49. Sentence starting with: "However…" suggested adding "used as drinking water sources" to the end of the sentence for clarification.

<u>Section 4. Effects on humans</u>

p. 26, lines 33-34, states "The test article was described as non-toxic at all dose levels tested."

It is not accurate that the test article was non-toxic at all doses tested. As mentioned by the USEPA SAB (2022) report:

> "An earlier abstract about this study ["this study" refers to Elcombe et al., 2013, also reported by Convertino et al., 2018] (Macpherson et al., 2010) stated that one of the patients dosed experienced drug related toxicity (DLT) consisting of 'grade 5 renal failure and transaminitis' (indicative of liver damage), and that these effects were noted as 'possibly drug related.' This indicates the potential for PFOA to cause renal and hepatic toxicity in humans, and it is unclear why the observation of 'possibly drug related' kidney and liver toxicity reported by Macpherson et al. (2010) was not mentioned by Convertino et al. (2018)."

p. 26, line 38. Section 4.2 on long term exposure. As a general comment, this section reviews many human toxicity endpoints with varying levels of evidence for association with PFOA and PFOS. To assist the reader, it is suggested that it be mentioned here that the subset of human

toxicity endpoints with the strongest evidence are summarized later in the document, in Section 7.3.

p. 26, sentence beginning on line 40. Liver function and serum lipid levels are key health effects that have been evaluated in many studies and should be included in the list of health effects that have been evaluated following long-term exposure.

p. 26, line 45. It is unclear why occupational studies are specifically mentioned for thyroid hormone levels since there are numerous studies of PFOS and PFOA and thyroid hormone levels from the general population. For example, USEPA (2021a,b) identified 32 recent human studies of PFOA and thyroid hormones and 34 for PFOS, none of which were occupational.

p. 26, line 48 – p. 27, line 1, states: "The analysis in these studies is typically based on correlating blood levels of PFOS and PFOA to the outcome of interest, and identifying odds ratios based on tertile or quartile of blood PFAS concentration."

This statement is not accurate because the vast majority of the human studies of health effects of PFOA and/or PFOS do not calculate the odds ratio for having a disease, health condition, or clinically defined abnormal level of a biomarker or other parameter. The endpoints evaluated in these studies are typically continuous (e.g., level of antibodies, cholesterol, liver enzymes; weight at birth) not dichotomous (e.g., clinically defined low birth weight, cancer, etc.). Also, PFAS are typically measured in serum, not "blood."

p. 27, line 12, section on Fecundity; this comment also applies to similar sections on other human health endpoints. While recognizing that this document is not intended to provide a comprehensive review of all available studies, conclusions from epidemiological data should be based on overall weight of evidence. As such, it is not helpful or informative to summarize a small subset of the available studies of a certain health endpoint without providing an overall conclusion on the weight of evidence for an association of that endpoint with PFOA and PFOS. For example, about half of the studies summarized in this section on fecundity appear to be positive and half appear to be negative. The reader would have no way of knowing whether these mixed results are representative of the entire body of evidence for this effect. Alternatively, there could be many additional studies that are positive and none that are negative, or vice versa. One potential way to address this issue is to cite the weight of evidence conclusions of other authoritative bodies regarding association of PFOA and PFOS with the endpoint. This was the approach taken in discussing human epidemiological evidence in the ITRC PFAS document, and it is recommended that this part of the ITRC document be reviewed by WHO (see Section 17.2.4 at https://pfas-1.itrcweb.org/17-additional-information/#17_2).

p. 27, line 14-19. Although it is implied, it should be stated for clarity that serum PFAS levels in the women (not the men) were evaluated in this study. This could be unclear since the discussion begins by saying that 501 couples were evaluated.

p. 28, lines 7-9. This sentence is paraphrased from the conclusions of Fei et al. (2009) ("…we studied TTP of pregnancies which led to the birth of a child, limiting conclusions that can be drawn regarding women who were unable to get pregnant"), but the intended meaning is not

clear unless the words "time to" are added, as shown in bold here: "However, this study only evaluated **time to** pregnancies that led to the birth of a child; therefore, the odds ratios stated above are not reflective of women unable to get pregnant."

p. 28, lines 20-23. EFSA (2018) is a draft that has been superseded by EFSA (2020), and EFSA (2020) should be cited. Additionally, the USEPA (2021a,b) conclusions that there is suggestive evidence for association of PFOA and PFOS and pregnancy induced hypertension and preeclampsia should be cited.

p. 30, line 26-29. This following sentence [bolding added] appears to be paraphrased from the conclusions section of the paper: "Bach et al. (2016) also noted that many of the studies on PFOS and PFOA exposure and male reproduction were cross-sectional, and **given the relatively long half-lives of PFOS and PFOA, it is uncertain whether the outcomes evaluated in cross-sectional studies and the measured blood plasma levels are causally related"**.

However, the associated sentence in the publication itself [bolding added] actually says the opposite of what is stated in the draft document, as follows: "Most of the studies on male reproduction were cross- sectional and thus assessed exposures simultaneously with the outcomes. However, because of the long half-lives of PFAS exposures**, samples are assumed to be representative for the time period where PFASs might have causally affected the semen production (several months back in time) or reproductive hormone homeostasis**."

In summary, the draft WHO document states that the authors said that the long half-lives of PFOA and PFOS <u>increase uncertainty about whether exposure and outcome are causally related</u> in this cross-sectional study. In reality, the authors actually said that the long half-lives of PFOA and PFOS mean that serum levels measured at the time of the study represent serum PFAS levels several months earlier (i.e., at the exposure timepoint of interest for the endpoints being evaluated) and <u>decrease uncertainty about a causal relationship</u>.

p. 30, line 4.2.2.1. Again, similar to the comment on the Fecundity section above, the conclusions of reviews such as EFSA (2020) and USEPA (2021a,b) should be cited first, rather than first citing a few of the many specific studies that are available.

p. 32, lines 18-21. The issue of the impact of plasma volume expansion and glomerular filtration rate (GFR) on the associations of PFOA/PFOS and birth weight/fetal growth should be discussed further since it is an important consideration for this endpoint. It should be mentioned that it has been concluded that some, but not all, of the decreased birthweight associated with PFOA/PFOS may be due to reverse causality related to GFR and that studies that measure serum PFAS levels earlier in pregnancy are less susceptible to this type of reverse causality.

As stated by USEPA SAB (2022):

> "… the possibility that physiological factors may potentially jointly affect PFAS and clinical outcome measures should be considered. Birthweight studies are clearly susceptible since it is well-established that greater plasma volume expansion is associated with greater birthweight (Salas et al., 2006) and likely also associated with lower (diluted) PFAS levels, which would create a spuriously elevated estimate of the

> quantitative impact of PFAS on birthweight. A recent examination of the literature (Steenland et al., 2018) provided indirect support for this hypothesized bias, with studies that measured PFAS exposure later in pregnancy when this would have the greatest effect showing the strongest association with reduced birthweight. Additionally, Verner et al. (2015) concluded that some, but not all, of the decreased in birthweight associated with maternal PFAS is accounted for by differences in maternal glomerular filtration rate and that this effect may be greater in studies based on blood serum PFAS measured later in pregnancy."

p. 32, lines 20-21. While ATSDR (2021) may have stated that "no studies found increases in the risk of low-birth-weight infants" associated with maternal PFOS serum levels (presumably based on their literature review which they stated to have been through 2018), it is important to note that the more recent USEPA (2021a) review identified 9 studies of PFOS exposure and different dichotomous fetal growth restriction endpoints, such as small for gestational age (or related intrauterine growth retardation endpoints), low birth weight, or both, and stated that "Collectively, the majority of small for gestational age and low birth weight studies were supportive of an increased risk with increasing PFOS exposures." USEPA (2021b) also stated that "Overall, seven of the ten different studies examining either small for gestational age or low birth weight or both showed some increased risks with increasing PFOA exposures."

p. 33, lines 17-20, states: "…EFSA (2020) selected immunotoxicity as the critical effect in their derivation of a tolerable daily intake for the major PFAS, further concluding that tolerable exposure limits should be based on preventing deficits of the humoral immune system in humans." It should be added that USEPA (2021a, b) also selected the same critical effect for PFOA and PFOS Reference Dose development.

p. 34, lines 38-41. "However, studies report inconsistencies in the relationship between PFAS exposure and infection propensity in early life (Antoniou et al., 2022; ATSDR, 2021; EFSA, 2020; Steenland et al., 2020; US EPA, 2021a; 2021b) and therefore, the clinical relevance of these findings [decreased antibody response to vaccination] is unclear."

Although the citations are placed after the first part of the sentence, the sentence may be interpreted to mean that the citations also apply to the conclusions in the second part of the sentence beginning with "…and therefore, …" It is important to make it clear that (as discussed below) most of the sources cited in the sentence did not question the clinical relevance of the decreased antibody response to vaccinations that is linked to PFOA and PFOS.

NJDEP emphasizes that it concurs with other authoritative bodies who disagree with the conclusion that decreased response to vaccination is not a clinically relevant effect. EFSA is one of the authoritative bodies relied upon by WHO in developing its draft. EFSA (2020) selected decreased response to vaccination as the critical endpoint for its Tolerable Weekly Dose for PFAS. EFSA (2020) reviewed the relevant human and animal studies and concluded that PFOS reduced resistance to infection in laboratory animals and that:

> "Based on the results from the [human] vaccination studies reviewed above, findings on increased propensity of infections would seem plausible. … Overall, there is some

> evidence to suggest that exposures to PFASs are associated with increased propensity of infections but more studies with objective measures of infections (not self-reports) are needed."

Like EFSA (2020), USEPA (another authoritative body relied upon by WHO) identified decreased immune response to vaccination as the critical effect for its draft Reference Doses for both PFOA and PFOS (USEPA, 2021a,b), and it did not question the clinical relevance of this effect in these evaluations. Similarly, ATSDR (2021), also relied upon by WHO, and Steenland et al. (2020), cited by WHO, do not question the clinical relevance of decreased vaccine response.

Furthermore, USEPA SAB (2022) supported the use of decreased antibody response as a critical non-cancer effect for PFOA and PFOS and stated that it is adverse, as follows:

> "Overall, the Panel agreed with the selection of … the critical effect, suppression of a vaccine response in children exposed during development, as appropriate for the derivation of chronic RfDs [Reference Doses] for PFOA and PFOS. …. Reduction in antibodies to a vaccine represents the failure of the immune system to respond to a specific challenge and is considered an adverse immunological outcome. The vaccine response is a functional response of the immune system to a specific challenge, in this case, an antigen (i.e., a non-self substance that stimulates a response from the immune system) delivered in the form of a vaccine. When a vaccine is delivered, cells of the immune system coordinate a response where the antigen is recognized, and cells of the adaptive immune system are ultimately stimulated to generate antibodies to the antigen. When the vaccine response is suppressed, it indicates that some part of the immune system is not performing at the level that it should. This form of immunosuppression could indicate impacts on one or more parts of the innate and/or adaptive immune systems."

WHO must also consider that the lack of an association of PFOA and PFOS with infectious disease has not been established. USEPA SAB (2022) noted that, although the evidence is not consistent, many studies reviewed by USEPA (2021a,b) and several newer studies (Timmermann et al., 2020, https://doi.org/10.1289/EHP6517; Dalsager et al., 2021, https://doi.org/10.1016/j.envint.2021.106395; Bulka et al., 2021, https://doi.org/10.1016/j.envpol.2021.116619) reported associations of PFOA and/or PFOS with infectious disease. USEPA SAB (2022) also noted that a recent review by Pachkowski et al. (2019) concludes that studies available through 2018 "provide evidence for an association between general population levels of PFOS exposure and infectious disease, a clinical meaningful measure of health risk."

p. 34, line 44. This section is about thyroid hormones, except for one sentence about sex hormones that could be moved to the reproductive effects section (see comment for p. 35, line 40, below), and the section does not discuss other hormones such as insulin. As such, it is suggested that this section be renamed "Thyroid hormones."

Also, as stated in a comment above, USEPA (2021a,b) reviewed a large number of general population (i.e., non-occupational) studies of thyroid hormones and PFOA and PFOS 0ncluding several studies of pregnant women and children. It is suggested this be mentioned instead of, or in addition to, USEPA (2016a,b).

p. 35, line 40-44. It is suggested that this text on sex hormones and reproductive endpoints be moved to the Reproductive Effects section.

p. 36, lines 4-7. USEPA (2021a,b) conclusions for endocrine outcomes are shown here. It is suggested that USEPA (2021a,b) conclusion also be included in sections on other endpoints.

p. 36, line 11. Suggest saying "serum lipids" rather than "cholesterol," because many studies included other serum lipids (e.g., triglycerides) not just cholesterol.

p. 36, lines 10-14. It is not appropriate or informative to provide a detailed discussion of the Veneto studies at the beginning of this section. The section should begin with a summary stating that there are many studies of these endpoints from many different locations, including studies of the general population, communities with drinking water exposure, and occupationally exposed workers. For examples of such summaries, see DWQI (2017a); USEPA (2016a,b): and USEPA (2021a,b).

p. 36, line 27. Blood pressure is mentioned here and elsewhere in Section 4.2.6, but it is not a metabolic outcome.

p. 37, lines 5-9, states: "Caution in the interpretation of the causal relationship between increased PFOS and PFOA exposure and increased cholesterol is discussed by the authors of the individual studies and by EFSA (2020), particularly as the findings in humans are contrary to those from animal studies where there is a PPARα-mediated decrease in serum lipids at high doses of PFOS and PFOA, implying that the mode of action in humans may be unrelated to peroxisomes."

NJDEP does not agree that differences in the effects of PFAS on serum lipids in high dose laboratory animal studies and human epidemiology studies with much lower exposures suggest that the increased cholesterol in humans is not caused by PFAS. Additionally, a PPAR-alpha-mediated mode of action does not mean that the mode of action is "related to peroxisomes." PPAR-alpha (peroxisome proliferator activated receptor-alpha) was originally so named because of the observation that activation of this receptor increases the number peroxisomes in rodent liver. However, PPAR-alpha is now known to be found in many tissues and to be regulate the expression of many genes with essential roles in cellular differentiation, development, and metabolism.

NJDEP concurs with the USEPA SAB (2022) report, which discusses several potential explanations for the fact that PFAS is linked to increased cholesterol in humans but caused decreased cholesterol in some rodent studies. USEPA SAB (2022) mentions that potential explanations noted by the USEPA (2021a,b) are the "difference in serum lipid composition between humans and commonly used rodent models" and that "food consumption may confound these results, as diet is a major source of lipids, yet studies do not consistently report a fasting period before serum collection." USEPA SAB (2022) also discusses additional potentially

important explanations for the human versus laboratory animal difference including "… much lower human exposure levels compared to the doses used in animal studies …, differences in the fat content of human diets versus rodent lab diet, and differences in the activity of PPAR-alpha in humans and laboratory animals" and mentions that "studies that investigated these issues include New Jersey Drinking Water Quality Institute (DWQI, 2017a), Tan et al. (2013), Rebholz et al. (2016), and newer studies such as Schlezinger et al. (2020)."

USEPA SAB (2022) further concludes that:

> "In general, human and rodent data suggest that the effects of PFOA and other PFAS on lipid formation and storage results from the balance of different effects which may act in opposite directions (Das et al., 2017). The decrease in serum lipids at the higher doses used in animal studies is believed to be due to activation of PPAR−alpha (DWQI, 2017a). PPAR−alpha is also active in humans, as demonstrated by the use of PPAR−alpha activating drugs to decrease high cholesterol in humans. However, PFOA, PFOS, and other PFAS do not activate PPAR−alpha in humans at lower environmentally relevant doses, and the increased serum lipids associated with PFOA, PFOS, and other PFAS may result from activation of other receptors and/or biological pathways involved with lipid metabolism that act in the opposite direction."

p. 37, lines 15-20, states: "Steenland et al. (2020) also suggested that the dose-response relationship between PFOS and PFOA exposure and elevated cholesterol may be non-linear, as the results from several cross-sectional studies indicate that the lower the range of PFOA exposure studied, the stronger the effect per unit exposure. Similarly, EFSA (2020) also noted that a maximum association with total cholesterol occurs at PFOA serum levels of 25 ng/mL and does not continue to increase as the serum level increases."

Regarding the steeper dose-response at lower serum PFOA and PFOS levels, it is important to consider that this lower range of serum PFAS levels is relevant to the serum levels that most commonly result from exposure to PFOS and PFOA in drinking water and that drinking water exposure is the focus of the draft WHO document.

p. 37, line 40 – p. 38, line 7. Section 4.2.7.1 on Liver. NJDEP disagrees with the statements and conclusions presented in this section. Specifically, NJDEP does not agree that the increases in ALT associated with PFOS and PFOA are not adverse because of their "low magnitude" and "lack of associated liver disease."

The draft document's discussion of the USEPA (2021a,b) conclusions about adversity of increased ALT and other hepatic effects is incomplete without also discussing the USEPA SAB (2022) conclusions on this topic. USEPA requested that its SAB provide an opinion on the validity of the USEPA (2021a,b) rationale for not considering increased ALT as a potential basis for PFOA and/or PFOS Reference Doses. USEPA also asked the SAB for additional studies that support ALT as a marker of adverse liver effects and whether other hepatic endpoints from human studies of PFOA and PFOS should be considered as the basis for Reference Dose development. It should be noted that USEPA has stated that it is currently revising USEPA

(2021a,b), which are draft documents, to reflect the recommendations of the USEPA SAB (2022) report that are discussed below.

USEPA SAB (2022) disagreed with USEPA's rationale for not considering increased ALT as a critical effect and provided detailed information to support the conclusion that the increased ALT associated with PFOA and PFOS is indicative of liver damage, an adverse health effect. USEPA SAB (2022) noted that: "A recent systematic review concluded that exposure to both PFOA and PFOS is associated with increased ALT in humans and that both compounds cause increased ALT and steatosis in rodents (Costello et al., 2022)," and that "California EPA (2021) selected increased risk of clinically elevated serum ALT as the basis for its draft RfD [Reference Dose] for PFOA."

USEPA SAB (2022) further noted that PFOA was associated with increased incidence of clinically defined elevated ALT (not just a numerical increase in ALT) in several studies and that it is well established that relatively small (<2-fold) increases in ALT are associated with pathology-confirmed liver disease such as non-alcoholic fatty liver disease. USEPA SAB (2022) also noted that the American Association for the Study of Liver Disease states that ALT may be a predictor for overall health and mortality, that the American College of Gastroenterology states that: "Multiple studies have demonstrated that the presence of an elevated ALT has been associated with increased liver-related mortality, as well as overall morbidity and mortality," and that multiple studies from the primary scientific literature report that ALT is associated with increased mortality. In summary, USEPA SAB (2022) stated that it "recommends the use of ALT as an endpoint" for PFOA and PFOS risk assessment. USEPA SAB (2022) further stated that available information suggests that patients with even slight elevations in ALT should be monitored for liver disease.

USEPA SAB (2022) also suggested that non-alcoholic fatty liver disease/steatosis be considered as an adverse liver endpoint for PFAS, noting that "a recent study of adults from a community with elevated exposure to PFOA from contaminated drinking water (Bassler et al., 2019) showed that PFOA 'was associated with cytokeratin 18 M30, a marker of hepatocyte apoptosis), and a mechanism of disease progression in non-alcoholic fatty liver disease.' "

p. 38, Section 4.2.8.1, Carcinogenicity of PFOS. Two additional recent studies that are relevant to this topic should be cited.

Goodrich et al. (2022) conducted a nested case-control study of serum PFAS and non-viral hepatocellular carcinoma (HCC). The study included 50 cases and 50 controls individually matched by age, sex, race/ethnicity, and study area from the Multiethnic Cohort, a prospective cohort of >200,000 California and Hawaii residents from a variety of racial/ethnic groups. PFAS and the metabolome were analyzed in plasma samples taken prior to cancer diagnosis. Geometric mean plasma concentrations of PFOA, PFOS, PFNA, PFHxS, and PFDA were similar in cases and controls. However, serum PFOS levels at or above the 90th percentile in NHANES (>55 ng/L) were associated with a statistically significant 4.5-fold increased risk of HCC.

Cao et al. (2022) reported an association of serum PFOS with increased risk of liver cancer (type not specified) in a Chinese study population (203 cancer patients, 203 controls). Serum PFAS

was measured after cancer diagnosis. Serum levels of PFOS and 6:2 chloro-polyfluoroalkyl ether sulfonic acid, a replacement for PFOS widely used in China, were significantly associated with increased risk of liver cancer after adjustment for various covariates, while associations with other PFAS including PFOA were not statistically significant.

p. 39, lines 41-43, states: "Evidence of carcinogenic effects of PFOA in epidemiology studies is derived primarily from studies that focused on a population who worked at a DuPont plant in West Virginia where PFOA was used from 1952 in the production of fluoropolymers."

This sentence is not accurate and needs to be revised. First, the epidemiology studies referred to here and discussed later in the paragraph (Barry et al., 2013; Vieira et al, 2013) are not studies of a population who worked at a DuPont plant. As stated later in the paragraph, they are studies of communities who had consumed drinking water contaminated by PFOA from the plant. Second, it is not accurate to say that evidence of the carcinogenic effects of PFOA in humans comes "primarily" from the studies of this population. The Shearer et al. (2021) study of the general population, conducted by the US National Cancer Institute, provides equally important information on the carcinogenicity of PFOA in humans.

p. 40, line 20. It is unclear why the EFSA (2018) draft is cited instead of the final EFSA (2020).

Section 5. Effects on Animals and In Vitro Test Systems

p. 40, line 40. As also mentioned in comments on the toxicokinetics section above, a general point that should be emphasized when interpreting toxicology studies of PFOA in rats (both short-term and chronic) is that PFOA is excreted much more quickly in female rats (half-life of 2-4 hours) than in male rats (half-life of 4-6 days). For this reason, the internal dose is much lower in females than males from the same administered dose. In contrast, PFOA has a long half-life in both sexes in humans. As stated by Butenhoff et al. (2012a), "This difference in pharmacokinetics likely accounts for the reduced responsiveness of [PFOA]-treated female rats as compared to males."

p. 40, lines 42-45. It is suggested that DWQI (2017a) and DWQI (2018) be added to the list of authoritative reviews of animal toxicology and *in vitro* studies. They provide comprehensive review and synthesis of information on these topics.

p. 41, lines 18-19. For clarity, suggest mentioning that other short-term ($\leq$90 day) studies of immunological, neurotoxic, and developmental effects are described separately in sections 5.4, 5.5, and 5.6 of the draft WHO document.

p. 41, lines 19-21. Sentence beginning with: "These studies…" Studies in mice should be mentioned, in addition to the rat studies. For example, DWQI (2018, p. 58) provides information on short-term mouse studies.

p. 41, lines 22-25 states: "Application of the criteria developed by Hall et al. (2012) to evaluate the human relevance of the liver changes [caused by PFOS in animal studies] suggested that several hepatic findings (including increases in liver weight, hepatocellular hypertrophy and

alterations in serum lipid levels in the absence of other degenerative lesions) were not relevant for human risk (ATSDR, 2021)."

NJDEP disagrees with the conclusion that application of the Hall et al. (2012) criteria indicates that hepatic effects of PFOA and PFOS in laboratory animals are not relevant to human risk; detailed comments are provided below. First, although NJDEP strongly disagrees with this conclusion and recommends that this sentence be removed, it is noted that the WHO document fails to mention that the Hall et al. (2012) criteria apply only to hepatic effects in rodents, not hepatic effects in laboratory animal species in general. It is also unclear why this statement is included in the section on short-term effects of PFOS, particularly in light of the duration of exposure considerations for the Hall et al. (2012) criteria discussed below. Furthermore, data from several of the studies of PFOS discussed later in this section of the WHO document contradict the information in this sentence.

The relevant text from ATSDR (2021) referred to in the WHO draft appears to be:

> "In laboratory animals, oral exposure to PFOS results in increases in liver weight, hepatocellular hypertrophy, and decreases in serum lipid levels. A small number of studies also reported focal necrosis and centrilobular hepatocytic vacuolization. The proposed mechanism of action for the increased liver weight, hepatocellular hypertrophy, and decreased serum lipid levels involves PPARα receptor activation. Due to species differences for this mechanism, these effects observed in rodents are not considered relevant to humans. The applicability of the hepatic hypertrophy and serum lipid alterations observed in rodent studies to humans has been questioned due to species differences in the presumed mechanism of action for these effects in rodents."

However, although ATSDR (2021) assumed that hepatic effects of PFOS occur through PPAR-alpha activation, this conclusion is not supported by the relevant toxicological data, as shown by the evaluation presented in DWQI (2018):

> "In contrast to the characteristic linkage between PPARα activation and liver weight increase seen with PPARα agonists such as bezafibrate and the WY compound, PFOS causes liver weight increases in PPARα-null mice (Qazi et al., 2009b; Rosen et al., 2010). In addition, Rosen et al. (2010) dosed WT and PPARα-null mice with WY or PFOS for 7 days. Both WT and PPARα-null mice exposed to PFOS showed hepatomegaly and increased incidence of hepatic vacuole formation. Profiling of gene expression was conducted with microarray analysis. Gross qualitative and quantitative differences in gene expression for fatty acid metabolism, inflammatory response, xenobiotic metabolism and ribosome biogenesis, as well as markers of PPARα activation, were found between WY and PFOS treated WT mice. These observations provide evidence that prototypical PPARα agonists (e.g., the WY compound) are not appropriate surrogates to predict the molecular and apical hepatic effects following PFOS exposure.
>
> Additionally, hepatic effects, including tumors, have been observed in rodents exposed to PFOS without evidence of peroxisome proliferating activity. For example, Butenhoff et al. (2012b) reported that chronic dietary exposure to 20 ppm PFOS resulted in liver

tumors as well as hepatocellular hypertrophy and necrosis in male and female rats. However, an increase in hepatic peroxisomal bodies was not observed based on transmission electron microscopy.

Further, increased palmitoyl CoA oxidase activity, a generally accepted marker of peroxisome proliferation induction and overall PPARα activation (Klaunig et al., 2003), has not been observed when hepatic effects were reported in PFOS-exposed rats. As part of the 2-year bioassay reported in Butenhoff et al. (2012b), Seacat et al. (2003) reported on interim sacrifices following 4 and 14 weeks of dietary exposure. When assessing the 20 ppm group, the dose that caused liver tumors in Butenhoff et al. (2012b), liver effects were limited to an increase in relative liver weight in male rats after 4 weeks of exposure. However, no significant increase in hepatic palmitoyl CoA oxidase activity was observed. Following 14 weeks of exposure, liver effects in the 20 ppm group included hepatocellular hypertrophy and vacuolation in males and females as well as increased relative liver weight in males with no observed significant increase in hepatic palmitoyl CoA oxidase activity.

Studies with shorter durations of exposure in rats by Elcombe et al. (2012a, 2012b) provide similar hepatic observations as those following chronic and subchronic PFOS exposures in rats as reported in Seacat et al. (2003) and Butenhoff et al. (2012b). Following cessation (i.e., on recovery day 1) of 7 days of dietary PFOS exposure at 20 ppm, increases in relative liver weight and hepatocellular hypertrophy along with changes in alanine aminotransferase, aspartate aminotransferase, and cholesterol were observed (Elcombe et al., 2012b). However, no increase was observed for hepatic palmitoyl CoA oxidase activity. Following 28 days of exposure to 20ppm PFOS, Elcombe et al (2012a) observed increased relative liver weight and hepatocellular hypertrophy along with a decrease in cholesterol. These hepatic observations were accompanied with only a marginal (i.e., 1.4-fold) increase in hepatic palmitoyl CoA oxidase activity.

To the extent that there is a relatively small amount of interaction with PFOS, PPARα may make a minor contribution to PFOS liver effects. This is in contrast to PPARα activators/peroxisome proliferators such as WY and the fibrates, for which liver effects, including carcinogenicity are clearly linked to PPARα activation.

In summary, PFOS effects on the rodent liver do not appear to primarily operate through a PPAR-dependent mode of action, including at doses resulting in liver tumors as in Butenhoff et al. (2012b). Thus, the lower abundance of PPARα and lower response to model PPARα activators in human liver as compared to rodent liver is not clearly relevant to the potential for PFOS to cause human hepatic effects including cancer."

An important general point that was not recognized by ATSDR (2021) about application of the criteria from Hall et al. (2012) is that increased liver weight and/or hepatocellular hypertrophy are adverse when they co-occur with or progress to other types of hepatic toxicity. Hall et al. (2012) emphasize that the *expected* duration of exposure must be considered in determining the

adversity of hepatic effects such as increased liver weight and hepatocellular hypertrophy. Specifically, Hall et al. (2012) state that:

> "[Increased liver weight and hepatocellular hypertrophy] may be reversible if the anticipated duration of exposure is short, while progression to more severe hepatic effects may occur from longer exposures to the same dose. However, prolonged exposure to a xenobiotic at levels that have previously been shown to be adaptive may eventually result in liver cell injury due to a failure of adaptive mechanisms. In this case, the combination of dose level and duration of exposure to the xenobiotic under the terms and conditions of the new experiment would now be considered adverse."

Therefore, the Hall et al. (2012) criteria cited by ATSDR (2021) are not relevant to the development of guidelines based on chronic exposure, including WHO drinking water guidelines. The primary focus of Hall et al. (2012) is hepatic effects observed in pre-clinical toxicity studies for drug development. In this scenario, drugs are normally administered for a limited time period (i.e., exposure is not chronic). Hepatic effects that result from exposure to the drug may be "adaptive," particularly if they are reversible when exposure ends. In contrast, drinking water guidelines such as those developed by WHO are intended to protect for lifetime exposure, and they must consider the potential for progression of initial effects to more severe effects. As such, reversibility after exposure ends (e.g., in "recovery" animals in studies that include a "recovery" component) is not a reason to discount the adversity of increased liver weight and hepatocellular hypertrophy in development of chronic drinking water guidelines. These lesions may persist and/or progress with the longer exposures that are relevant to lifetime exposure to a contaminant in drinking water.

For PFOS, data from chronic, subchronic, and shorter duration studies indicate that hepatocellular hypertrophy caused by PFOS in rodents progresses to more severe liver toxicity, including tumors. As indicated above, Hall et al. (2012) do not recommend dismissing the adversity of effects such as hepatocellular hypertrophy when they are known to progress. (Additionally, as discussed above, the same studies indicate that hepatic toxicity of PFOS in rodents is not dependent on PPAR-alpha.) At the same dose that caused liver tumors after 2 years of exposure, increased relative liver weight was the only hepatic effect observed at interim sacrifice after 4 weeks of exposure, while hepatocellular hypertrophy and vacuolation in males and females and hepatic effects and increased relative liver weight in males occurred at this same dose at interim sacrifice at 14 weeks (Seacat et al., 2003, https://doi.org/10.1016/S0300-483X(02)00511-5). Results were similar in other rat studies with shorter durations of PFOS exposure (Elcombe et al., 2012, https://doi.org/10.1016/j.tox.2011.12.014; 2012b, https://doi.org/10.1016/j.tox.2011.12.015).

p. 42, line 38, states: "No subchronic repeated dose studies in mice with PFOS exposure durations of 90 or more days were located." The intent of this statement is unclear since studies of 90 or more days are, by definition, not subchronic.

p. 42, lines 39-41 states: "In several shorter-duration studies (10 – 30 days) [of PFOS] in mice, similar hepatic effects were observed but generally at higher PFOS doses compared to rats (approximately 2 – 5 mg/kg bw per day in mice)." However, the rest of this paragraph does not support this statement. Specifically, there was a significant effect at the lowest dose (2.5 mg/kg/day) with no NOAEL identified in the Xing et al. (2016) study that is provided as an example. Therefore, is not known if the effects would also have occurred in mice exposed to the lower doses used in the rat studies.

p. 43, lines 12-15. These sentences refer to the hepatic and lipid metabolism effects of PFOS as "primary" effects, and additional effects, including immunological effects and others, as "other" effects. This wording is misleading and should be revised. While liver and lipid metabolism effects may have been the primary effects of short-term exposure among the endpoints evaluated in the studies reviewed in this section, studies reviewed in other sections of the draft WHO document demonstrate that immune system toxicity is a primary effect of PFOS and that it is the most sensitive effect reported in animal studies. Additionally, developmental effects are also sensitive endpoints of PFOS toxicity, and they should be included in the effects listed here.

p. 43, lines 20-23. This sentence should be revised to say that the liver is one of the main sites of systemic toxicity of PFOA (not that it is the main target organ overall). Developmental and immunological effects are also sensitive endpoints for PFOA. Additionally, "…potentially occurring through PPARα-mediated peroxisome proliferation, enhanced lipid peroxidation, or other mechanisms" should be revised to "…potentially occurring through activation of PPAR-alpha and other receptors, enhanced lipid peroxidation, or other mechanisms." As discussed above, hepatic and other effects mediated by PPAR-alpha activation are not appropriately described as occurring through "peroxisome proliferation." Additionally, as discussed in detail below, PPAR-alpha independent effects are equally or more important contributors to the hepatic effects of PFOA.

p. 43, lines 23-26. It should be mentioned that hepatic necrosis occurred in rats as well as mice. See USEPA (2021b; Table 7).

p. 45, lines 26-29, states: "Application of the criteria developed by Hall et al. (2012) to evaluate the human relevance of the liver changes suggested that doses associated with liver hepatocyte hypertrophy and cytoplasmic vacuolation in the absence of any necrotic changes were not relevant for human risk (ATSDR, 2021)."

NJDEP has several comments on this sentence. First, it is unclear why this sentence is included in the section on short-term effects. Also, as mentioned above, the Hall et al. (2012) criteria only apply to hepatic effects in rodent studies, not hepatic effects in non-rodent animal species. Additionally, Hall et al. (2012) does not say that vacuolation is not adverse or not relevant to human risk assessment. Rather, vacuolation is included in the Hall et al. (2012) list of irreversible effects as follows: "… irreversible toxicity, such as fibrosis, necrosis, vacuolization, fatty degeneration, and even neoplasia."

Most importantly, as discussed in detail in the comments on p. 41, lines 22-25, above, the Hall et al. (2012) criteria cited by ATSDR (2021) are relevant to short-term exposures where effects may be reversible when exposure ends, but they are not relevant to the development of guidelines intended to protect for chronic exposure including WHO drinking water guidelines. For PFOA, it is well established that hepatic effects increase in severity with continuing exposure. DWQI (2017a) reviewed this issue in detail and concluded: "Although studies of PFOA that report increased liver weight do not always include evaluation of other hepatic endpoints, numerous studies of PFOA have demonstrated that increased liver weight co-occurs with and/or progresses to more severe hepatic effects including increased serum liver enzymes, hepatocellular necrosis, fatty liver, and/or hyperplastic nodules."

For example, histopathological data from a chronic rat study (Butenhoff et al., 2012a) suggest that hepatic changes caused by PFOA in rodents progress to more severe hepatic toxicity and neoplastic lesions. In this study, the incidence of focal hepatocellular necrosis and vacuolation was increased in male rats exposed to PFOA for one year, while the incidence of hepatic hyperplastic nodules, stated to result from a regenerative process, was increased in male rats after two years, but not after one year, of exposure. Butenhoff et al. (2012) stated that the observations at one year and two years in this study suggest a progression of lesions "from hepatocellular hypertrophy to fatty degeneration to necrosis followed by regenerative hyperplasia."

p. 46, line 16 – p. 47, line 12. Discussion of the NTP (2020) chronic rat study. The discussion of the two other chronic studies that follows the discussion of NTP (2020) includes information on tumor incidence. As such, tumor incidence from NTP (2020) should also be mentioned in this section. Also, the paragraph (p. 47, lines 2-12) on the 16-week interim evaluation should be placed before the discussion of the end-of-study evaluation.

p. 47, line 18. The Butenhoff et al. citation should be changed from 2012b to 2012a.

p. 47, lines 28-29. The authors of the paper discussed here (Butenhoff et al., 2012) did not state the increase in Leydig cell adenomas was a "potential" increase, and the word "potential" should be removed. There was a dose-related increase in these tumors (0, 4, and 14% in control, low dose and high dose groups, respectively) that was statistically significant in the high dose group.

p. 47, line 36. Regarding hepatic tumors in Biegel et al. (2011), "carcinomas" should be changed to "adenomas." Biegel et al. (2001) states: "Dietary administration of C8 [PFOA] produced a statistically significant increase in the incidence of hepatocellular adenomas (13% vs. 3% or 1% in the ad libitum or pair-fed control groups, respectively), but no carcinomas were observed in the C8 treated rats."

p. 49, lines 1-2. The Butenhoff et al. (2002) citation is incorrect. Butenhoff et al. (2002) is a 26 week study of cynomolgus monkeys, not a 90 day study of rhesus monkeys. Also, Butenhoff et al. (2002) is not discussed in the draft document, and it should be discussed in the PFOA general toxicology section.

p. 49, line 22. A general comment on Section 5.5 on reproductive and developmental effects is that there is inconsistency in the effects discussed under reproductive versus developmental effects throughout this section. Also, the PFOA and PFOS sections should be structured in the same way. For example, there is a section on neonatal mortality for PFOA, but there is no similar section for PFOS.

p. 50, lines 33-34, discussion of pup survival in the Lau et al. (2003) study of reproductive and developmental effects of PFOS. The important observation that pups with gestational exposure to PFOS were born alive and appeared to be healthy but died soon thereafter needs to be discussed. As stated by Lau et al. (2003): "All animals were born alive and initially appeared to be active. In the highest dosage groups (10 mg/kg for rat and 20 mg/kg for mouse), the neonates became pale, inactive, and moribund within 30-60 min, and all died soon afterward. In the 5 mg/kg (rat) and 15 mg/kg (mouse) dosage groups, the neonates also became moribund but survived for a longer period of time (8-12 h). Over 95% of these animals died within 24 h. Approximately 50% of offspring died at 3 mg/kg for rat and 10 mg/kg for mouse. Cross-fostering the PFOS-exposed rat neonates (5 mg/kg) to control nursing dams failed to improve survival."

p. 51, lines 8-10. The potential relevance of maturation of the lung to the neonatal mortality observed soon after birth following gestational exposure to PFOS (Lau et al., 2003; mentioned above) should be discussed.

p. 51, lines 11-18. The study described in this paragraph should be reviewed if it is to be discussed, rather than citing the discussion in HC (2018a) which is not a primary reference.

p. 53, lines 16-17. Instead of saying that "PFOS was the only member of the sulfonate family to exhibit this effect," the sentence should be clarified to state that the two other shorter chain perfluoroalkyl sulfonates tested (PFBS, PFHxS) in the study did not cause the effect.

p. 53, lines 24-25. As mentioned above, the monkey study, which is cited here (Thomford, 2001; Butenhoff et al., 2002) in the context of reproductive effects, should be discussed in the section on general toxicology studies.

p. 53, line 31 – p. 54, line 8. As mentioned above, when discussing reproductive and developmental studies in rats, it must be emphasized that female rats excrete PFOA very quickly (half-life of 2-4 hours) whereas PFOA bioaccumulates in females of other laboratory animal species (e.g., mice, monkeys) and humans. For this reason, female rats are not an ideal model for human developmental effects of PFOA.

p. 55, line 11. Section on effects of PFOA on mammary gland development. In general, this section does not provide a synthesis of the information from the various studies of this endpoint, and it does not mention that this is the most sensitive adverse effect of PFOA in laboratory animals. It is suggested that WHO review the comprehensive discussions of this topic in Post et al. (2012) and DWQI (2017a).

p. 55, line 23. In general, the effects of toxicants on mammary gland development are highly dependent on the lifestage at which exposures occur (see: Fenton, S.E. (2006). Endocrinology 147: 518-524). The draft document should mention that PFOA exposure in Yang et al. (2009) was peripubertal, while exposure in many of the other studies discussed below was prenatal and/or postnatal. Therefore, the results of Yang et al. (2009) cannot be compared to the results of the prenatal/postnatal exposure studies.

p. 55, lines 26-28. "…suggesting substantial delay (possibly up to 10 days) in gland differentiation on PND 20 and alterations in milk protein gene expression on PND 20 (White et al. 2007)." As written, it is unclear which effects occurred in the dam and which occurred in the offspring.

p. 55, line 30. Suggest clarifying by replacing the word "postnatal" with "lactational."

p. 56, lines 2-15. As for Yang et al. (2009) above, it should be clarified that exposure in Zhao et al. (2010) was peripubertal, not prenatal or lactational. Yang et al. (2009) and Zhao et al. (2010) should be discussed together, and WHO should consider the problematic issues with interpretation of results of these two studies, as well as Zhao et al. (2012), another peripubertal exposure study from the same research group. These issues are discussed by DWQI (2017a) as follows:

> "Three studies reported effects of peripubertal (during periods between 3 and 7 weeks of age) exposure to PFOA on mammary gland development in female mice…. Two studies used C57Bl/6 and Balb/C mice (Yang et al., 2009; Zhao et al, 2012). One of these studies (Zhao et al., 2012) and an additional study (Zhao et al., 2010) evaluated C57Bl/6 PPAR-alpha null mice. Data on serum PFOA levels from all three of these studies are presented in Zhao et al. (2012). Interpretation of the combined results of these three studies is problematic because each PFOA dose level was used in each strain in only one of the three studies, and any dose-response interpretations must be made based on combining data from different studies. Because conditions (e.g., animals, housing conditions, time) may vary during different studies, dose-response curves based on combining data from different studies are difficult to interpret and conclusions based on such dose-response curves are highly uncertain…
>
> In C57Bl/6 PPAR-alpha null mice, mammary gland development was not affected by 7.5 mg/kg/day PFOA (Zhao et al., 2012), and was reported to be stimulated at 5 mg/kg/day, although quantitative data are not shown by Zhao et al. (2010). As was the case for the studies of C57Bl/6 wild type mice and Balb/C mice discussed above, each dose was used in PPAR-alpha null mice in only one of the studies (Zhao et al., 2010; Zhao et al., 2012). The effects on mammary gland development in the PPAR-alpha null mice therefore were similar to those in wild type mice of the same strain at 5 mg/kg/day and differed at 7.5 mg/kg/day. As above, interpretation of these data is problematic because only one dose was used in each study."

For the reasons discussed by DWQI (2017a), the data from these three peripubertal studies cannot be used to make firm conclusions about the involvement of PPAR-alpha in mammary gland effects of PFOA such as those presented on lines 17-18 of the draft WHO document. Additionally, it should be made clear that the "inconsistent effects of PFOA on mammary gland development in different strains and dose levels" mentioned on lines 20-22 refer only to the three peripubertal exposure studies and not to the prenatal/lacational exposure studies (White et al. 2007, 2009, 2011; Macon et al., 2011) discussed on p. 55, lines 25-48. As discussed in DWQI (2017a), the adverse effects of prenatal and lactational exposure to PFOA on mammary gland in mice are well established and are considered to be adverse and relevant to humans.

p. 56, line 37. The age at which the mice were dosed should be mentioned.

p. 57, lines 8-9. As above, reproductive versus developmental effects are not clearly or consistently distinguished throughout. Here, increased resorption of litters, a reproductive effect, is discussed in the section on developmental effects.

p. 57, lines 12-13. The effects mentioned here (delayed eye opening and accelerated sexual maturation) are not relevant to the topic of this section, neonatal survival.

p. 57, lines 28-33 state: "In a similar experiment using wild-type, PPARα-null mice and PPARα-humanized mice exposed to a single dose of 3 mg/kg PFOA, Albrecht et al. (2013) also found that the frequency of litter resorptions was independent of PPARα status, whereas PPARα status had no effect on the timing of eye-opening in neonates. Albrecht et al. (2013) further reported that pup survival was decreased only in PFOA-exposed wild-type mice, but not in PFOA-exposed PPARα-null or PPARα humanized mice."

WHO must consider the problematic issues with the Albrecht et al. (2013) study that are relevant to the conclusions presented here. These issues are discussed on p. 142-143 of DWQI (2018), as follows:

> "Although postnatal lethality in wild type mice treated with 3 mg/kg/day (the only dose used in the study) on PND 20 was reported as statistically significant ($p<0.05$), this conclusion appears to be based on an inappropriate statistical comparison. In evaluating postnatal lethality, the number of pups per litter on PND 20 in the control and PFOA-treated groups of wild type mice were compared. However, this comparison does not appear to be valid because the control and PFOA treated litters initially had different numbers of pups on PND 0. The appropriate evaluation of this parameter is a comparison of the number of pups within the same litter on PND 0 and PND 20 (i.e., percent mortality within the litter between PND 0 and PND 20). In wild type pups, 96% of controls and 70% of PFOA-treated survived from PND 0 to PND 20. From the analysis presented, it is unclear whether postnatal lethality is actually significantly increased by PFOA in wild type pups. For this reason, the basis for the conclusion that wild type, but not humanized PPAR-alpha, mice are sensitive to developmental effects of PFOA is uncertain.

An important concern is that Albrecht et al. (2013) state that elevated PFOA levels (up to >1000 ng/ml) were found in liver and serum from some control fetuses, pups, and dams. However, no further information such as which groups of animals these samples came from, how many samples had elevated PFOA concentrations, or statistical data for serum levels in the control samples is provided. Importantly, data from control animals with elevated PFOA exposures do not appear to have been excluded in the comparisons of endpoints of toxicity in control and treated groups. Inclusion of these data from the control animals could have affected the results of these comparisons, especially since serum levels in some of the treated groups were only a few fold higher than those in some of the controls.

Developmental effects observed in the same strain of mice (SV/129) in another study (Abbott et al., 2007) at lower doses (0.6 and 1 mg/kg/day) were not observed at the higher dose (3 mg/kg/day) used by Albrecht et al. (2013). Abbott et al. (2007) observed significantly increased postnatal lethality in wild type pups exposed gestationally to 0.6 and 1 mg/kg/day PFOA. Additionally, eye opening was significantly delayed in the 0.6 and 1 mg/kg/day wild type pups in Abbott et al. (2007), but not at 3 mg/kg/day in Albrecht et al. (2013).

Although both studies used SV/129 mice, Albrecht et al. (2013) obtained them from NIH and Abbott et al. (2007) obtained them from Jackson Laboratories. Albrecht et al. (2013) suggest that pharmacokinetic differences in the wild type mice from the two different sources may explain the differences in effects of PFOA in these mice in the two studies. However, a close review of the data from the two studies (Table 3 of Abbott et al., 2007; Figure 10 of Albrecht et al., 2013) indicates that the serum levels in wild type pups in Albrecht et al. (2013) at which no developmental effects occurred were higher than the serum levels in wild type pups at which delayed eye opening and postnatal mortality were reported by Abbott et al. (2007). Furthermore, the serum PFOA data for wild type dams on PND 20 appear to be inconsistent within the publication. Maternal serum levels in wild type dams on PND 20 are stated to range from 2066 – 6812 ng/ml, and no statistical parameters (e.g., median, mean, S.D.) are provided. However, the estimated serum level from the bar graph of maternal serum levels is 6700+3600 in the wild type dams (higher than what would be expected from the range provided in the text)."

p. 57, lines 33-35, state: "…Nakamura et al. (2009) showed that increased expression of PPARα-related genes was observed in wild-type mice, but not in humanized PPARα or PPARα-null mice, exposed for 2 weeks to ≥ 0.1 mg/kg bw per day."

Nakamura et al. (2009) is a study of adult mice, not a developmental study; therefore, it is not relevant to this section. Additionally, the differences in hepatic effects of PFOA on wild type versus humanized PPAR-alpha and PPAR-alpha null mice in Nakamura et al. (2009) were not as clearcut as suggested here. As summarized by DWQI (2017a):

> "Expression (mRNA and protein levels) of five genes that are targets of PPAR-alpha was measured in the three strains. Expression of four of these genes was significantly increased only in the wild type mice, while expression of the fifth gene was significantly increased only in the humanized PPAR-alpha mice. None of the PPAR-alpha target genes were affected by PFOA in the PPAR-alpha null mice. Histopathological examination showed mild hepatocellular hypertrophy in both wild type and humanized PPAR-alpha mice. Cytoplasmic vacuoles indicating lipid accumulation were observed in PPAR-alpha null mice, consistent with those described in this strain by Wolf et al. (2008)."

p. 57, lines 44-48, state: "Both PFOA-induced mammary gland inhibition and reduced neonatal survival in mice appears to be at least partially dependent on PPARα expression and phenotype, with PPARα knockout mice being less susceptible to mammary gland inhibition, and humanized phenotypes being less susceptible to neonatal mortality. The relevance of these effects in the tested strains of mice to humans is therefore uncertain and likely requires further study."

The premise of these WHO conclusions is that, in general, toxicological effects of PFAS in laboratory animals that are mediated by PPAR-alpha (including developmental effects) are not relevant to humans. This conclusion is not scientifically supportable, and it is not accepted by the authoritative agencies (USEPA, ATSDR, Health Canada, EFSA) whose reviews are relied upon by WHO. Therefore, this statement and similar statements elsewhere in the document should be removed.

Regarding human relevance of developmental effects in laboratory animals that are mediated by PPAR-alpha, DWQI (2017a) states (bolding added):

> "PPAR-alpha, -beta, and -gamma are expressed in many fetal and adult tissues in rodents and humans. Abbott et al. (2010, 2012) found that PPARs are present in nine human fetal tissues examined (liver, heart, lung, kidney, intestine, stomach, adrenal, spleen, and thymus) from embryonic days 54 to 125. They found that the levels may increase or decrease with age of the fetus, or between the fetus and the adult. In some fetal tissues, PPARs were expressed at levels equivalent to or higher than in adults. Although the role of PPAR-alpha and other PPARs in human and animal development is not well characterized, based on their physiological actions, they are expected to have important roles in reproduction and development (Abbott et al., 2010). **For these reasons, it can be assumed that PPAR-alpha mediated effects on development are relevant to humans.**"

Additionally, as discussed above, the conclusions that PPAR-alpha knockout (null) mice are less susceptible to mammary gland inhibition (comments on p. 56, lines 2-15) and humanized PPAR-alpha mice are less susceptible to neonatal mortality (comments on p. 57, lines 31-33) are not scientifically supportable.

p. 59, lines 24-25 state: “As simultaneous hepatotoxicity was also apparent [in Vetvicka and Vetvickova, 2013], the [immunotoxic] effects have the potential to be indirect rather than direct effects.”

This statement is not supportable and should be deleted. The only hepatic effect reported by the authors of this study was increased relative liver weight. The authors (Vetvicka and Vetvickova, 2013) did not state that the immunotoxic effects, which affected many different endpoints of immune system function, were potentially secondary to increased liver weight. Furthermore, WHO does not present any information to support this hypothesis.

p. 62, lines 24-26, states: “In summary, findings from the long-term carcinogenicity study confirm that the liver is a potential target organ for chronic toxicity and carcinogenicity [of PFOS], which may be attributable to a mode of action involving activation of PPARα and other PPARα-independent modes of action.” However, as discussed in detail in comments on p. 41, lines 22-25, above, the hepatic effects of PFOS are primarily PPAR-alpha independent, with little if any contribution from PPAR-alpha mediated effects.

p. 62, lines 29-31. As discussed in the remainder of this section, PFOA also caused tumors (hepatic and pancreatic) in rats in NTP (2020). This study should be included along with the other two chronic rat studies in this introductory discussion of carcinogenicity of PFOA in laboratory animals.

p. 63, lines 17-20. When discussing the NTP (2020) pancreatic tumor data, it is important to mention that the incidence of both benign and malignant pancreatic tumors was increased by PFOA. Also, similar to other comments above, it should be mentioned that occurrence of tumors at much higher doses in female rats than in male rats is likely due to the much more rapid excretion in female rats, meaning that a much higher dose must be administered to females than to males to obtain the same internal dose.

p. 63, lines 22-25. This is a general discussion of the results of NTP (2020) and should not be placed in the section on pancreatic acinar tumors.

p. 63, lines 24-25 states that “It is therefore not possible using current evidence to exclude PFOA as a human carcinogen” in the context of the NTP conclusion of “clear evidence of carcinogenic activity in male rats” for PFOA. This is an inaccurate interpretation of NTP conclusions about the carcinogenicity of agents tested in NTP bioassays. NTP conclusions about agents tested in their bioassays refer to evidence for carcinogenicity in each specific type of test animal (e.g., male rat, female rat, male mouse, or female mouse), and NTP bioassay study reports do not make conclusions about evidence for human carcinogenicity. That being said, NJDEP agrees that the human and animal data, considered together, support the conclusion that PFOA is a likely human carcinogen, as determined by USEPA (2021b) and supported by USEPA SAB (2022).

Section 6. Mode of Action

p. 63, line 27. Regardless of whether or not it was included by ATSDR (2021), carcinogenicity should be added to the list of primary effects of PFOS and/or PFOA listed here.

p. 63, line 35 – p. 64, line 13. This section on mode of action of hepatic effects is confusing and not readily understandable to even an informed reader. It appears that all or most of this section is paraphrased from ATSDR (2021) without clear explanations of the general concepts and conclusions. As just one of numerous examples, peroxisomal beta-oxidation is mentioned (p. 64, line 11), but it is not explained that peroxisomal beta-oxidation is a marker for PPAR-alpha activity.

For PFOA, it is suggested that WHO review the clear and comprehensive evaluation of PPAR-alpha dependent and independent hepatic effects in DWQI (2017a, p. 181-190). In summary, this evaluation concluded that data from non-human primates (monkeys), standard strains of rats and mice, PPAR-alpha null mice, and humanized PPAR-alpha mice support the conclusion that hepatic effects of PFOA in experimental animals are relevant to humans for the purposes of risk assessment. As just one example of the numerous analyses provided by DWQI (2017a), the dose-related increase in palmitoyl CoA oxidase activity (a marker for PPAR-alpha activity) in non-human primates (i.e., non-rodent species in which human relevance of hepatic effects is not in question) was similar in magnitude to the increase in rats, demonstrating that PFOA causes similar hepatic PPAR-alpha activity in non-human primates as in rodents.

For PFOS, as explained above, available information indicates minimal contribution of PPAR-alpha to hepatic effects.

p. 64, lines 23-25. While effects on mammary gland development have not been studied in animal species other than mice or humans, it is suggested that it be mentioned that at least five human epidemiology studies from different locations have reported adverse effects of PFOA on breast feeding (duration and/or initiation) while no studies negative for this effect were identified. The five publications reporting effect on breast feeding are Fei et al. (2010, https://www.sjweh.fi/show_abstract.php?abstract_id=2908; Nielsen et al. (2022, https://doi.org/10.1016/j.envres.2021.112206; Romano et al. (2016, https://doi.org/10.1016/j.envres.2016.04.034; Timmermann et al. (2017, https://doi.org/10.1016/j.reprotox.2016.07.010; and Timmermann et al. (2022, https://doi.org/10.1210/clinem/dgab638)

p. 65, line 20. The conclusions of lack of human relevance for Leydig cell tumors and pancreatic acinar tumors caused by PFOA are inconsistent with the conclusions of USEPA (2016b, 2021b). The USEPA (2005, https://www.epa.gov/sites/default/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf) guidelines for carcinogen risk assessment assume human relevance of animal tumors unless a mode of action that is not relevant to humans has been definitively established, and the mode of action for these tumors has not been definitively established (Klaunig et al., 2012, https://doi.org/10.1016/j.reprotox.2011.10.014;

USEPA, 2016b; DWQI, 2017a). Additionally, animal tumors do not need to occur at the same sites as human tumors to be considered relevant to humans according to the USEPA (2005) guidelines. While Health Canada (cited by WHO) may use different criteria than USEPA (2005) for forming conclusions on human relevance of animal tumors, it is not appropriate for WHO to preferentially cite a conclusion based on the guidance used by one specific nation as the definitive conclusion on this or other topics.

Section 7. Summary of Health Effects

p. 65, line 43-45 states: "For PFOS and PFOA specifically, this database comprises evaluations of health outcomes in subjects exposed in occupational settings, and residents living near a PFOA plant who had relatively high exposure via drinking-water and other environmental sources."

This statement is not accurate. It should be revised to indicate that the large majority of epidemiology studies of PFOA and PFOS are from the general population without exposure from a specific source of environmental contamination, not from occupationally exposed workers or communities with "relatively high environmental exposures."

p. 66, lines 12. In this summary of information on toxicokinetics, it is unclear what is meant by "non-linear nature of toxicokinetics of PFOS and PFOA."

p. 66, lines 13-15. In this summary of information on toxicokinetics, it should be noted that, for animal studies where serum PFOA or PFOS data are available, interspecies extrapolation can be based on use of clearance factor to predict the Human Equivalent Dose from measured serum levels (described above in comments on p. 24, lines 9-12).

It should also be noted that toxicokinetic models that consider prenatal and lactational exposure (e.g., Goeden et al., 2019 and the model used by EFSA, 2020) are also used to predict exposures to infants in assessments based on human epidemiological studies, not only in assessments based on animal studies where interspecies extrapolation is required.

p. 66, lines 17-22. In this summary of information on toxicokinetics, the very rapid excretion of PFOA in female rats, which results in fluctuations in internal dose over short periods of time (in contrast to bioaccumulation in male rats, and males and females of other laboratory animal species and humans), should be mentioned. This concept is important in interpretation of toxicological data for PFOA. The half-lives in rodents other than female rats should be characterized as several days to several weeks for PFOA and one to two months for PFOS, not "hours to days."

p. 66, line 29. Here and/or elsewhere, it should be emphasized that there is concordance between laboratory animals and humans for most of the major toxicological effects of PFOA and PFOS, including developmental, immunological, hepatic, and carcinogenic effects.

p. 66, line 36. Suggest noting decreased immune response as a specific type of immune effect caused by PFOA and PFOS.

p. 66, line 37. In addition to delayed mammary gland development in mice, it is suggested that other developmental effects in rodents including decreased offspring body weight and delayed development (e.g., delayed eye opening) be mentioned.

p. 66, lines 42-45. "The applicability of the adverse health effects reported in animals to human health is uncertain, recognizing species and sex-related differences in the toxicokinetics of PFAS. In addition, the mode(s) of action for PFOS and PFOA-induced toxicities are not fully elucidated, although both PPARα- dependent and -independent pathways have been proposed."

These statements are not scientifically supportable, are not accepted by the scientific community, and appear to be intended to cast doubt on the generally accepted human relevance of toxicological effects of PFOA and PFOS in experimental animals. As such, they should be removed.

Specifically, the general conclusion that the human relevance of adverse health effects reported in animals is uncertain contradicts the conclusions of all the authoritative agencies whose reviews were heavily relied upon by WHO (e.g., USEPA, Health Canada, EFSA, ATSDR).

Furthermore, toxicokinetic differences between humans and animals are not germane to whether or not adverse effects observed in animals are relevant to humans. Rather, they are considered in determining the administered dose at which effects seen in animals are expected to occur in humans, with a lower human administered dose needed to obtain the same internal dose that occurred in the animals. Toxicity factors (e.g., Reference Doses and similar non-cancer values; cancer slope factors) based on animal data for all contaminants (not just PFAS) consider interspecies toxicokinetic differences. As discussed in detail in the draft WHO document, modeling approaches have been specifically developed to address this issue for PFOA and PFOS.

Finally and importantly, it is unclear why PPAR-alpha is mentioned here in the context of human relevance. As discussed in detail in multiple comments above, the available scientific information demonstrates that health effects of PFOA and PFOS that are mediated by PPAR-alpha, as well as those that are PPAR-alpha independent, are relevant to humans. This conclusion is generally accepted by other authoritative agencies and the scientific community.

p. 67, line 1. This section is called "Repeated dose toxicity," but specific types of repeated dose toxicity such as neurotoxicity and immune system toxicity are covered in other sections. For the benefit of the reader, suggest stating this at the beginning of the section.

p. 67, lines 6-8 states: "However, increased liver weight is not by itself an adverse effect unless accompanied by necrosis, fibrosis, steatosis or other clinically relevant signs of liver damage (Hall et al., 2012)."

As discussed above, this statement from Hall et al. (2012) is not applicable to chronic exposure, which is the relevant exposure duration for WHO drinking water guidelines. In summary, the Hall et al. (2012) criteria conclusions about adversity of increased liver weight assume that this

effect may be reversible when exposure to the agent (e.g., a drug) ends. However, as discussed by Hall et al. (2012; see above) these reversibility considerations are not relevant to chronic exposure since it does not end after a certain period of time. Importantly, increased liver weight from short-term exposure to PFOA and PFOS has been shown to progress over time to more severe forms of hepatic toxicity. Furthermore, it is unclear why this statement is even included here since many studies of PFOA and PFOS demonstrate the types of hepatic toxicity that are noted as adverse in the statement.

p. 67, lines 13-15. As mentioned multiple times above, evaluation of the relevant data indicates that hepatic effects of PFOS are primarily PPAR-alpha independent, with little or any contribution from PPAR-alpha mediated effects.

p. 67, lines 16-18. "PFOA-induced transactivation of PPAR-alpha" is not mentioned elsewhere in the document, and it is unclear which study is referred to here. This information is also too specific (including the dose at which this occurred) for this summary, and its overall relevance is unclear.

p. 67, lines 34-36 state, regarding delayed mammary gland development in mice, that: "Other strains (i.e. C57Bl or Balb/3) experienced similar effects but at much higher PFOA doses (1 or 5 mg/kg); the reason for this difference is not known."

As discussed in detail above, the studies in C57Bl and Balb/3 mice mentioned here used peripubertal exposure, while the other studies (mentioned earlier in the paragraph) in which effects occurred at lower doses used prenatal and/or lactational exposure. Because effects on mammary gland development are highly life-stage dependent, the results of the peripubertal exposure studies are not inconsistent with the results of the prenatal/lactational exposure studies.

p. 67, line 37. It is unclear who proposed a prenatal developmental LOAEL of 1 mg/kg/day.

p. 68, line 9-11. It should be mentioned that the same study that showed neurotoxicity for PFOA also showed neurotoxic effects of PFOS at 0.75 mg/kg. This information should also be included in the discussion of neurotoxicity of PFOS earlier in the document.

p. 68, lines 27-30 states: "As [immunotoxic] effects were usually seen at doses that also induced general toxic effects including those related to food intake and body weights, indirect effects of PFOA on the immune system cannot be ruled out."

This is not a valid conclusion and should be removed. Specifically, studies that included lower doses (e.g., Dewitt et al., 2008, https://doi.org/10.1289/ehp.10896; Dewitt et al., 2009, https://doi.org/10.1093/toxsci/kfp040) demonstrated immunotoxicity at doses at which there were no effects on food intake or body weight. Additionally, it is noted that the systematic review of immune system toxicity of PFOA and PFOS performed by NTP (2016) did not make this conclusion.

p. 68, line 44. It should be mentioned that PFOA caused both benign and malignant acinar cell tumors in male rats.

p. 68, line 46-47. It should be mentioned that the fact that PFOA did not cause pancreatic tumors in female rats may be related to the very rapid excretion and lack of bioaccumulation of PFOA in female rats. This rapid excretion in female rats is discussed in detail above.

p. 69, lines 3-4. It is not clear what is meant by "the most common adverse effects in humans." The effects with the strongest/most consistent evidence should be listed, not the most "common."

Also, this introduction to the section on human toxicity endpoints should say that most human studies that report these adverse effects are from the general population who do not have additional exposure from a point source (e.g., contaminated drinking water) and that the effects are associated with the exposure range prevalent within the general population.

Importantly, the fact that PFOA and/or PFOS is associated with increased risk of clinically defined abnormal values for each of the endpoints discussed below should be added to the information on each specific endpoint. Additionally, as noted by the USEPA SAB (2022):

> "In studies where the number of subjects with clinically abnormal values was not specifically evaluated, an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. While the clinical relevance of exposure to PFOA or PFAS cannot be predicted on an individual basis, the increased number of individuals within a population with clinically defined abnormal values is of public health concern."

The specific studies that reported an increased risk of clinically defined abnormal values for each endpoint are provided by USEPA SAB (2022), as follows:

> "While most of these studies did not evaluate the number of subjects with a clinically abnormal value for biomarkers, one or more studies, for each of the four effects [increased serum cholesterol, increased serum ALT, decreased antibody response to vaccination, decreased birth weight] reported an association of PFOA and/or PFOS with increased risk of a clinically abnormal value. Examples of studies that reported an increased risk of clinically abnormal values are as follows: Grandjean et al. (2012) reported tetanus or diphtheria antibodies levels below a clinically protective level; Looker et al. (2014) found an increased risk of not attaining the antibody threshold considered to offer long-term protection to A/H3N2 influenza virus; multiple studies reviewed in the draft MCLG documents found clinically defined low birth weight or small for gestational age; Steenland et al. (2009) observed clinically defined high cholesterol; Gallo et al. (2012) and Darrow et al. (2016) reported clinically defined elevated ALT."

p. 69, lines 6-7. This sentence on the decreased antibody response to diphtheria and tetanus vaccines in children should mention that PFOA and PFOS were associated with increased risk of antibody levels below the clinically protective threshold (Grandjean et al., 2012).

p. 69, lines 6-12, state: "Epidemiological studies suggest that exposure to PFOS and PFOA adversely affects antibody response to vaccination against diphtheria and tetanus in children,

with evidence of PFOA having a stronger association compared to PFOS. However, there is limited evidence of an association between PFOS and PFOA serum levels and increased incidence of illness in children; for example, according to CDC (2019) data the number of new cases of diphtheria in the United States over a 40-year period was less than one per year on average."

As discussed in detail in the comment on p. 34, lines 40-41, of the draft WHO document above, NJDEP strongly disagrees with WHO's conclusion that decreased antibody response to vaccinations is not adverse or appropriate as the basis for risk assessment unless there is an increased incidence of the diseases prevented by vaccination. The same comment also notes that the lack of an association of PFOA and PFOS with increased incidence of infectious disease has not been established. These comments are also relevant here.

p. 69, lines 22-23. This sentence should mention that association with increased risk of clinically defined high cholesterol has been reported (Steenland et al., 2009).

p. 69, lines 25-27. As above, the range of serum levels up to 25 ng/ml is relevant here, since it is within the range commonly associated with drinking water contamination.

p. 69, lines 29-32 states: "Although epidemiological studies provide evidence for an association between exposure to PFOS and PFOA and increased serum ALT, the magnitude of the associations is small, with ALT levels rarely being outside the reference range and no evidence of liver disease."

This sentence is misleading and should be modified or removed. Most studies did not evaluate the number of subjects above the reference range, and Gallo et al. (2012, https://doi.org/10.1289/ehp.1104436) and Darrow et al. (2016, https://doi.org/10.1289/ehp.1510391) reported associations of PFOA with clinically defined elevated ALT.

Additionally (as discussed above), "an increase in the number of subjects with a clinically abnormal value is…expected from the overall change (shift in the distribution curve) in the abnormal direction," (USEPA SAB, 2022).

The extensive additional comments on p. 37, line 40 – p. 38, line 7 (Section 4.2.7.1), above that support the conclusion of adversity of increased ALT are also relevant here.

p. 69, lines 43. It should also be mentioned that multiple studies have reported associations of PFOA and/or PFOS with increased risk of low birth weight or small for gestational age. See USEPA (2021a, b).

p. 69, line 46. The word "partially" should be added as follows: "…the overall association may be partially confounded by…"

p. 70, lines 2-7. This text should be revised to indicate that there is evidence for association of PFOA and kidney cancer in occupationally exposed workers, communities with drinking water

exposure, and the general population, while the association with testicular cancer was only reported in occupationally exposed workers and communities with drinking water exposure.

p. 79, lines 7-10 state. “WHO considered that the uncertainties in identifying the key endpoint applicable to human health following exposure to PFOS and/or PFOA are too significant to derive a HBGV with confidence.”

The fact that PFAS causes many different types of health effects is not a valid reason not to develop a HBGV. Rather, the large number of health effects highlights the need for concern about exposure to PFAS and the need for health-based guidelines that are protective for the most sensitive health endpoint(s). It is noted that WHO has developed drinking water guidelines for many other contaminants, and that some of these are based on non-cancer effects and others on lifetime cancer risk at the 10^{-5} (1 in 100,000) risk level. There is ample data from both humans and experimental animals to develop a health-based guideline for cancer and non-cancer effects of PFOA and non-cancer effects of PFOS.

Section 8. Practical Considerations

p. 75, line 1. Section 8.4.6. on examples of PFAS removal from full-scale and pilot studies. In establishing New Jersey’s Maximum Contaminant Levels (MCLs) of 14 ng/L (0.014 μg/L) for PFOA and 13 ng/L (0.013 μg/L) for PFOS, New Jersey considered the availability of treatment technologies to reduce levels of PFAS in drinking water and did not find it to be a limiting factor. In making its MCL recommendations for PFAS, the New Jersey Drinking Water Quality Institute (DWQI) conducted a comprehensive review of literature and case studies to identify available treatment technologies for removal of these compounds from drinking water. The findings of the DWQI were presented in the report “Recommendation on Perfluorinated Compound Treatment Options for Drinking Water” and subsequent addendum documents (DWQI 2015, 2016, 2017b). The DWQI reports found that PFOA and PFOS could be reliably removed by drinking water treatment facilities utilizing granular activation carbon (GAC) or an equally efficient treatment technology to below New Jersey’s MCLs of 14 ng/L for PFOA and 13 ng/L for PFOS.

This conclusion is further supported by the treatment removal achieved by New Jersey drinking water treatment plants. Based on NJDEP records, 12 facilities in New Jersey have installed permanent treatment for removal of PFAS. Of these, seven facilities have utilized GAC treatment and five facilities have utilized ion exchange (IEX) treatment. Regulated drinking water systems began submitting standardized compliance data for PFOA and PFOS to NJDEP in January 2019. These compliance samples are collected post-treatment, prior to entering the water distribution system. Between January 23, 2019 and October 3, 2022, close to 200 samples were submitted for PFOA and PFOS by the 12 facilities utilizing permanent PFAS treatment. An analysis of these data shows that these facilities were able to achieve levels of PFOA and PFOS below detection in the vast majority (>99.9%) of treated water samples. Detection limits for these samples ranged from 0.53-5 ng/L.

DWQI (2015, 2016, 2017b) found GAC to be "a common and effective (>90% removal) treatment for long-chain [PFAS] contamination." Of the 138 samples submitted by the seven treatment plants that utilized GAC, only three samples had detectable levels of PFOA (between 3.4-5.4 ng/L), and no samples had detectable levels of PFOS. These facilities were able to reduce PFOA and PFOS to below the detection levels in 96-100% of samples.

An analysis of the 60 samples submitted by the five facilities with IEX treatment shows only three detections of PFOA ranging from 2.2-7.6 ng/L and two detections of PFOS ranging from 2.0-3.2 ng/L. Based on these data, the facilities that utilized IEX treatment for removal of PFAS were able to remove PFOA and PFOS to below detectable levels in 90-93% of all finished water samples.

The analysis presented above shows that facilities can reliably and consistently achieve levels of PFOA and PFOS close to or below detection levels (0.53-5 ng/L) through carefully designed and operated PFAS treatment. All detections of PFOA or PFOS were below New Jersey's MCLs of 14 ng/L for PFOA and 13 ng/L for PFOS for both GAC and IEX treatment technologies.

In addition to these 12 facilities with permanent treatment for PFAS, permits have been submitted to NJDEP for installation of PFAS treatment at approximately 90 additional facilities. These facilities are in various stages of completing the permitting process and constructing PFAS treatment. Records indicate that these facilities are designing their treatment systems to achieve finished water levels of PFAS ranging from <1 ng/L - 40 ng/L, with over 94% < 14 ng/L.

NJDEP recommends that WHO consider additional case studies and the data on regulated New Jersey drinking water systems presented above. Additionally, the DWQI reports cited above present several case studies of full and pilot-scale applications of PFAS treatment, which show that PFOA and PFOS can be reliably and consistently removed from drinking water to below the NJDEP drinking water standards of 14 ng/L and 13 ng/L, respectively. These include case studies with relatively high levels of PFAS in the raw water, such as in Oakdale, Minnesota, where PFOS at 540-620 ng/L was reliably removed to <13 ng/L, and Horsham, Pennsylvania, where PFOS at 293-1297 ng/L was reliably removed to <2.5 ng/L (DWQI, 2017b).

p. 75, lines 3-12, discussion of Belkouteb et al. (2020). The draft document cites the long-term removal efficiencies for PFOS at 30,000 treated bed volumes presented in Belkouteb et al. (2020) as 80-100%. NJDEP notes that Belkouteb et al. (2020) also calculated relatively high long-term removal efficiencies for PFOA to be between 68-100% at the greatest common operation time, approximately 22,000-22,500 treated bed volumes.

p. 75, lines 13-26, unpublished report from Italy. NJDEP was unable to obtain and review the unpublished report from Italy. From the information presented in the draft WHO document, it is unclear whether the installation and improvement of GAC filtration at this facility had any effect on the reduction of PFAS in finished water, as the date when treatment was placed online is not provided.

p. 75, lines 28-40, discussion of Appleman et al. (2014). It should be noted that the treated water PFAS concentrations from Appleman et al. (2014) include data from facilities that utilized treatments generally considered ineffective for reduction of PFAS, including coagulation, oxidation, aeration, and disinfection (Appleman et al., 2014). Additionally, many of these utilities did not have treatment installed specifically for removal of PFAS. Of the four full-scale GAC treatments discussed in Appleman et al. (2014), Utility 20 is mentioned as being of particular interest because it is designed for PFAS treatment. Appleman et al. (2014) state that Utility 20 had higher levels of raw water PFAS, as the source was highly contaminated by an industrial source, and that this utility installed GAC vessels in a lead lag configuration. Over the course of one year, this utility had an average removal for the lag basin of >95% for PFOS and >92% for PFOA (Appleman et al., 2014).

p. 75, lines 35-36. The background report also states that the use of IEX technologies is not as certain, but that IEX is expected to be effective for removal of "charged species of PFAS." Relevant to this point, the two PFAS that are the focus of the draft WHO document (PFOA and PFOS), as well as the homologues with other chain-lengths, exist as charged species (anions) in water. It is noted that the two anion exchange treatments presented in Appleman et al. (2014) exhibited 92% removal for PFOS and 75% removal for PFOA, and that these IEX treatments were not specifically designed for PFAS removal. USEPA states that IEX has up to or greater than 99% removal effectiveness for PFOA and greater than 90-99% removal effectiveness for PFOS (USEPA, 2022 Drinking Water Treatment Database. https://tdb.epa.gov/tdb/home). Data from New Jersey drinking water facilities also show IEX to be an effective treatment for removing PFAS to below the New Jersey MCLs of 14 ng/L for PFOA and 13 ng/L for PFOS.

Section 9. Conclusions

p. 79, lines 10-11 states: "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."

Again, as discussed in detail above (comment on p. 34, lines 40-41), the points stated here about infection and clinical implications are not a valid rationale to dismiss decreased antibody response to vaccination as the basis for PFOA and PFOS health-based guidelines. The comments on p. 69, lines 6-12, are also relevant here.

More generally, a detectable increase in human disease is clearly not a requirement for use of a toxicological endpoint as the basis for a drinking water guideline, as per WHO's own guidance for developing health-based drinking water values (p. 170 of WHO, 2022. Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda. Geneva: World Health Organization) which states that health-based guidelines are established when: "There is credible evidence of occurrence of the chemical in drinking-water, combined with evidence of actual or potential toxicity." Relevant to this point, the extent of the human and animal health effects database and the level of certainty for human health effects of PFOA and PFOS far

exceed the health effects evidence for many or most of the other contaminants for which WHO has developed drinking water guidelines. Most of the drinking water guidelines listed in Chapter 12 of WHO (2022) are based on animal data, and there are little or no relevant human health effects data for some of the contaminants with WHO drinking water guidelines.

p. 79, lines 13-17 states: "Although animal data would generally be utilised in the absence of adequate human data for risk assessment purposes, there are also areas of uncertainty around the suitability of animal studies for assessing the effects to human health for PFOS and PFOA as discussed earlier, including interspecies differences in kinetic parameters such as elimination half-life and clearance rate."

The comments about these same points on p. 66, lines 42-45, are applicable here and should be reviewed by WHO in the context of this statement.

Additionally, human epidemiology data are the basis for recent toxicity factors for PFOA and PFOS developed by several authoritative agencies (EFSA, 2020; USEPA, 2021a, b; California EPA, 2021). When human data are used, interspecies extrapolation is unnecessary.

p. 79, lines 17-19. "Additionally, diverging estimates of the human half-life of PFOA may also add uncertainty to animal-to-human dosimetric adjustments, as well as PBPK-based conversions of human plasma PFAS concentrations to external doses."

This is not a scientifically supportable reason for not developing a health-based value, and it appears to be included to inappropriately create the appearance of unacceptable uncertainty. The variability within the range of potential human half-lives for PFOA is not large. It is similar or less than the variability of other factors and assumptions routinely used in the development of drinking water guideline including body weight, drinking water ingestion rate, and others.

p. 79, lines 19-23 states: "Finally, the uncertainty and lack of consensus in the critical health end point to derive a HBGV is evident from the diverse range of endpoints utilised by other agencies to derive tolerable daily intakes or similar values, and the resulting range in proposed drinking-water values described in Table A.1 (see appendix)."

The different health endpoints chosen by different agencies is not a valid reason not to develop a health-based guideline. Values from different agencies were developed over a period of time based on the information available at that time they were developed. Additionally, different agencies have different statutory requirements and approaches for development of health-based drinking water guidelines. Note: comments on Table A.1 are provided below.

p. 79, lines 23-24 states: "Although the values derived by several different organizations vary significantly, all have margins of safety."

This statement is not logical. If the lower values that have been derived are scientifically supportable, the higher values do not have margins of safety. Perhaps this was meant to say that all of the values were <u>intended</u> to have a margin of safety by those who developed them.

p. 79, lines 27-32, states: "Health-based drinking water values derived by authoritative agencies range from 0.05 to 0.6 µg/L for PFOS and from 0.05 to 0.56 µg/L for PFOA.[8]"

This sentence is inaccurate and misleading because: (1) The Danish value of 2 ng/L for the total of PFOA, PFOS, PFNA, and PFHxS that is much lower than the ranges mentioned in this sentence is omitted, and it is also not included in Table A.1; (2) The sentence about ranges on lines 27-32 does not include the much lower values from USEPA (2021a,b); they are included only as a footnote to the sentence; and (3) WHO does not recognize U.S. states, several of which have developed detailed scientific evaluations to support development of lower values, as "authoritative agencies." These states should be recognized as "authoritative agencies" by WHO and their health-based drinking water values should be included here.

Regarding the Danish value of 2 ng/L for the total of PFOA, PFOS, PFNA, and PFHxS, see information at https://tox.dhi.dk/en/news/news/article/danish-epa-more-tough-on-pfas-in-drinking-water/ and Danish "Criteria for Soil and Groundwater Used as Water Supply" at https://mst.dk/media/223446/liste-over-jordkvalitetskriterier-juli-2021_final1.pdf.

Please note that the Interstate Technology & Regulatory Council (ITRC) maintains a complete and up to date table (Excel spreadsheet) of U.S. and international PFAS drinking water guidelines (https://pfas-1.itrcweb.org/wp-content/uploads/2022/09/ITRCPFASWaterandSoilValuesTables_AUG2022-Final.xlsx), posted at https://pfas-1.itrcweb.org/fact-sheets/). As shown on the ITRC spreadsheet, Denmark established its drinking water guideline of 2 ng/L for the total of PFOA, PFOS, PFNA, and PFHxS in 2021. However, only the older (2015) and much higher Danish values of 100 ng/L for PFOS and 300 ng/L for PFOA are shown on Table A.1 of the WHO document.

Importantly, USEPA has stated repeatedly that it plans to propose National Primary Drinking Water Standards and MCLGs (health-based drinking water values) for PFOA and PFOS before the end of 2022. Since it is unlikely that WHO will complete its revision of its draft document before the end of 2022, the proposed USEPA values (assuming they are proposed in 2022 as planned) should be included in the revised WHO document.

p. 79, line 32. The EFSA (2020) health-based value (i.e., Tolerable Weekly/Daily Intake; TWI/TDI) is mentioned along with the ATSDR (2021) values (i.e., Minimal Risk Levels, MRLs) here. However, in the next paragraph, drinking water values based on the ATSDR (2021) MRLs and WHO drinking water assumptions are presented, but a drinking water value based on the EFSA (2020) TDI is not presented. It should be added that EFSA (2020) developed a TWI of 4.4 ng/kg/week, equivalent to a TDI of 0.62 ng/kg/day, for the total of PFOA, PFOS, PFNA, and PFHxS, and that applying default WHO parameters results in a drinking water value of 4 ng/L for the total of these four PFAS.

p. 79, line 43-44. The sentence beginning with "According to Post (2021)…" should be updated to reflect current information. The older information on state drinking water guidelines from Post (2021) was updated by Post in an August 2022 presentation (posted at

https://dnr.wisconsin.gov/sites/default/files/topic/PFAS/tech/Presentation20220819.pdf; PDF p. 10 of 49). Currently, 10 U.S. states have guidelines of 0.007 - 35 ng/L for PFOA individually and 1-18 ng/L for PFOS individually, and five states have guidelines of 20 - 30 ng/L for the total of PFOA, PFOS, and 3 or 4 other PFAS.

p. 79, line 44 - p. 80, line 1. This sentence refers to "US EPA's health advisory of 70 ng/L for combined PFOA/PFOS." However, as stated above in comments on p. 9, line 48, the current USEPA Health Advisory for PFOA and PFOS is no longer 70 ng/L. It should be stated in the text (not just in a footnote) that USEPA has issued newer interim Health Advisories for PFOA and PFOS and has said that these recent interim Health Advisories supersede the 2016 Health Advisories of 70 ng/L. See: United States Environmental Protection Agency. Lifetime Drinking Water Health Advisories for Four Perfluoroalkyl Substances. Fed. Reg. 87(18): 36848-36849. June 21, 2022. https://www.govinfo.gov/content/pkg/FR-2022-06-21/pdf/2022-13158.pdf

p. 80, lines 2-4. As shown in the ITRC table (Excel file) cited above, the higher values mentioned in this sentence are groundwater remediation levels, not drinking water guidelines. Cordner et al. (2019) may have inadvertently referred to them as drinking water guidelines.

p. 80, lines 11-13 states: "Acknowledging the significant uncertainties and absence of consensus with identifying the critical health endpoint to calculate a HBGV and the rapidly evolving science, a pragmatic solution is therefore proposed for the derivation of provisional guideline values (pGVs)."

This sentence is misleading because it implies that a provisional guideline cannot be based on health effects. WHO has developed many provisional guidelines based on health effects and could potentially do so for PFAS as well. In other words, "provisional guideline" does not mean "treatment-based guideline.

It should also be noted that most WHO health-based drinking water guidelines are solely based on animal data, and there is little or no health effects information from human studies for many contaminants. When human data are available, they are often from highly exposed workers who are exposed occupationally. It is rare to have so much data indicating multiple health effects in the general population exposure range, along with a comprehensive database of animal toxicity studies, as are available for PFOA and PFOS. The fact that existing guidelines are based on a variety of health effects reflects the large number of different health endpoints identified in the extensive PFOA and PFOS database. The large number of health effects of concern should not be used as an excuse not to develop a protective health-based guideline.

p. 80, lines 9-29, Section 9.2 on derivation of provisional guideline values.

The provisional guideline values (pGVs) of 0.1 µg/L (100 ng/L) for PFOS and PFOA presented in the draft document are not based on health-effects, but rather are stated to be based on consideration of occurrence, analytical methods, and treatment achievability for PFAS. It is further stated that optimized treatment can reliably reduce PFOA and PFOS levels to "below 0.1

μg/L" and that the pGVs are derived partially based on this achievability of treatment. NJDEP disagrees with this statement which implies that lower levels cannot be reliably achieved. Based on NJDEP's experience and understanding, current treatment technologies are capable of reducing PFOA and PFOS much lower than 0.1 μg/L (100 ng/L). Furthermore, NJDEP notes that the draft background document does not provide any specific calculation or supporting evidence as to how or why these specific values of 0.1 μg/L (100 ng/L) were chosen. In summary, the derivation of these pGVs appears arbitrary and are not supported by the data presented in the draft document and other relevant data reviewed in the comments on Section 8, above.

Additionally, the draft document states that the pGVs of 0.1 ug/L (100 ng/L) correspond to greater than 90% removal efficiency of PFAS treatments such as granular activated carbon (GAC), ion exchange (IEX), and membrane filtration when considering that the upper-bound concentrations of PFAS in drinking water have been in the low μg/L range. However, using the upper-bound concentration of PFAS in drinking water to derive a guidance value is not a protective approach, especially since health effects information from both humans and experimental animal studies clearly supports health-based values far below 0.1 ug/L (100 ng/L).

The draft document (Section 2.1.2) states that PFOA and PFOS are frequently detected at low concentrations (in the nanograms per liter [ng/L] range) in surface and groundwater in many countries, and it goes on to cite several studies reporting levels of PFAS in various regions of the world. The highest levels mentioned are 0.046 μg/L (46 ng/L) for PFOS and 0.044 μg/L (44 ng/L) for PFOA, both of which are below 0.1 μg/L (100 ng/L), with the exception of higher values reported in the UCMR 3 monitoring study conducted in the U.S. between 2013 and 2015. However, although the maximum UCMR3 results for PFOA and PFOS cited are 0.349 μg/L (349 ng/L) and 7.0 μg/L (7000 ng/L), respectively, the range of average UCMR3 levels for public water systems with different types of source water (ground water, ground water under direct influence of surface water, surface water, or a combination of these, are much lower), 0.026-0.045 μg/L (26-45 ng/L) for PFOA and 0.042-0.199 μg/L (42-199 ng/L) for PFOS.

Additionally, it is important to recognize that UCMR3 used older Reporting Levels (20 ng/L for PFOA; 40 ng/L for PFOS) that are much higher than current USEPA Reporting Levels of 4 ng/L for both PFOS and PFOA. Therefore, the UCMR3 data are not comparable to other studies reviewed in the WHO draft that used lower Reporting Levels. It is noted that concentrations of PFOA and PFOS below the UCMR3 Reporting Levels have been frequently reported throughout the U.S. in more recent monitoring and that these lower detections are not included in the UCMR3 data. Therefore, the average UCMR3 values presented above do not represent current information on the occurrence of PFAS in the U.S.

Most detections of PFOA and PFOS in source water are < 100 ng/L. If pGVs of 0.1 ug/L (100 ng/L) are established, a major concern is that the many facilities that use source water with PFOA and PFOS levels below 100 ng/L may choose not to take any measures to reduce levels of these PFAS in their finished water, even though the health risks of exposure to concentrations

below 100 ng/L are clear. Drinking water guidelines that assume the same removal efficiency (90%) used to determine the draft pGVs for the lower PFOA and PFOS concentrations that are much more common in source water would be far lower than 0.1 µg/L (100 ng/L).

7. Additional comments

NJDEP's general comments on the draft document, including a summary of the most important points from the line-by-line comments above, are as follows:

- The draft WHO document must undergo extensive revision if it is to be finalized. The current draft includes numerous factual errors and often misquotes/misinterprets the conclusions of its sources. Overall, the draft document appears to inappropriately minimize and/or dismiss numerous conclusions about health effects and drinking water treatment removal of PFAS that are generally accepted by the scientific and technical community.

- The WHO website states that PFOA, PFOS, and PFAS are being "considered" for a drinking water quality guideline. If WHO does not develop a stringent public health-protective guideline, it would be preferable for WHO to remain silent on this topic instead of finalizing a drinking water quality guideline (provisional or otherwise) for PFAS. The information on treatment removal reviewed above demonstrates that such a stringent public health-protective guideline is readily achievable.

- The draft document lacks an overview of the fundamental reasons why PFOA, PFOS, and other PFAS differ from other drinking water contaminants and why they are of high concern when present in drinking water. This information is provided in the section on "Additional key points not included in the draft document that should be emphasized by WHO" below.

- The draft document often emphasizes relatively unimportant details from one or a few studies (which may not be the most important ones) without overall synthesis of the available information on the topic. It is recognized that this document is not intended to provide a comprehensive review of all available studies. However, conclusions, particularly for epidemiological data, must be made based on overall weight of evidence. As such, it is not helpful or informative to summarize a small subset of the available studies of a certain health endpoint without providing an overall conclusion on the weight of evidence.

- The extent of the human and animal health effects database and the level of certainty for human health effects of PFOA and PFOS far exceed the evidence for many or most of the other contaminants for which WHO has developed drinking water guidelines. The data set for PFOA and PFOS includes numerous health effects studies from the general population, communities with drinking water exposure, and occupationally exposed workers, as well as comprehensive information on animal toxicity. In contrast, the WHO drinking water guidelines for many other contaminants are based solely on animal data. There is little or no

human health effects information for many other contaminants with WHO drinking water guidelines, and even when human data are available, they are often from highly exposed workers, not the general population or impacted communities whose exposures are most relevant to drinking water guidelines.

- The large number of different health endpoints identified in the extensive PFOA and PFOS database is not a valid reason not to develop protective health-based drinking water guidelines. Rather, the numerous health effects of PFOA and PFOS highlight the need for concern about exposure to PFAS in drinking water and the need for health-based guidelines that are protective for the most sensitive health endpoint(s). There are ample data from both humans and experimental animals to develop a health-based guideline for cancer and non-cancer effects of PFOA and non-cancer effects of PFOS, as has been done by a number of authoritative bodies.

- Current drinking water treatment technologies are capable of reducing PFOA and PFOS to much lower than 0.1 µg/L (100 ng/L). The draft background document does not provide any specific calculation or supporting evidence as to how or why the treatment-based pGV values of 0.1 µg/L (100 ng/L) were chosen. These pGVs are not supported by the treatment removal and occurrence data presented in the draft document or other relevant data reviewed in the line-by-line comments above. As such, the derivation of these pGVs appears to be arbitrary.

- WHO's use of upper-bound concentrations of PFOA and PFOS in drinking water (1 µg/L; 1000 ng/L) to derive guidance values based on a certain percent treatment removal (90%) is not a protective approach, especially since health effects data support health-based values far below 0.1 µg/L (100 ng/l). If pGVs of 0.1 ug/L (100 ng/L) are established, a major concern is that the many facilities that use source water with PFOA and PFOS levels below 100 ng/L may choose not to take any measures to reduce levels of these PFAS in their finished drinking water. Drinking water guidelines that assume the same removal efficiency (90%) that was used to determine the draft pGVs for the lower PFOA and PFOS concentrations that are much more common in source water would be far below 0.1 µg/L (100 ng/L).

Additional key points not included in the draft document that should be emphasized by WHO are:

- PFAS differ from other drinking water contaminants because they are persistent, bioaccumulative, and toxic (PBT) chemicals that are soluble in water, and for which drinking water is an important exposure route. These properties distinguish PFAS from other well-known PBT chemicals (such as PCBs and dioxins) that have low water solubility. For other PBT chemicals, drinking water is not an important exposure source; most exposure is through fat in the diet (e.g., meat, dairy) not drinking water.

- Due to their long human half-lives (several years), PFOA, PFOS, and other long-chain PFAS bioaccumulate from drinking water to human blood serum. For example, the PFOA level in an individual's blood serum builds up to more than 100 times the level in the person's drinking water. Ongoing exposure to even low levels in drinking water dominates exposures from generally prevalent sources (e.g., food/food packaging, consumer products). It takes many years for elevated body burdens to decrease after exposure to contaminated drinking water ends.

- Exposure to even low levels of PFOA and PFOS in drinking water is of concern because multiple human health effects are observed within the exposure range prevalent in the general population, even without additional exposure from drinking water.

- PFOA and PFOS also cause multiple types of toxicity in laboratory animals, including at low doses. The toxicological effects in experimental animals (e.g., hepatic, developmental, immunologic, tumorigenic) are generally concordant with the health effects observed in human epidemiology studies. Based on mode of action analysis, toxicological effects in laboratory animals are considered relevant to humans.

- Infants receive higher exposures than older individuals from contaminated drinking water. This is because: 1) infants (breastfed and formula-fed) ingest more water on a body weight basis than older individuals, and 2) PFOA and PFOS levels are higher in breast milk than in the mother's drinking water. Serum levels of PFOA and PFOS increase several-fold during the first 6 months after birth, with peak serum PFOA levels in a breastfed infant predicted to be six times higher than in their mother. These higher exposure and serum levels are of particular concern because infants are a sensitive subpopulation for developmental effects of PFOA and PFOS.

- Relatively small changes in the health parameters associated with PFOA and PFOS in humans are of public health concern. Most epidemiological studies of PFOA and PFOS evaluate changes in the numerical values of the health parameters of interest rather than increased risk of clinically defined abnormal values. However, associations of PFOA and/or PFOS with increased risk of clinically defined abnormal values have been reported for each of the most well-established non-cancer health effects (increased cholesterol, increased ALT, decreased birth weight, decreased response to vaccination). Additionally, even when the number of subjects with clinically abnormal values is not specifically evaluated, an increase in the number of subjects with a clinically abnormal value is expected from the overall change (shift in the distribution curve) in the abnormal direction.

Key conclusions presented in the draft WHO document with which NJDEP disagrees include:

- NJDEP does not agree with WHO that a detectable increase in human disease is required for use of a health endpoint as the basis for a drinking water guideline. Relevant to this point, WHO (2022) guidance for developing health-based drinking water values states (p. 158) that

health-based guidelines are established when: "There is credible evidence of occurrence of the chemical in drinking-water, combined with evidence of actual or potential toxicity." Few if any drinking water guidelines for other chemical contaminants are based on measurable increases in human disease.

- NJDEP disagrees with WHO that decreased vaccine response associated with PFOA and PFOS should be dismissed from consideration for risk assessment because it is "not clinically relevant." NJDEP concurs with the conclusions of other authoritative bodies, including EFSA and USEPA, that decreased antibody response to vaccinations linked to PFOA and PFOS is adverse and that it is an appropriate basis for risk assessment. As discussed in detail in the line-by-line comments above, WHO's statement that increased incidence of infection is not definitively associated with PFOA and PFOS is not a valid reason to dismiss the use of decreased vaccine response as the basis for drinking water guidelines. NJDEP also emphasizes that the lack of an association of PFOA and PFOS with increased incidence of infectious disease has not been established.

- NJDEP disagrees with WHO's conclusion that the increased serum levels of the liver enzyme ALT associated with PFOS and PFOA in humans are not adverse because of their "low magnitude" and "lack of associated liver disease." As discussed in the line-by-line comments above, the increased ALT associated with PFOA and PFOS is indicative of liver damage, an adverse health effect. NJDEP further notes that PFOA was associated with clinically-defined elevated ALT (not just a numerical increase in ALT) in several studies, that relatively small increases in ALT are associated with pathology-confirmed liver disease such as non-alcoholic fatty liver disease, and that authoritative medical groups have concluded that elevated ALT is associated with overall health and mortality.

- NJDEP disagrees with WHO that differing results in laboratory animal and human studies of the effects of PFOA and PFOS on serum lipids suggest that the increase in cholesterol in humans is not causal. The basis for NJDEP's conclusion is discussed in line-by-line comments above.

- NJDEP disagrees with WHO's general conclusion that the human relevance of adverse health effects reported in animals is uncertain. This WHO conclusion is not scientifically supportable and contradicts the conclusions of all of the authoritative agencies whose reviews were heavily relied upon by WHO (e.g., USEPA, Health Canada, EFSA, ATSDR). Specifically, NJDEP and the authoritative agencies mentioned above disagree with WHO's premise that PPAR-alpha mediated toxicological effects of PFOA and PFOS (including non-hepatic effects such as developmental effects) are not relevant to humans.

- NJDEP disagrees with WHO's premise that toxicokinetic differences between humans and animals preclude the development of health-based guidelines based on animal data. All health-based drinking water guidelines based on animal data consider interspecies toxicokinetic differences. Models that consider these interspecies differences have been developed for PFOA and PFOS and have been used in development of toxicity factors and drinking water guidelines for these PFAS.

- NJDEP disagrees with the conclusion that application of the Hall et al. (2012) criteria indicates that hepatic effects of PFOA and PFOS in laboratory animals are not relevant to human risk; detailed line-by-line comments are provided above. The Hall et al. (2012) criteria's conclusions about adversity of increased liver weight and hepatocellular hypertrophy assume that these effects may be reversible when exposure to the agent (e.g., a drug) ends. However, these reversibility considerations are not relevant to chronic drinking water guidelines in which exposure is not assumed to end after a certain period of time. Importantly, increased liver weight from short term exposure to PFOA and PFOS has been shown to progress over time to more severe forms of hepatic toxicity.

- NJDEP disagrees with the WHO conclusion that immunotoxicity caused by PFOA in laboratory animals "usually" occurs only at higher doses that cause decreased food intake and body weight, suggesting indirect effects of PFOA on the immune system. In several studies that included lower doses, PFOA caused immunotoxicity at doses that did not affect food intake or body weight.

- NJDEP notes that the range of "health-based drinking water values derived by authoritative agencies" of "0.05 to 0.6 µg/L for PFOS and from 0.05 to 0.56 µg/L for PFOA" in the draft document (p. 79, lines 27-32) is not accurate and omits at least two values that are far below the ranges provided. The drinking water guideline of 2 ng/L for the total of PFOA, PFOS, PFNA, and PFHxS established by Denmark in 2021 was not considered in WHO's ranges of values and is omitted from WHO's table of health-based drinking water values. Additionally, although the interim Health Advisories established by USEPA in 2022 are mentioned in WHO's table, they are not considered in the ranges of values in the text and are included only as a footnote to the text.

- Finally, NJDEP strongly disagrees that a drinking water guideline based on the assumption of 90% treatment removal of a very high concentration (1 µg/L; 1000 ng/L) of PFOA or PFOS that rarely occurs in source water is technically supportable or public health-protective. Detailed supporting information is provided above.